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**Title:** Autochthonous organic matter promotes DNRA and suppresses N<sub>2</sub>O production in sediments of the coastal Baltic Sea

**Year:** 2021

**Version:** Accepted version (Final draft)

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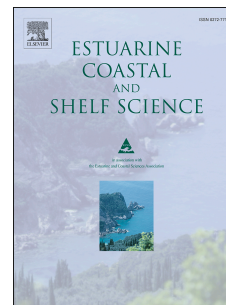
**Please cite the original version:**

Aalto, S. L., Asmala, E., Jilbert, T., & Hietanen, S. (2021). Autochthonous organic matter promotes DNRA and suppresses N<sub>2</sub>O production in sediments of the coastal Baltic Sea. *Estuarine, Coastal and Shelf Science*, 255, Article 107369. <https://doi.org/10.1016/j.ecss.2021.107369>

# Journal Pre-proof

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PII: S0272-7714(21)00222-5

DOI: <https://doi.org/10.1016/j.ecss.2021.107369>

Reference: YECSS 107369

To appear in: *Estuarine, Coastal and Shelf Science*

Received Date: 14 May 2020

Revised Date: 31 January 2021

Accepted Date: 6 April 2021

Please cite this article as: Aalto, S.L., Asmala, E., Jilbert, T., Hietanen, S., Autochthonous organic matter promotes DNRA and suppresses N<sub>2</sub>O production in sediments of the coastal Baltic Sea, *Estuarine, Coastal and Shelf Science* (2021), doi: <https://doi.org/10.1016/j.ecss.2021.107369>.

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# 1 Autochthonous organic matter promotes DNRA and suppresses N<sub>2</sub>O 2 production in sediments of the coastal Baltic Sea

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## 14 Abstract

15 Coastal environments are nitrogen (N) removal hot spots, which regulate the amount of land-  
16 derived N reaching the open sea. However, mixing between freshwater and seawater creates  
17 gradients of inorganic N and bioavailable organic matter, which affect N cycling. In this study, we  
18 compare nitrate reduction processes between estuary and offshore archipelago environments in the  
19 coastal Baltic Sea. Denitrification rates were similar in both environments, despite lower nitrate and  
20 carbon concentrations in the offshore archipelago. However, DNRA (dissimilatory nitrate reduction  
21 to ammonium) rates were higher at the offshore archipelago stations, with a higher proportion of  
22 autochthonous carbon. The production rate and concentrations of the greenhouse gas nitrous oxide  
23 (N<sub>2</sub>O) were higher in the estuary, where nitrate concentrations and allochthonous carbon inputs are  
24 higher. These results indicate that the ratio between nitrate and autochthonous organic carbon  
25 governs the balance between N-removing denitrification and N-recycling DNRA, as well as the end-  
26 product of denitrification. As a result, a significant amount of the N removed in the estuary is  
27 released as N<sub>2</sub>O, while the offshore archipelago areas are characterized by efficient internal recycling  
28 of N. Our results challenge the current understanding of the role of these regions as filters of land-  
29 to-sea transfer of N.

30 Keywords: denitrification; DNRA; DOM; estuary; N<sub>2</sub>O; sediment organic matter

## 31 **1 Introduction**

32 Coastal systems are transitional zones where riverine freshwater mixes with saline seawater. They  
33 are important hot spots in the nitrogen (N) cycle, as N transformations in coastal ecosystems regulate the  
34 amount of land-derived N reaching the open sea (Bouwman et al., 2013). Various coastal processes,  
35 including assimilation to biomass and subsequent microbial degradation of organic matter, modulate  
36 land-to-sea transfer of N. Crucially, N may be removed from biogeochemical cycling in estuaries by a  
37 sequence of sedimentary microbial processes terminating in denitrification, which releases dinitrogen gas  
38 (N<sub>2</sub>) into the atmosphere. Denitrification is a critical part of the 'coastal filter'; the set of biogeochemical  
39 processes regulating the impact of riverine nutrient inputs on coastal eutrophication (Asmala et al.,  
40 2017).

41 Denitrification rates in coastal environments depend on nitrate concentrations, which typically  
42 decrease from near-shore to offshore areas (Asmala et al., 2017). However, heterotrophic  
43 denitrification also depends on the presence of bioavailable organic carbon (OC) in coastal  
44 sediments (Helleman et al., 2017; Hietanen and Kuparinen, 2008). Higher OC bioavailability has  
45 been suggested to promote denitrification in freshwater stream sediments (Barnes et al., 2012;  
46 Stelzer et al., 2014), raising the question of whether the same is true in coastal marine systems.  
47 Coastal systems often display strong gradients in both nitrate concentrations, and in sedimentary OC  
48 sources and characteristics, with distance away from river mouths. Typically, the relative amount of  
49 terrestrial OC in sediments decreases gradually along the coastal salinity gradient, while the amount  
50 of fresh, autochthonous phytoplankton-derived OC increases (Fellman et al., 2011; Goñi et al., 2003;  
51 Spencer et al., 2007). Combined, these observations suggest that coastal nitrate removal efficiency  
52 through denitrification could be related to the availability of both nitrate and bioavailable OC  
53 (Asmala et al., 2017).

54 The balance in the availability of nitrate and bioavailable carbon may also influence rates of  
55 alternative nitrate reduction pathways. Heterotrophic dissimilatory nitrate reduction to ammonium  
56 (DNRA), which retains N as biologically reactive ammonium in the aquatic system (e.g., Giblin et al.,  
57 2013), is the prominent pathway under conditions of high OC availability relative to nitrate (Hardison et  
58 al., 2015; Kraft et al., 2014). This phenomenon may occur because under nitrate-limited conditions, DNRA  
59 makes more efficient use of the available electron acceptors (6 electrons transferred per mole of N  
60 reduced compared to 3 for denitrification), and therefore maximizes entropy production (Algar and  
61 Vallino 2014). Furthermore, OC composition is as important as OC availability in controlling the nitrate  
62 reduction end-product (Carlson et al., 2020). From this, it follows that the importance of DNRA in net  
63 nitrate reduction may increase towards the open sea where terrestrial influence decreases (lower nitrate

64 and higher bioavailable carbon concentrations). Indeed, high contributions of DNRA to total nitrate  
65 reduction were recently observed in the Baltic Sea offshore region (Hellemann et al., 2020) and in  
66 Australian estuaries (Kessler et al., 2018). Therefore, outer coastal areas may recycle nitrate more  
67 efficiently than remove it, in comparison with near-shore areas with a lower bioavailable OC to nitrate  
68 ratio, which favours denitrification.

69 Incomplete denitrification leads to the production of nitrous oxide ( $N_2O$ ). The proportion of  $N_2O$   
70 production from total denitrification can increase with DIN concentrations (Murray et al., 2015), and  
71 decrease with increased bioavailable carbon (Zhao et al., 2014). This suggests that among other variables  
72 (e.g. oxygen, temperature, salinity, and rates of nitrogen fixation and nitrification; Foster and Fulweiler,  
73 2016; Silvennoinen et al., 2008; Zhao et al., 2014), OC bioavailability is an important factor controlling  
74 denitrification-derived  $N_2O$  production in coastal ecosystems, and  $N_2O$  production the rates may be  
75 higher in near-shore estuarine environments with low amounts of bioavailable OC and high nitrate  
76 concentrations. Hence, OC characteristics and especially bioavailability may play a key role in many  
77 aspects of coastal sedimentary N cycling. These factors must be deconvolved from the effects of  
78 nitrate gradients to properly understand the coastal N cycle.

79 The overall bioavailability of aquatic OC can be assessed with optical proxies of dissolved organic  
80 matter (DOM), derived from the absorbance and fluorescence properties of the colored dissolved  
81 organic matter (CDOM) (Asmala et al., 2013). A range of optical proxies (e.g. the humification index  
82 (HIX) and the index of recent autochthonous contribution (BIX)) have been derived to characterize  
83 the DOM pool (Huguet et al., 2009; Murphy et al., 2008). We assume DOM in sediment porewaters  
84 to reflect the broad overall organic matter composition of sediments, and optical analysis of  
85 porewater DOM composition provides a tool for characterizing the source and bioavailability of  
86 sedimentary carbon. Porewater DOM characterization potentially provides additional information to  
87 traditional approaches such as C/N ratios or  $\delta^{13}C$  of bulk organic matter.

88 Here, we investigate the combined influence of nitrate availability and organic matter composition  
89 on nitrate reducing processes in coastal sediments in the northern Baltic Sea. The Baltic is a semi-  
90 enclosed shallow brackish water basin with significant anthropogenic N loading. In 2010, the total N load  
91 to the Baltic was 977 000 tons, of which 758 000 tons was waterborne (Helcom 2015), yielding a  
92 waterborne N load from the catchment of 0.44 tonnes/km<sup>2</sup>. The Baltic Sea coastal zone (29% of total  
93 Baltic Sea area) was estimated to remove 16% of land-derived N inputs, the N removal efficiency varying  
94 between different types of coastal ecosystems (Asmala et al. 2017). Denitrification dominates  $N_2$   
95 production in Baltic Sea coastal ecosystems, with anammox playing only a minor role (Bonaglia et al.,  
96 2014; Hietanen, 2007; Thamdrup and Dalsgaard, 2002). Knowledge on the balance between

97 denitrification and DNRA is limited for this region, but results from an anthropogenically impacted Baltic  
98 Sea estuary suggest that denitrification is the main process (Bonaglia et al., 2014) due to the high DIN  
99 availability, and that the contribution of DNRA increases to 30-50% of total nitrate reduction in the  
100 offshore region (Hellemann et al., 2020). The limited data from oligotrophic coastal sediments of the  
101 Baltic Sea, where availability of labile organic carbon limits the denitrification process, also indicate that  
102  $N_2O$  production from benthic denitrification is low ( $N_2O:N_2 < 0.02$ ) (Hellemann et al., 2017).

103 In this study, we measured porewater DOM characteristics, nitrous oxide concentrations, and N  
104 processes along a gradient encompassing near-shore (estuary) and offshore archipelago stations in a  
105 coastal region of the Baltic Sea to examine the effects of both nitrate availability and OC  
106 characteristics on nitrate reduction processes. We hypothesized that higher nitrate availability and  
107 terrestrial dominance of the carbon pool (i.e. low quantities of bioavailable carbon compared to  
108 nitrate) would promote denitrification and possibly  $N_2O$  production at the near-shore estuarine  
109 stations. Conversely, we hypothesized that the significance of DNRA as a nitrate reduction process  
110 would increase at the offshore archipelago stations due to a higher amount of bioavailable carbon  
111 and/or lower nitrate concentrations.

## 112 **2 Materials and methods**

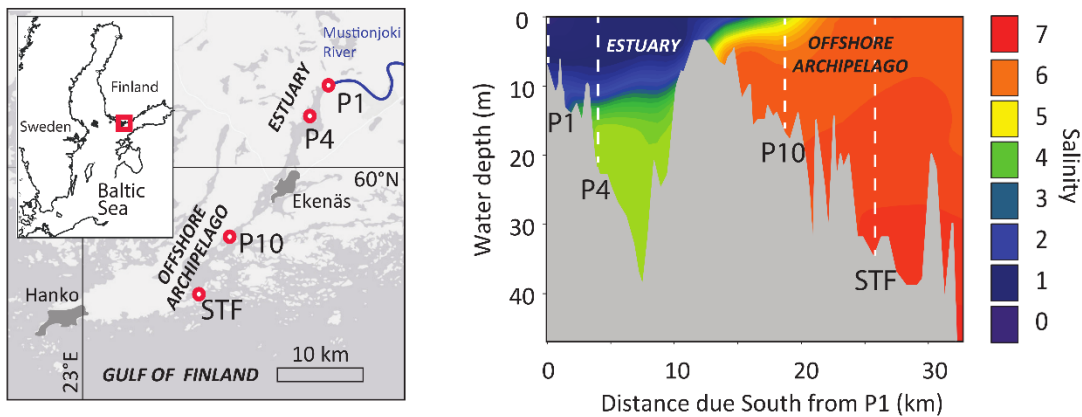
### 113 **2.1 Study area, sampling, and water column analyses**

114 The study was conducted in the Finnish coastal area of the Gulf of Finland, Baltic Sea.  
115 Pohjanpitäjänlahti is a long and narrow embayment that receives freshwater input from the river  
116 Mustionjoki and brackish water input from the adjacent coastal archipelago of the Baltic Sea (Fig. 1).  
117 A shallow (2-3 m) sill area, with a dredged 6 m channel through it, separates the estuary from the  
118 offshore region connecting to the open Baltic Sea, limiting the water exchange between the estuary  
119 and the offshore region. The inner basin is salinity-stratified, with a pronounced pycnocline at 10-15  
120 m water depth, which leads to seasonal hypoxia in summer and autumn. Inflows of brackish water  
121 over the sill usually occur in late autumn – early winter, leading to temporary ventilation of the basin  
122 (Malve et al., 2000). The adjacent offshore region experiences temperature stratification in summer,  
123 leading to the development of hypoxia in isolated areas. However, much of that remains oxic  
124 throughout the annual cycle due to sufficient vertical mixing and exchange of water masses. The  
125 catchment of the Mustionjoki has a large proportion of lakes (11%; Mattsson et al., 2005) and  
126 several hydropower plants regulating the flow. This characteristic leads to extensive processing of  
127 the riverine nutrients and organic matter already within the lotic system and relatively low area-  
128 specific loading of organic carbon to the estuary (Räike et al., 2012).

129 To monitor water column N<sub>2</sub>O concentrations, water column sampling was conducted at stations P4  
130 (“estuary”, see Fig. 1) and STF (“offshore archipelago”) at 5 m depth intervals using a 5L Limnos  
131 sampler on multiple occasions during 2015-2017. Subsamples for determination of dissolved N<sub>2</sub>O  
132 were collected in triplicate by filling 60mL plastic syringes directly from a Limnos water sampler on  
133 board. In the laboratory, the water volume in the syringe was reduced to 30 mL, and 31mL of 5.0  
134 purity N<sub>2</sub> gas was injected to create a headspace. Syringes were left at 20 C for 30 min and then  
135 vigorously shaken for 3 min, after which 25mL of the headspace was injected into a pre-evacuated  
136 12mL gastight glass vial (LabCo Exetainer model 839W). Nitrous oxide concentrations in the  
137 headspace were determined using an Agilent Technologies 7890B gas chromatograph equipped with  
138 electron capture detector (ECD) and the results calculated as in Myllykangas et al. (2017).

139 Sampling was carried out at two stations in the estuary (stations P1 and P4) on 6<sup>th</sup> of June 2017 and  
140 15<sup>th</sup>–16<sup>th</sup> of August 2017 and at two stations in the offshore archipelago region (stations P10 and  
141 STF; Fig. 1) only on 15<sup>th</sup>–16<sup>th</sup> of August 2017. Sampling occasions were chosen to represent situations  
142 with high (June) and low (August) amount of fresh, recently deposited phytoplankton-derived  
143 material on the sediment surface (Heiskanen and Kononen, 1994). Temperature, salinity and oxygen  
144 were determined using a YSI CTD equipped with an optical oxygen sensor. Sediment cores were  
145 collected using a Gemax twin sampler (core diameter 9 cm, length of a core 30 - 50 cm) from each  
146 sampling station. Water samples were collected using a 5L Limnos water sampler from 1 m depth to  
147 1 m above the sediment at 2-5 m intervals, and from the overlying water of the sediment cores.  
148 Oxygen samples for Winkler titration (150 ml) were treated immediately with fixing reagents and  
149 analyzed the following day. Dissolved inorganic nitrogen (ammonium, nitrite and nitrate) samples  
150 were collected in acid-washed plastic bottles, filtered through 0.2µm polycarbonate filters and  
151 stored dark at 4°C. Concentrations were measured using a discrete photometric analyzer (Thermo  
152 Scientific Aquakem 250) the following day. Theoretical 3-sigma detection limits were as follows:  
153 ammonium 0.11 µM, nitrate and nitrite 0.08 µM.

154 Figure 1. (left) Sampling locations in the Pohjanpitäjänlahti system on the Finnish coast of the Gulf of  
155 Finland, northern Baltic Sea. Stations P1 and P4 are classified as “estuary” stations, while P10 and  
156 STF are classed as “offshore archipelago”. The Mustionjoki river discharges into the  
157 Pohjanpitäjänlahti estuary close to station P1. (right) Bathymetric detail of the transect through the  
158 sampling locations, showing typical salinity distribution (data shown here from June 2015, redrawn  
159 from Jilbert et al., 2018). A shallow sill close to the city of Ekenäs restricts exchange of brackish  
160 deeper waters between the offshore archipelago and estuary.



161

## 162 2.2 Sediment and porewater analyses

163 Sediment cores were collected using a Gemax twin sampler (core diameter 9 cm, length of core 30 -  
 164 50 cm) from each sampling station. Sediment water content and porosity were determined from the  
 165 upper portion of each core (0–6 cm) (Burdige, 2006). Sediment total C and N content (%C, %N) of the  
 166 upper portion was determined by Thermal Combustion Elemental Analysis (TCEA) at Tvärminne  
 167 Zoological Station with precision and accuracy of < 2.5% RSD. Sedimentary inorganic carbon and  
 168 nitrogen are assumed insignificant in this setting, hence %C<sub>tot</sub> and %N<sub>tot</sub> are assumed equal to  
 169 organic carbon and nitrogen, respectively (%C<sub>org</sub> and %N<sub>org</sub>).

170 Porewater DOC and CDOM samples were taken from the surface sediment layer (0-1 cm) of three  
 171 replicate cores. In the laboratory, pore water was extracted with centrifuging (1500 rpm for 10 min),  
 172 and filtered through a combusted (4 h 450 °C) glass fiber filter (47 mm, VWR collection GF/F). DOC  
 173 concentration in porewaters was measured with a Shimadzu TOC-V<sub>CPH</sub> analyzer. The detection limit  
 174 for DOC analysis was 40 μmol L<sup>-1</sup>. CDOM absorption was measured using a Shimadzu 2401PC  
 175 spectrophotometer with 1 cm quartz cuvette over the spectral range from 200 to 800 nm with 1 nm  
 176 intervals. Ultrapure water served as the blank for all samples. Excitation-emission matrices (EEMs) of  
 177 fluorescent DOM (FDOM) were measured and corrected as in Asmala et al., (2018). For assessing the  
 178 terrestrial signature of the porewater DOM, fluorescence peaks (peaks A, C, M, and T; Coble, 1996),  
 179 humification index (HIX; Zsolnay et al., 1999) and biological index (BIX; Huguet et al., 2009) were  
 180 calculated from the measured and corrected EEMs. Processing of the EEMs was done using the  
 181 eemR package for R software (Massicotte, 2018).

## 182 2.3 Sedimentary nitrogen process rates

183 Samples for benthic nitrate reduction rate measurements (n=8 per sampling station) were collected  
 184 into acrylic cores (∅ 2.3 cm, length 15 cm), which were pushed gently into the sediment so that 1/3  
 185 of each core was filled with sediment and the rest with overlying water, capped and placed in a



186 water bath at *in situ* temperature. The four cores were immediately enriched with  $^{15}\text{N}$ -labelled  
187 nitrate to a final concentration of  $100\ \mu\text{M}\ ^{15}\text{N-NO}_3^-$  ( $\text{K}^{15}\text{NO}_3$  Sigma Aldrich, 98%  $^{15}\text{N}$ -atm), closed and  
188 incubated under stirring at *in situ* temperature in dark for 3-4 h. Enrichment with  $200\ \mu\text{M}\ ^{15}\text{N-NH}_4^+$   
189 ( $^{15}\text{NH}_4\text{Cl}$  Cambridge Isotope Laboratories, 99%  $^{15}\text{N}$ -atm; 4 replicate cores) was used to exclude  
190 anammox and measure nitrification (data not shown). After incubation, sediment and overlying  
191 water in the samples were mixed and 12 mL subsamples were transferred into gas-tight glass vials  
192 (Labco Exetainer model 739W) with 0.5 mL  $\text{ZnCl}_2$  (100 % w/v, Merck) after a brief sediment settling  
193 period. Isotopic composition of  $\text{N}_2$  and  $\text{N}_2\text{O}$  was analysed with a TraceGas preconcentrator system  
194 interfaced with an IsoPrime 100 continuous flow isotope ratio mass spectrometer (CF-IRMS;  
195 Isoprime Ltd, Cheadle Hulme, UK) at the Department of Environmental Sciences, University of  
196 Jyväskylä, Finland as in Helleman et al., (2017). The detection limits were  $320\ \text{nmol L}^{-1}$  for  $^{29}\text{N}_2$ ,  $11$   
197  $\text{nmol L}^{-1}$  for  $^{30}\text{N}_2$ ,  $397\ \text{pmol L}^{-1}$  for  $^{45}\text{N}_2\text{O}$ , and  $322\ \text{pmol L}^{-1}$  for  $^{46}\text{N}_2\text{O}$ .

198 The remaining  $^{15}\text{NO}_3^-$ -enriched slurry was mixed again, and 20 mL samples for  $^{15}\text{NH}_4^+$  analysis were  
199 collected into 50 mL centrifuge tubes, treated with 1 mL of  $\text{ZnCl}_2$ , and frozen immediately. Before  
200  $^{15}\text{NH}_4^+$  analysis,  $\text{NH}_4^+$  attached to the sediment particles was desorbed using KCl extraction. The  
201 isotopic composition of  $\text{NH}_4^+$  in the samples was analyzed after conversion to  $\text{N}_2$  using alkaline  
202 hypobromite iodine solution (Risgaard-Petersen et al., 1995) as in Helleman et al., (2020). A  
203 standard series of  $^{15}\text{NH}_4^+$  (5; 10; 15  $\mu\text{M}$ , 5%  $^{15}\text{N}$ -atm from  $^{15}\text{NH}_4\text{Cl}$  Cambridge Isotope Laboratories,  
204 98%  $^{15}\text{N}$ -atm) was prepared, treated and analyzed parallel with samples to calculate conversion  
205 efficiency and  $^{15}\text{N}$  recovery, which was > 85 %.

206 The  $\text{N}_2$  and  $\text{N}_2\text{O}$  producing denitrification rates were calculated from the production rates of  $^{29}\text{N}_2$ ,  
207  $^{30}\text{N}_2$  and  $^{45}\text{N}_2\text{O}$ ,  $^{46}\text{N}_2\text{O}$ , and partitioned to denitrification based on water column nitrate ( $D_w$ ) and  
208 coupled nitrification-denitrification ( $D_n$ ) (Nielsen, 1992). DNRA rates were calculated from the  
209 production rates of  $^{15}\text{NH}_4^+$  and the production rates of  $^{29}\text{N}_2$ ,  $^{30}\text{N}_2$  and  $^{45}\text{N}_2\text{O}$ ,  $^{46}\text{N}_2\text{O}$  in the same  
210 incubation cores according to Christensen et al., (2000). It was assumed that DNRA takes place in the  
211 same layers as denitrification, meaning that the  $^{15}\text{N}$  labeling of  $\text{NO}_3^-$  reduced to ammonia equals the  
212  $^{15}\text{N}$  labeling of  $\text{NO}_3^-$  reduced to  $\text{N}_2/\text{N}_2\text{O}$ . Total  $\text{N}_2$  production ( $\sum\text{N}_2$ ) was calculated as  $\sum\text{N}_2 = D_w\text{-N}_2 +$   
213  $D_n\text{-N}_2$  and total  $\text{N}_2\text{O}$  production ( $\sum\text{N}_2\text{O}$ ) as  $\sum\text{N}_2\text{O} = D_w\text{-N}_2\text{O} + D_n\text{-N}_2\text{O}$ . The total denitrification was  
214 then defined as  $\sum\text{N}_2 + \sum\text{N}_2\text{O}$  and total nitrate reduction as  $\sum\text{N}_2 + \sum\text{N}_2\text{O} + \text{DNRA}$ . The hourly rates were  
215 scaled to day by multiplying with 24h. The  $\text{N}_2\text{O}$  produced in coupled nitrification-denitrification was  
216 divided into the rate of  $\text{N}_2\text{O}$  produced in the nitrification stage and the denitrification stage of the  
217 coupled nitrification-denitrification according to Dong et al., (2006).

## 218 2.4 Statistical analysis

219 The data analysis was conducted using R (version 3.6.3; R Core Team, 2020). The differences in the  
 220 porewater DOM characteristics, and N processes between estuary and offshore archipelago region  
 221 were examined with one-way ANOVA, or if the assumptions on the normality and equal variances  
 222 were not met, with Mann-Whitney U test. The relationship between DOM variables and N processes  
 223 were examined with Pearson correlation analysis, and relative DNRA (%DNRA) and N<sub>2</sub>O (%N<sub>2</sub>O) and  
 224 DOC and bioavailable carbon fraction (protein-like DOM fluorescence) were further examined with  
 225 linear regression.

## 226 3 Results

### 227 3.1 Hydrography

228 In both estuary and offshore archipelago, the water column was well oxygenated during the  
 229 sampling campaigns despite being stratified, with a thermocline present at all stations between 3.5-  
 230 10 m depth (Table 1; Suppl. Fig. 1). At the estuary stations, closer to the direct influence of the  
 231 Mustionjoki River, a pronounced halocline was present (Suppl. Fig. 1).

232 Table 1. Temperature (T), salinity, oxygen concentration (O<sub>2</sub>), and DIN concentrations (NO<sub>x</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) in  
 233 near-bottom water and sediment C:N at the estuary and offshore archipelago sampling stations.

	Station	Sampling time	T °C	salinity	O <sub>2</sub> μM	NO <sub>x</sub> <sup>-</sup> μM	NH <sub>4</sub> <sup>+</sup> μM	C:N
Estuary	P1	June 2017	5.8	4.0	234	11.1	0.7	18.7
	P4	June 2017	3.3	5.1	236	13.0	1.5	12.4
	P1	August 2017	13.8	3.3	155	1.7	3.2	21.6
	P4	August 2017	4.7	5.0	126	14.1	6.1	12.7
Offshore archipelago	P10	August 2017	10.1	6.2	176	1.6	5.8	11.2
	STF	August 2017	8.8	6.4	216	1.4	4.0	10.1

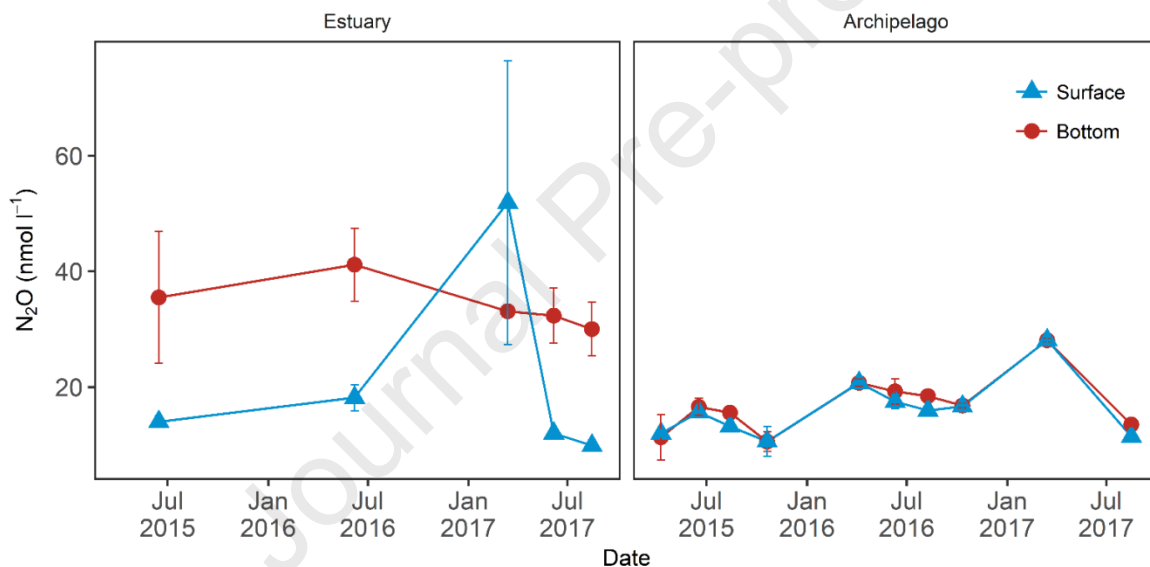
234

### 235 3.2 Dissolved inorganic nitrogen and nitrous oxide

236 The near-bottom combined nitrite+nitrate (NO<sub>x</sub><sup>-</sup>) concentrations decreased as expected from near-  
 237 shore estuary to offshore archipelago stations. At the estuary stations P1 and P4, near-bottom NO<sub>x</sub><sup>-</sup>

238 concentrations varied between 3-14  $\mu\text{M}$  (Table 1; Suppl. Fig. 2). Near-bottom  $\text{NO}_x^-$  concentrations  
 239 were consistently low ( $\leq 1.6 \mu\text{M}$ ) at the offshore archipelago stations P10 and STF. Near-bottom  
 240 ammonium ( $\text{NH}_4^+$ ) concentrations (1-6  $\mu\text{M}$ ) were similar at all sampling stations. Dissolved nitrous  
 241 oxide ( $\text{N}_2\text{O}$ ) concentrations were consistently high (25-50 nM at P4, Fig. 2) below the halocline in the  
 242 estuary. Surface waters at the offshore archipelago stations, P4 and STF, and deeper waters at STF,  
 243 had lower  $\text{N}_2\text{O}$  concentrations (10-30 nM), except for a high value in the surface waters of P4 under  
 244 ice cover in March 2017.

245 Figure 2. Nitrous oxide ( $\text{N}_2\text{O}$ ) concentration in water column above (blue triangles) and below  
 246 halocline (red circles) between April 2015 and August 2017 at the near-shore estuary (station P4)  
 247 and offshore archipelago (station STF) stations. Points indicate mean value and error bars  $\pm 1$   
 248 standard deviation. Number of observations per each mean value in the figure ranges between 3  
 249 and 24, the median number of observations being 10.



250

### 251 3.3 Organic carbon source proxies

252 All the sampled sediments were muddy, with surface (0-1 cm) porosities ranging from 0.94 to 0.97.  
 253 Sediment C:N ratio decreased from the estuary ( $16 \pm 5$ ) to the offshore archipelago stations ( $11 \pm 1$ )  
 254 (Table 1). The amount of bulk dissolved organic matter in the porewater, as indicated by the DOC  
 255 concentration, was almost twice as high at the estuary stations as at the offshore archipelago  
 256 stations (Fig. 3a). The ratio (mean  $\pm$  SD) between DOC concentrations in the uppermost sediment  
 257 layer (0-1 cm) and near bottom  $\text{NO}_x$  ( $\text{DOC}:\text{NO}_x^-$ ) was  $2.5 \pm 2.9$  at the estuary stations and  $4.8 \pm 1.3$  at  
 258 the offshore archipelago stations. Organic matter characteristics were on average more terrestrial-  
 259 like at the estuary than at the offshore archipelago stations (one-way ANOVA,  $p < 0.05$ ), as indicated  
 260 by optical proxies: higher CDOM absorption at 254 nm ( $a_{(\text{CDOM}254)}$ ), DOC-specific UV absorbance  
 261 ( $\text{SUVA}_{254}$ ), humic- and protein-like DOM fluorescence (peak C and T, respectively) and higher

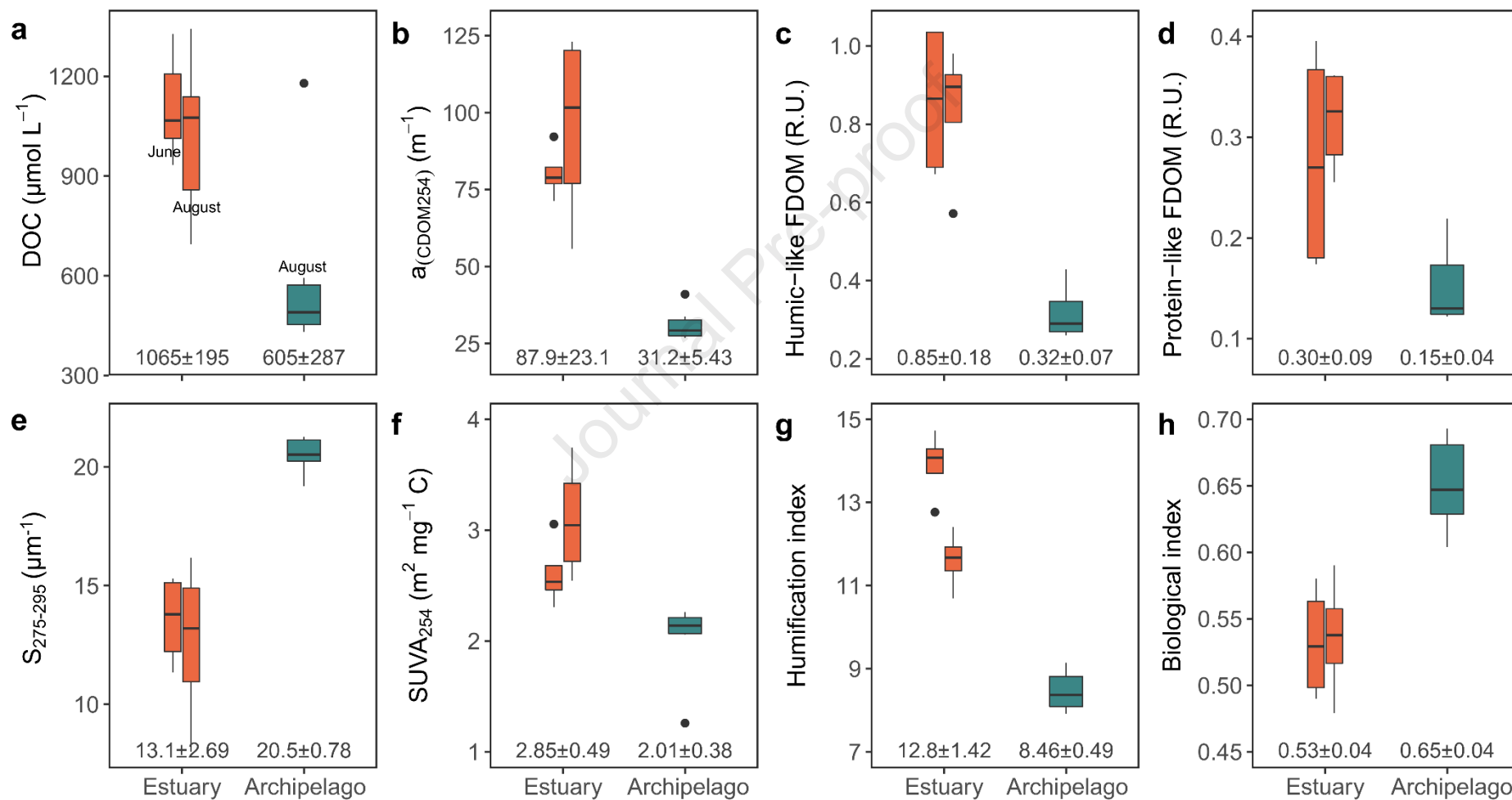
262 humification index (HIX). Also, at the offshore archipelago stations, UV absorption slope ( $S_{275-295}$ ) and  
263 biological index (BIX) were higher than at the estuary stations ( $p < 0.05$ ; Fig. 3), indicating higher  
264 contribution of autochthonous bioavailable carbon with smaller molecular size.

### 265 **3.4 Nitrogen transformation rates in estuary and offshore archipelago sediments**

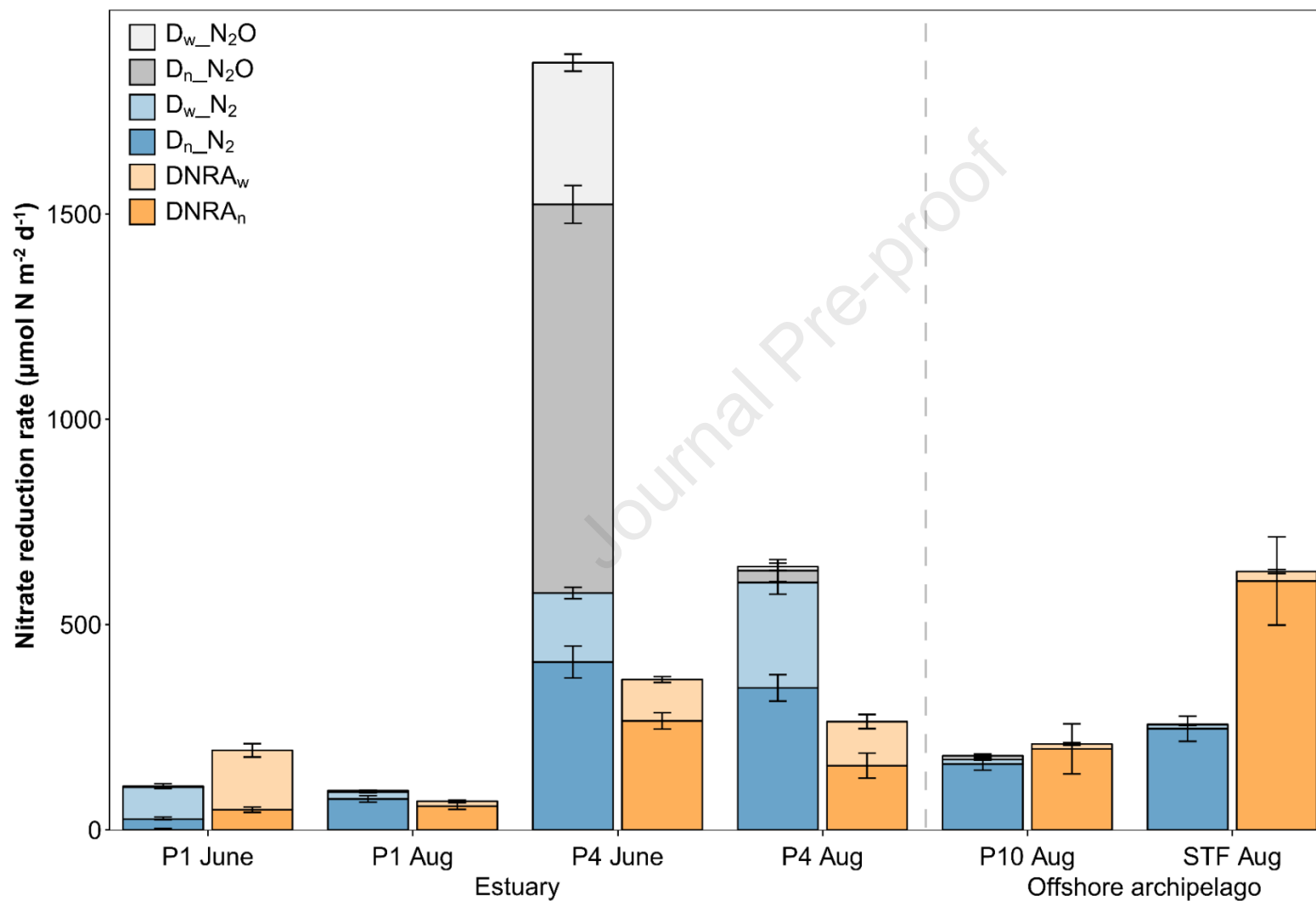
266 All nitrate reduction rates varied substantially between the sampling stations, as both the highest  
267 and lowest rates were measured at the estuarine stations (Fig. 4, Suppl. Fig. 3). Total denitrification  
268 ( $\sum N_2 + \sum N_2O$ ) rates and total nitrate reduction ( $\sum N_2 + \sum N_2O + DNRA$ ) rates did not differ significantly  
269 between the estuary and offshore archipelago stations (Mann-Whitney U test,  $p > 0.05$ , Fig. 4, Suppl.  
270 Fig. 3). No anammox was detected (data not shown). Denitrification rates based on water column  
271 nitrate ( $D_{w\_N_2}$ ,  $D_{w\_N_2O}$ ) were higher at the estuary stations ( $D_{w\_N_2}$ : one-way ANOVA,  $p = 0.004$ ;  
272  $D_{w\_N_2O}$ :  $p < 0.001$ ), but the coupled nitrification-denitrification process rates ( $D_{n\_N_2}$ ,  $D_{n\_N_2O}$ ) were  
273 dominant and equal between the estuary and offshore archipelago stations ( $p > 0.05$ , Fig. 4, Suppl.  
274 Fig. 3). Similarly, DNRA rates based on water column nitrate ( $DNRA_w$ ) were higher at the estuary  
275 stations ( $p = 0.006$ ), while total DNRA rates ( $p = 0.024$ ) and the proportion of DNRA of total nitrate  
276 reduction (%DNRA;  $p = 0.03$ ) and nitrification-fed DNRA ( $DNRA_n$ ;  $p = 0.003$ ) rates were higher at the  
277 offshore archipelago stations (Fig. 5, Suppl. Fig. 3). The proportion of  $N_2O$  produced in nitrate  
278 reduction (% $N_2O$ ) as well as the proportion of  $N_2O$  produced from the denitrification stage of  
279 coupled nitrification-denitrification were higher at the estuary stations than at the offshore  
280 archipelago stations (% $N_2O$ :  $p < 0.001$ , % $N_2O$  from denitrification:  $p = 0.006$ ), being especially high at  
281 P4 in August (Fig. 5). Significant relationships between organic carbon characteristics (source  
282 proxies) and both %DNRA (decreasing with higher terrestrial OM share) and % $N_2O$  (increasing with  
283 higher terrestrial OM share) were observed (Suppl. Table 1), while no relationship was found with  
284 total denitrification rates. Notably, the variance of either %DNRA or % $N_2O$  was not explained by bulk  
285 carbon concentration (DOC) (Fig. 6a–b). Rather, protein-like DOM fluorescence (a common proxy for  
286 biologically labile organic carbon) had a strong negative relationship with %DNRA and strong positive  
287 relationship with % $N_2O$  (Fig. 6c–d).

288

289 Figure 3. Porewater (0–1 cm) DOM quantity and quality characteristics at the estuary stations in June (left orange bar, n = 6)  
 290 = 6) and August (right orange bar, n = 6) and at the offshore archipelago stations in August (n = 6): a) dissolved organic carbon (DOC), b) CDOM absorption coefficient at 254 nm ( $a_{(\text{CDOM}_{254})}$ ), c)  
 291 humic-like DOM fluorescence (Peak C), d) protein-like DOM fluorescence (Peak T), e) CDOM spectral slope between 275–295 nm ( $S_{275-295}$ ), f) DOC-specific  
 292 UV absorbance at 254 nm ( $\text{SUVA}_{254}$ ), g) humification index (HIX) and h) biological index (BIX). Mean values  $\pm$  standard deviation for estuary and offshore  
 293 archipelago groups are also given. The two groups are significantly different for each variable (one-way ANOVA,  $p < 0.05$ ).

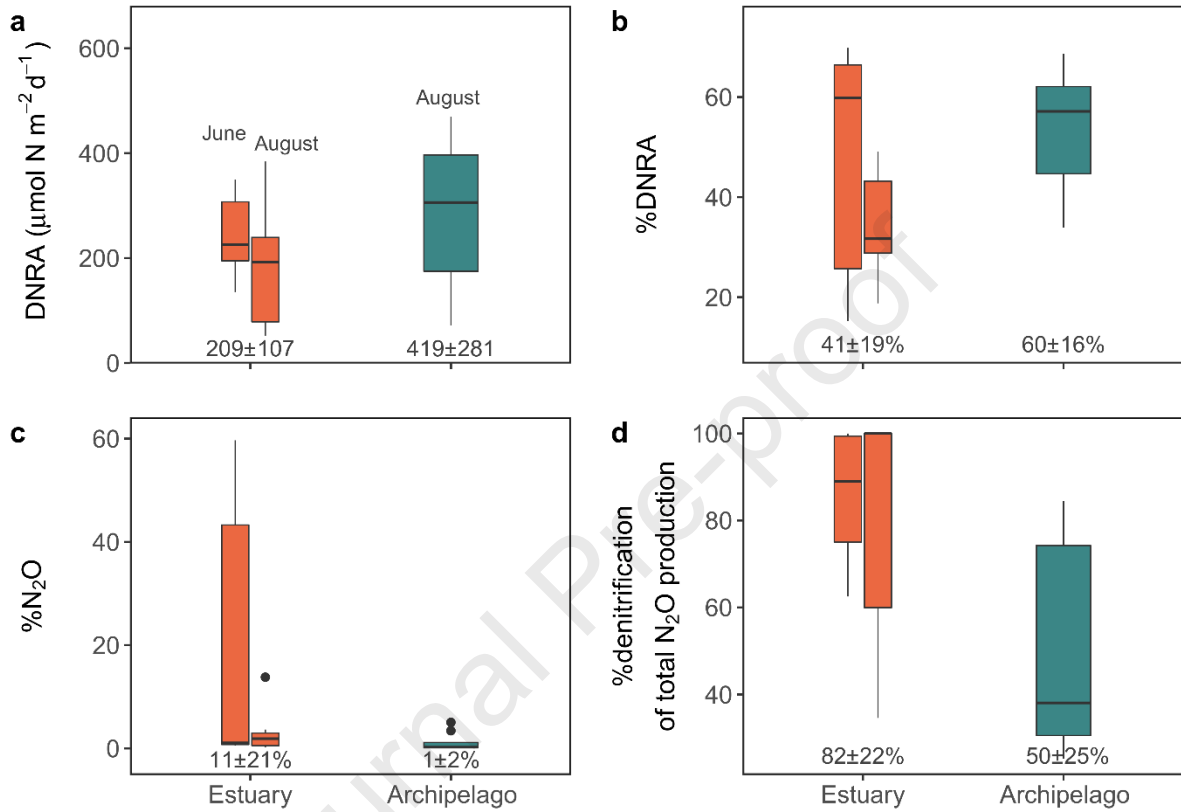


295 Figure 4. DNRA and denitrification rates at the estuary (P1, P4) and offshore archipelago (P10, STF) stations.  $D_w$  denotes water column nitrate based process  
 296 and  $D_n$  process based on the nitrate produced through sediment nitrification. Bars represent mean values  $\pm$  standard error for four sediment core  
 297 replicates.



298

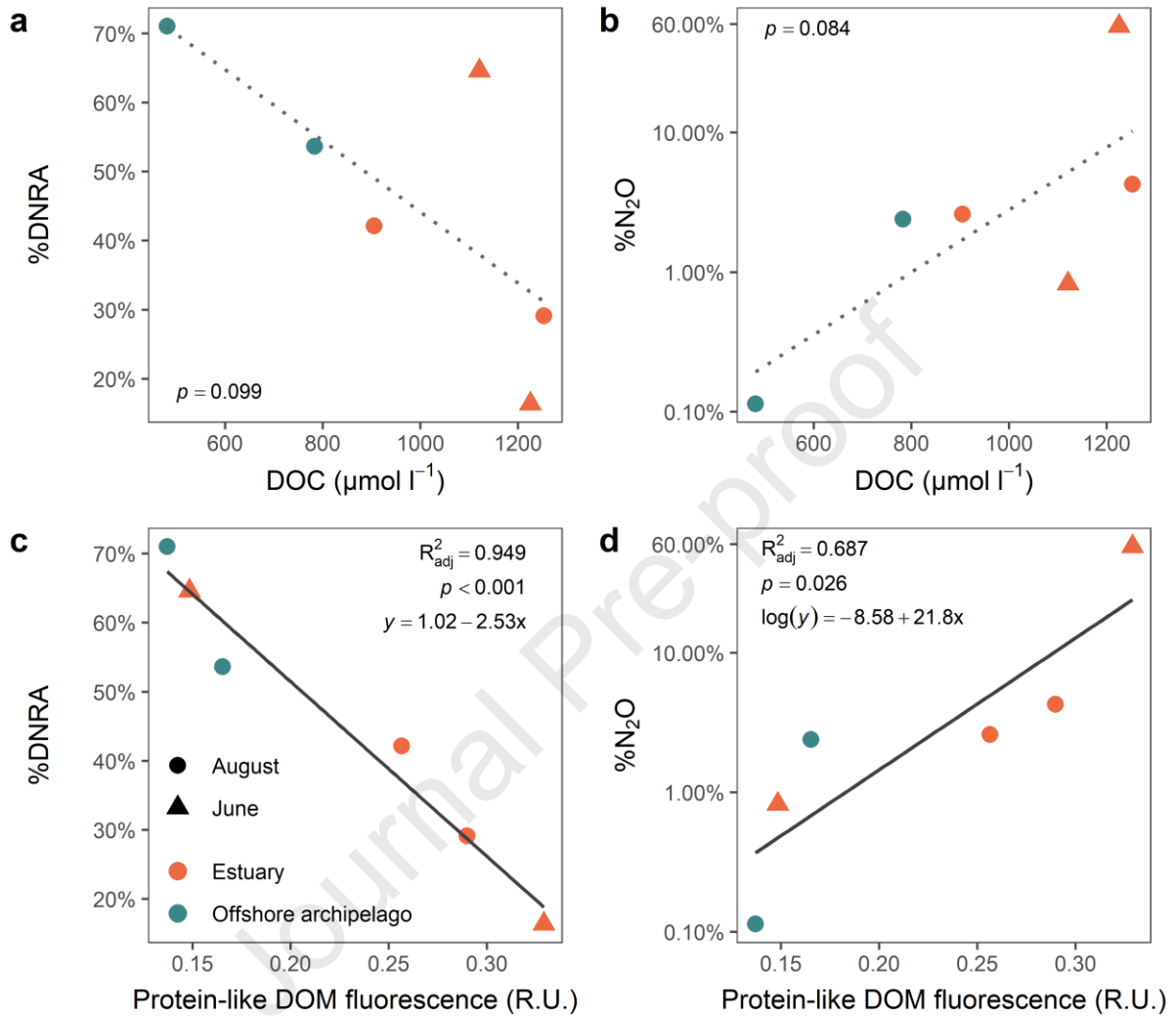
299 Figure 5. Differences in the a) absolute and b) relative rates of DNRA, and the proportion of N<sub>2</sub>O of c)  
 300 total nitrate reduction, and d) originating from denitrification stage of total N<sub>2</sub>O production during  
 301 coupled nitrification-denitrification process between the estuary stations in June (left orange bar, n  
 302 = 8) and August (right orange bar, n = 8) and at the offshore archipelago stations in August (n = 8).  
 303 Mean values  $\pm$  standard deviation for estuary and offshore archipelago groups are given. The two  
 304 groups are significantly different for each variable (one-way ANOVA/Mann-Whitney U test,  $p < 0.05$ ).



305

306

307 Figure 6. Relationships between dissolved organic carbon (DOC) and relative a) DNRA and b) N<sub>2</sub>O  
 308 production, and between bioavailable organic matter fraction (protein-like fluorescence; peak T) and  
 309 relative c) DNRA and d) N<sub>2</sub>O production at the estuary and offshore archipelago stations. The linear  
 310 regression equations of the significant ( $p < 0.05$ ) relationships only are presented.



311

312



## 313 4 Discussion

314 Our results show that the dominant microbial nitrate reduction process switched from N-removing  
315 denitrification to N-recycling DNRA when moving from the terrestrially-dominated estuary to  
316 offshore archipelago region. This can be explained by changes in both DIN concentrations and  
317 organic carbon bioavailability. As expected, nitrate concentrations were generally higher at the  
318 estuarine than at the offshore archipelago stations, due to the diminishing impact of high-DIN  
319 riverine water (Asmala et al., 2017). In parallel, we observed strong contrasts in the DOM  
320 characteristics between estuary and offshore archipelago. High humic-like fluorescence,  
321 humification index and  $SUVA_{254}$  in porewater DOM at the estuarine stations indicate a pronounced  
322 terrestrial contribution to the DOM pool (Asmala et al., 2013). These proxies suggest low DOM  
323 bioavailability in these areas, while high  $S_{275-295}$  and BIX values at the offshore archipelago stations  
324 indicate a higher contribution of recently produced autochthonous, likely more bioavailable DOM  
325 (Lee et al., 2018). A similar gradient in the source of sedimentary particulate OM was observed by  
326 Jilbert et al., (2018), where sedimentary N:C values of 0.05-0.06 (C:N of 17-20) observed in the  
327 estuary indicated a higher contribution of terrestrially sourced material, while in the offshore region,  
328 the N:C of 0.13-0.14 (C:N of 7-8) reflected the dominance of phytoplankton-derived material.

329 In previous studies, denitrification has been shown to decrease with decreasing water-column  
330 nitrate concentrations in the coastal Baltic Sea (Asmala et al., 2017). Our data show that rates of all  
331 nitrate reduction processes using water column nitrate ( $D_w_{N_2}$ ,  $D_w_{N_2O}$ ,  $DNRA_w$ ) decrease from  
332 estuary to offshore archipelago (Suppl. Fig. 3). However, because total nitrate reduction was mainly  
333 based on the nitrate provided through nitrification rather than water column nitrate, total nitrate  
334 reduction rates ( $\sum N_2 + \sum N_2O + DNRA$ ) were not significantly different between estuary and offshore  
335 archipelago stations. We suggest that the low amount of bioavailable carbon was limiting  
336 denitrification in the estuary, whereas decreasing nitrate availability started to limit the process  
337 offshore archipelago. The low bioavailable organic carbon-to-nitrate ratio at the estuarine stations  
338 was reflected in the higher denitrification-to-DNRA ratio, whereas DNRA dominated nitrate  
339 reduction under high bioavailable carbon-to-nitrate ratio at the offshore archipelago stations. A  
340 preference of the sediment microbial community for DNRA under nitrate-limited conditions has  
341 previously been explained in terms of the efficiency with which DNRA makes use of nitrate as an  
342 electron acceptor, with a higher rate of electron transfer per mole of N reduced despite the higher  
343 free energy yield of denitrification (Algar and Vallino, 2014). Interestingly, the DOM characteristics  
344 was directly related to N processes, while the amount of bulk organic carbon (as indicated by the  
345 porewater DOC concentration) was not (Fig. 6). We acknowledge that several alternative factors may

346 influence rates and pathways of nitrate reduction processes in coastal sediments. For instance, the  
347 presence of hydrogen sulfide ( $\text{H}_2\text{S}$ ) close to the sediment-water interface promotes %DNRA  
348 (Plummer et al., 2015). However, upper-sediment sulfide concentrations in the range of 1–3 mM are  
349 required for a clear impact on N processes, while sulfide in the upper sediments of our study area  
350 were consistently  $< 0.1$  mM (Jilbert et al., 2018). These low concentrations result from the titrating  
351 effect of sedimentary Fe oxides in the coastal Baltic Sea, suggesting that sulfide is a minor driver of  
352 the observed changes in %DNRA in our dataset. Furthermore, the presence of abundant Fe oxides  
353 producing  $\text{Fe}^{2+}$ , an alternative electron donor, may promote DNRA (Kessler et al., 2018; Robertson et  
354 al., 2016). Again, our study area shows only mild enrichments of porewater  $\text{Fe}^{2+}$  in the upper  
355 sediments (up to 0.2 mM, Jilbert et al., 2018) in comparison to the sites studied by Robertson et al.,  
356 (2016) (up to 0.8 mM), decreasing the potential significance of Fe. The anomalously high rates of  
357 DNRA at P1 in June may however relate to porewater  $\text{Fe}^{2+}$ , since this is the most Fe-rich of our  
358 sampling stations (see Station A in Jilbert et al., 2018).

359 In addition, our results demonstrate that the overall difference in potential organic carbon  
360 bioavailability between estuary and offshore archipelago regions is likely to influence the end-  
361 product of denitrification. At the near-shore estuarine stations, denitrification produced high  
362 proportions of  $\text{N}_2\text{O}$  (1-58% of total nitrate reduction;  $3\text{-}1230 \mu\text{M N m}^{-2} \text{d}^{-1}$ ). This result implies that  
363 nitrate was preferred over  $\text{N}_2\text{O}$  as an electron acceptor under conditions of high nitrate to  
364 bioavailable carbon the nitrate-replete conditions of the estuary (Richardson et al., 2009), allowing  
365  $\text{N}_2\text{O}$  to accumulate in bottom waters. In contrast, the share of  $\text{N}_2\text{O}$  in denitrification was lower in the  
366 offshore archipelago stations (0.1-2%,  $1\text{-}9 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ), where the bulk carbon concentrations  
367 were low but the contribution of bioavailable autochthonous carbon to the carbon pool was high  
368 and nitrate concentration low. In accordance,  $\text{N}_2\text{O}$  concentrations in the bulk water column samples  
369 collected between 2015 and 2017 were higher at the estuarine stations than in the offshore  
370 archipelago, agreeing with the previous results in coastal environments with high freshwater impact  
371 and fluctuating environmental conditions (e.g. Foster and Fulweiler, 2016; Nielsen et al., 2009;  
372 Silvennoinen et al., 2008). While part of the accumulated  $\text{N}_2\text{O}$  can originate from nitrification or  
373 coupled nitrification-denitrification (Foster and Fulweiler, 2016), we measured rather equal rates of  
374 sediment nitrification at the estuary and offshore archipelago stations (estuary:  $841\pm 378$ , offshore  
375 archipelago:  $1089\pm 193 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ; data not shown), arguing against an important role for  
376 nitrification in  $\text{N}_2\text{O}$  production in the estuary. Furthermore, our data show that  $\text{N}_2\text{O}$  produced in  
377 coupled nitrification-denitrification was mainly derived from denitrification. Although part of the  
378 water-column  $\text{N}_2\text{O}$  pool in the estuary is likely advected with riverine water (Bange et al., 1998), the

379 majority appears to derive from sediment processes, since N<sub>2</sub>O concentrations were generally higher  
380 in the bottom water than at the surface (Fig. 2).

381 Coastal systems are considered as important nutrient filters, reducing N loading from catchment  
382 areas towards the open sea. Although our results confirm that the main N removal process in the  
383 studied coastal environment is N<sub>2</sub>-producing heterotrophic denitrification, they also highlight the  
384 importance of N-recycling DNRA. In the outer offshore archipelago region with decreasing influence  
385 of riverine water, DNRA can produce substantial amounts of bioavailable ammonium, enhancing the  
386 N recycling between sediments and surface water, especially in summer with the highest  
387 autochthonous biomass production and sedimentation. Intensifying eutrophication increases  
388 bioavailable carbon availability through higher algal biomass production, which in turn may promote  
389 DNRA and increase the role of estuaries as hotspots for N recycling, over N removal. This  
390 phenomenon has already been observed in some eutrophied systems (Bernard et al., 2015; Song et  
391 al., 2014), and could delay the recovery of water quality of the open sea in the Baltic Sea region.

392 The future role of eutrophic coastal systems as sources of N<sub>2</sub>O to the atmosphere depends on the  
393 balance of N processes in coastal sediments. In systems such as Pohjanpitäjänlahti, the DIN pool of  
394 the estuary is dominated by nitrate, favouring production of N<sub>2</sub>O during denitrification under  
395 nitrate-replete conditions. Hence, further increases in nutrient loading to this system is likely to  
396 enhance N<sub>2</sub>O-producing denitrification, especially under scenarios of increased annual runoff and  
397 higher summer temperature, which will enhance stratification and hypoxia throughout the Baltic Sea  
398 (Meier et al., 2011), contributing to the predicted rise in emissions of this greenhouse gas in the  
399 future (Murray et al., 2015). Our results highlight the need to consider the intricate balance of  
400 processes in the nitrogen cycle along coastal gradients, especially in relation to organic carbon  
401 characteristics. Also their spatial variation and temporal evolution needs to be further clarified in  
402 order to properly understand the role of coastal ecosystems as filters of land-to-sea transfer of N.

## 403 **5 Acknowledgements**

404 We are grateful to the technical staff of Tvärminne Zoological Station and the Ecosystems and  
405 Environment Research Program at University of Helsinki for assistance during fieldwork and  
406 laboratory analyses. This work was supported by the Academy of Finland (projects 267112, 309748,  
407 310302, and 317684)

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## Highlights

- The availability of bioavailable carbon defines nitrate reduction end-product
- Estuaries with low bioavailable organic carbon can release high amounts of N<sub>2</sub>O
- Nitrogen is recycled through DNRA in the archipelago areas

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Author statement

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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