

# Benthic nitrogen cycling in the North Sea

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## Abstract

We present new data on the rates of sedimentary denitrification and its component processes (canonical denitrification, anammox and dissimilatory nitrate reduction to ammonium) for intertidal and subtidal sites in the North Sea using nitrogen isotope addition methods. We find overall average denitrification rates of 6.3 (range 0.4-10.6)  $\mu\text{mol m}^{-2}\text{h}^{-1}$ , similar to those previously reported for this region and other temperate shelf environments. We find canonical denitrification to be the dominant (>90%) process of the three. At the subtidal sites, most of the denitrification is supported by nitrate generated within the sediments, while at the intertidal site the main source is from the water column. We go on to consider the impact of these rates on nitrogen cycling within the North Sea region and compare the sediment core incubation rate results to estimates derived from modelling approaches. Model rates are somewhat higher than those directly measured and we consider possible reasons for this.

## Key words

**Denitrification, Anammox, DNRA, sediments, North Sea**

## Highlights

**New measurements of denitrification, anammox and DNRA rates in North Sea sediments.**

**Denitrification is the dominant process and rates measured compare well to other direct measurements.**

**Model estimates suggest rather higher rates of nitrogen loss compared to direct measurements and some reasons for this difference are considered.**

31 Fixed nitrogen (i.e. nitrogen present in the marine environment in forms other than  $N_2O$  and the  
32 relatively inert gas  $N_2$ ) is a key limiting nutrient in large areas of the oceans and one whose inputs to  
33 the ocean due to human activity have increased markedly (Moore et al., 2013, Sharples et al., 2017,  
34 Voss et al., 2013). These inputs potentially contribute to a range of environmental problems in coastal  
35 waters including increased phytoplankton biomass (Howarth and Marino, 2006), hypoxia (Diaz and  
36 Rosenberg, 2008) and possibly harmful algal blooms (Davidson et al., 2014). However, such linkages  
37 between nutrient loadings and ecosystem responses are not at all straightforward (Cloern, 2001,  
38 Howarth and Marino, 2006, Paerl et al., 2014) and depend on a wide variety of other physical, chemical  
39 and biological factors, as illustrated in the responses of phytoplankton abundance to nutrient inputs  
40 seen in the North Sea (McQuatters-Gollop et al., 2007).

41 The main loss mechanism for nitrogen from the ocean is via a suite of microbial processes sometimes  
42 collectively referred to as denitrification, including canonical denitrification and anammox which  
43 convert fixed nitrogen to  $N_2$  gas (Seitzinger et al., 2006, Dalsgaard et al., 2005, Devol, 2015, Ward,  
44 2013, Trimmer and Engström, 2011, Thamdrup, 2012). These processes occur under low oxygen  
45 conditions, and shelf sea sediments are a globally important environment for these processes within  
46 the context of the marine nitrogen cycle, and also in terms of mitigating the effects of fluvial inputs to  
47 the oceans (e.g. Voss et al., 2013, Seitzinger et al., 2006, Trimmer and Nicholls, 2009, Devol, 2015,  
48 Sharples et al., 2017). Canonical denitrification is a heterotrophic process involving nitrate acting as  
49 an alternative electron acceptor for the bacterial oxidation of organic matter, ferrous iron and  
50 hydrogen sulphide in the near absence of oxygen, yielding  $N_2$  gas and  $N_2O$  as a by-products. In  
51 sediments, the source of the nitrate may be from the water column or from nitrification of ammonium  
52 released during the degradation of organic matter within the sediments; the latter is sometimes called  
53 coupled nitrification-denitrification. Anammox was only identified as a biochemical process about 20  
54 years ago (Thamdrup, 2012, Mulder et al., 1995) and involves chemoautotrophic bacteria oxidising  
55 ammonium with nitrite as an energy source. Additionally the reduction of nitrate and nitrite may  
56 proceed through the process of DNRA, dissimilatory nitrate reduction in which bacteria reduce nitrate  
57 to ammonium, a process by which fixed nitrogen is retained within the sediments. The relative  
58 importance of anammox, canonical denitrification and DNRA and the controls on this balance are  
59 uncertain, and given the central importance of the marine nitrogen cycle for life in the sea, resolving  
60 this uncertainty is clearly important (Voss et al., 2013, Devol, 2015, Trimmer et al., 2013). In  
61 subsequent discussion we will refer to the general process of denitrification and where appropriate  
62 the three different biochemical pathways of canonical denitrification, anammox and DNRA.

63 Rates of denitrification have been estimated in several different ways ranging from biogeochemical  
64 models (Seitzinger and Giblin, 1996, Fennel et al., 2006) and shelf sea budgeting (Hydes et al., 1999)  
65 to direct measurements of rates using a variety of techniques of core incubations (Trimmer and  
66 Nicholls, 2009, Devol, 2015, Rysgaard et al., 1993, Kitidis et al., 2017). Recently direct sediment core  
67 incubation studies using  $^{15}\text{N}$  additions have allowed accurate estimation of rates with fewer  
68 uncertainties than previous approaches. This approach allows the relative importance of canonical  
69 denitrification, anammox and DNRA to be determined as well as providing some information on the  
70 source of the nitrogen species being converted to  $\text{N}_2$ . However, these measurements are time  
71 consuming and labour intensive, meaning there is rather limited data available from coastal seas.  
72 Given the natural heterogeneity of shelf seas, it is important to build the data set of direct  
73 measurements and compare it to model and budget approaches to build confidence in shelf sea  
74 nitrogen budgets and better understand the process controlling these budgets and hence improve  
75 models.

76 The North Sea is a large semi-enclosed coastal sea with water residence time of a few years and subject  
77 to large inputs of terrigenous nutrients, particularly nitrate. (Patsch et al., 2017, Queste et al., 2013).  
78 Previous budget and model studies (Hydes et al., 1999, Seitzinger and Giblin, 1996) have suggested  
79 that there are relatively high denitrification rates in this region, and these ameliorate the potential  
80 harmful ecological effects of terrestrial nutrient inputs.

81 In the work reported here we have conducted measurements of denitrification rates within the North  
82 Sea using the  $^{15}\text{N}$  tracer method. The sampling was targeted to representatively sample widely  
83 occurring sediment types within the North Sea in terms of sediment grain size and organic carbon  
84 content (Diesing et al., 2017). We also report measurements of denitrification rates from the Wash  
85 region, a large shallow and intertidal area where denitrification has been suggested to be acting to  
86 substantially reduce nitrate inputs to the open North Sea (Jickells et al., 2014, Trimmer et al., 1998).  
87 We compare the results obtained to other data from the North Sea and other temperate shelf systems  
88 and also to model and budget estimates of denitrification, with the goal of trying to improve overall  
89 estimates of the significance of shelf sea denitrification and the biogeochemical controls on the  
90 process.

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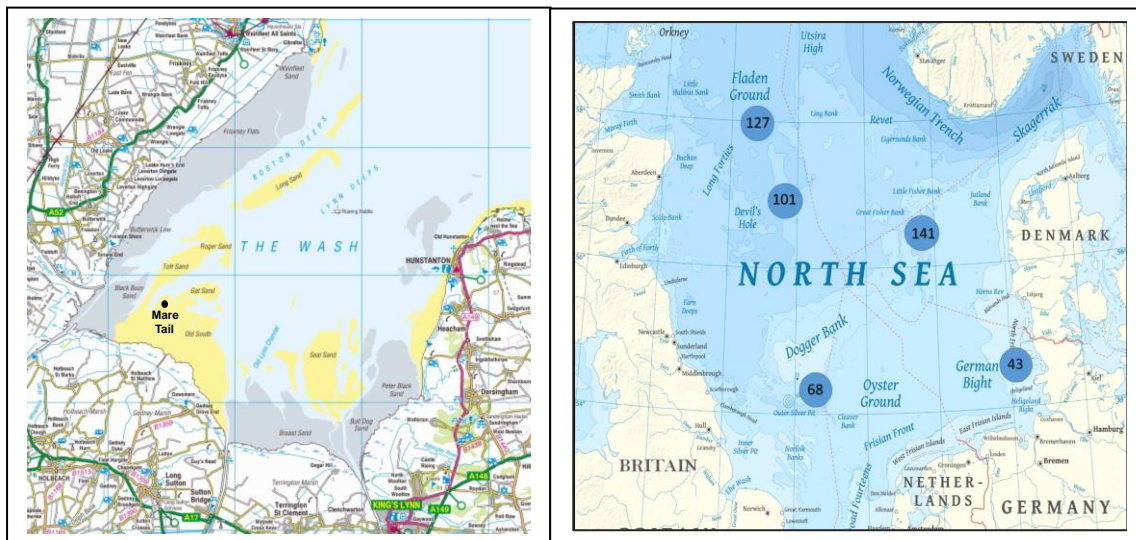
93 **2. Methods**

94 Full details of all methods are presented in Villa 2016 (Rosales Villa, Alida (2016) *Insight into the*  
 95 *nitrogen cycling in the North Sea*. Doctoral thesis, University of East Anglia. Available at  
 96 <https://ueaeprints.uea.ac.uk/61021/>).

97 The sampling sites for this survey are in The Wash (Fig 1a), a large area of intertidal sand and mud  
 98 banks and the North Sea (Fig 1b).

99 Needs revised figures Alida

100



101 Figure 1. Sampling sites: The Wash (a),  
 102 [http:// upload.wikimedia.org/wikipedia/ commons/c/c8/ Ordnance\\_Survey\\_1-250000 - TF.jpg](http://upload.wikimedia.org/wikipedia/commons/c/c8/Ordnance_Survey_1-250000_-_TF.jpg)) and  
 103 The North Sea (b), showing the sampling sites in blue circles and the station numbers refer to sites in  
 104 Table 1. Latitude and Longitude of the sampling stations are listed below

Stn	Lat	Long
43	52° 58.277	000° 19.141 E
68	54° 08.475	000° 12.757 E
101	56° 51.522	000° 18.290 E
127	57° 49.977	000° 25.639 W
141	56° 57.047	000° 23.928 E
The Wash		
May	52° 54.730	00° 08.800 E
June	52° 53.560'	00° 11.180' E
Sept	52° 55.129 N	00° 12.121E
Oct	52° 55.968 N	00° 11.981E

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107

108 In the Wash the sampling was limited to one station called Mare Tail with a total of four sampling visits  
109 carried out during May, June, September and October 2013. The site was accessed on board of the RV  
110 Three Counties from EIFCA (Eastern Inshore Fisheries Conservation Authority) and then by foot around  
111 low tide with samples collected by hand. After their collection, the samples were placed in buckets  
112 filled with site water to keep them at the ambient temperature, and then transported promptly  
113 (within about 3 hours) by boat and road to UEA laboratories for subsequent analysis.

114 Samples were also collected during August 2013 at 5 sites in the North Sea (Figure 1b) in the water  
115 depth range 28-116 m. Sediment samples were collected using a NIOZ cylindrical box corer deployed  
116 from the RV Cefas Endeavour. The depth of sediment in the box corer ranged between 40 and 50 cm  
117 and was collected with 15 to 25 L of the original bottom water still in place. Subsamples for the  
118 sediment incubations were taken by hand from the box corer along with overlying water. Samples  
119 collected on board the Cefas Endeavour were processed on board.

120 A total of 30 intact sediment core samples were collected at each site in 300 mm plexiglass tubes (6  
121 cm i.d). The sediment column in the core were about 15 cm length with about 420 ml overlying  
122 volume of water filling the remainder of the tube. After sampling, the cores were placed in 200 L  
123 water containers in a room at in situ temperature and were left in the dark overnight with gentle  
124 aeration of the overlying water of the core tubes.

125

126 Experimental design and calculations

127 Two types of experiments were conducted in the dark, so far as practical, firstly (a) time-series  
128 experiment using intact sediment cores (based on Trimmer et al., 2006, Nielsen, 1992) and secondly  
129 (b) end-point anaerobic sediment slurry (Thamdrup and Dalsgaard, 2002). The time-series experiment  
130 was designed to determined rates of denitrification, anammox and DNRA, while the aim of the  
131 anaerobic sediment slurry experiment was to confirm the presence of anammox.

132

133 (a) *Time series experiments.* After being left overnight, the overlying water in the core tube was  
134 enriched with  $^{15}\text{NO}_3^-$  (by adding  $\text{Na}^{15}\text{NO}_3$  [99%  $^{15}\text{N}$  atom%] Sigma-Aldrich) to a give a final  
135 concentration of about 50  $\mu\text{M}$ , approximately twice the ambient concentration. Six of the  
136 cores were not enriched to allow measurements to be made for correction for natural  
137 abundance. After leaving for 30-60 minutes to allow exchange between sediment and  
138 overlying water, three cores were sacrificed as time zeroes, and the remaining cores sealed  
139 with rubber bungs and incubated in the dark with gentle magnetic stirring (60 rpm) to ensure

140 water mixing and minimal disturbance of the sediments. Three cores were subsequently  
141 sacrificed every hour for 5 hours. When sampling sacrificed cores, samples of overlying water  
142 were first taken for nutrient measurements and the water and sediment were gently slurried.  
143 One slurry sub-sample for DNRA rate determination by  $^{15}\text{NH}_4^+$  analysis was collected and  
144 frozen. Other slurry sub-samples collected for isotopic analysis of  $\text{N}_2$  and  $\text{N}_2\text{O}$  production were  
145 taken in a gas tight vial, poisoned with formaldehyde (100  $\mu\text{L}$ , 38%) and stored sealed at room  
146 temperature.

147 (b) *Confirmation of anammox.* Sediment samples for the end-point slurry experiment to confirm  
148 the presence of anammox were subsampled from cores by extruding a 3-cm thick slice (both  
149 oxic and anoxic layers were included). The sediment was homogenised and subdivided into  
150 18 subsamples of 2 mL that were placed in gas-tight vials (Exetainer, Labco), filled with helium  
151 degassed seawater and incubated in the dark to eliminate all nitrate, nitrite and oxygen. After  
152 24 hours, 15 vials were spiked with  $^{15}\text{NO}_3^-$  and  $^{15}\text{NH}_4^+$  to achieve a final concentration of 500  
153  $\text{nmol cm}^{-3}$  of  $^{15}\text{NH}_4^+$  and 100  $\text{nmol cm}^{-3}$  for  $^{14}\text{NO}_3^-$  (by injecting 50  $\mu\text{L}$  of  $^{15}\text{NH}_4\text{Cl}$  120 mM; [98  
154  $^{15}\text{N}$  atom%] Sigma-Aldrich) and 50  $\mu\text{L}$  of  $\text{Na}^{14}\text{NO}_3$  (25 mM). The remaining three samples were  
155 left un-spiked for natural N abundance measurements. At time zero, three of these vials and  
156 the three vials with no isotope addition were sacrificed by adding  $\text{ZnCl}_2$ . Samples were stored  
157 upside down at ambient temperature with the remaining samples incubated with gentle  
158 rotation for 24 hours at *in situ* temperatures, before also being sacrificed as above.

159 *Isotopic Measurements.* A helium headspace was created in the stored gas-tight vials to allow  
160 extraction of gas samples for isotopic measurements of  $\text{N}_2$  (Dalsgaard et al., 2003) at University of  
161 Southern Denmark and  $\text{N}_2\text{O}$  (Trimmer and Nicholls, 2009, Trimmer et al., 2006) at Queen Mary  
162 University, London. In order to measure DNRA, ammonium was first converted to  $\text{N}_2$  by the  
163 hypobromite method and then its isotopic abundance measured (Risgaard-Petersen et al., 1995).  
164 Calibration was carried out with oxygen free nitrogen (99.998%  $^{14}\text{N}_2$ ) with correction for instrument  
165 drift and mass effects using repeat standard analyses throughout the analytical run. The  
166 measurements of nitrogen isotopes of  $\text{N}_2$  were carried out according to Dalsgaard et al (2012). Isotopes  
167 of  $\text{N}_2\text{O}$  were measured using a Cryo-Focusing; Precon, Thermo-Finnigan. These measurements of  $\text{N}_2\text{O}$   
168 can provide a means to separate the contributions of denitrification and anammox based on the  
169 assumption that  $\text{N}_2\text{O}$  is only produced by denitrification and not by anammox (Trimmer et al., 2006).

170 Anammox rates in the slurries were calculated as in Thamsdrup and Dalsgaard (2002). Denitrification  
171 rates and the source of  $\text{NO}_3^-$  for denitrification were calculated using the equations of Nielsen (Nielsen,  
172 1992) when anammox were not present and Risgaard-Petersen et al. (2003) and Trimmer et al. (2006)  
173 when anammox was present:

174

175

176

$$p_{14} = 2r_{14} \times (p^{29}N_2 + p^{30}N_2 \times (1 - r_{14}))$$

177

178

$$r_{14} = p^{45}N_2O / 2p^{46}N_2O$$

179

180 Where,  $p_{14}$  is the total  $N_2$  production, i.e total denitrification,  $r_{14}$   $^{14}NO_3^-$  and  $^{15}NO_3^-$  in the  $NO_3^-$  reduction  
181 zone and  $p^{29}N_2$  and  $p^{30}N_2$  are the measured production rates of the  $^{15}N$ -labelled  $N_2$ . The production of  
182  $N_2$  due to anammox ( $p_{14anammox}$ ) was calculated as:

183

184

185

$$p_{14anammox} = 2r_{14} \times (p^{29}N_2 + 2r_{14} \times 2p^{30}N_2)$$

186

187 Denitrification rates supported either by the unlabelled  $NO_3^-$  diffusing from the overlying water ( $p_{14w}$ )

188 or rates of coupled nitrification-denitrification ( $p_{14n}$ ) within the sediments were calculated as follows:

189

190

$$p_{14w} = p_{14} \times r_{14w} / r_{14}$$

191

192

$$p_{14n} = p_{14} - p_{14w}$$

193

194  $r_{14w}$  is the ratio of  $^{14}NO_3^-$  and  $^{15}NO_3^-$  in the overlying water. Concentrations of unlabelled  $NO_3^-$  were  
195 measured in water samples before adding the  $^{15}NO_3^-$ . As co-occurrence of anammox and DNRA was  
196 not observed (see results), there was no need for corrections taking a coupling of these processes into  
197 account (Song et al., 2016)

198 The nutrients analysis were carried out in the Analytical Instrument Laboratory facilities of the University  
199 of East Anglia. Analysis for  $NO_3^-$  and  $NH_4^+$  were determined by colorimetric methods. The protocols and  
200 standards preparation were based on the methods described by Grasshoff *et al.* (1983).

201 Standard deviation on the rate estimates for denitrification ranged between 0.5 and 3.7  $\mu\text{mol m}^{-2} \text{h}^{-1}$ .

202

203 **3. Results**

204 Results are presented in summary form in Table 1. The characteristics of the sediments sampled in  
205 this study are included in a wider compilation of sediment characteristics in this region (Diesing et al.,  
206 2017 and Parker et al. this volume) and only briefly summarised here. The sediments sampled, ranged  
207 from sand (>90%) to muddy sand (~80% sand) with average grain sizes ranging from 76-165  $\mu\text{m}$  and  
208 the organic carbon content ranged from 0.05-0.48%. The Wash site characteristics were similar on  
209 each sampling occasion and intermediate between the North Sea sites in which the deeper sites  
210 tended to be finer grained and more organic carbon rich. The sediment oxygen penetration depth  
211 measured by electrode ranged from 2-8 mm. Hence, the oxygen penetration depth was small at all  
212 sites, but in the Wash sediments (2-4 mm) tended to be shallower than at the North Sea stations (6-8  
213 mm). Ambient water column nitrate concentrations ranged from 0.7-6  $\mu\text{M}$ .

214 In terms of the sedimentary nitrogen cycling measurements, there were no obvious difference  
215 between the measurements made on the Wash sediments and those made in the North Sea, so  
216 initially we consider the results from the whole data set, while noting difference where they are  
217 evident. Based on anoxic slurry measurements, anammox was detectable at all sites at rates ranging  
218 from 0.004-0.73  $\text{nmol cm}^{-3}\text{h}^{-1}$  which confirms the potential for this process in these sediments.  
219 Measured denitrification rates in intact sediment cores range from 0.4-10.6  $\mu\text{mol m}^{-2}\text{h}^{-1}$ . These rates  
220 are comparable to other results from this region and from other marine sediments including (Trimmer  
221 et al., 2003, Bale et al., 2014, Trimmer and Engström, 2011, Bonaglia et al., 2017). In the intact  
222 sediment cores, which may better reflect the ambient environmental conditions than the slurry  
223 measurements (Trimmer and Engström, 2011), our measurements found at all times and all sites that  
224 canonical denitrification was the dominant process, compared to anammox. Anammox was only  
225 detected at two North Sea locations, and even there contributed less than 10% to the total nitrogen  
226 loss. At the Wash site the denitrification appeared to be sustained predominantly by nitrate from the  
227 overlying water, while at the North Sea sites, nitrification was consistently the dominant (80-90%)  
228 source of nitrate for denitrification. DNRA rates were rather variable and the process was only  
229 detected when anammox was absent. The DNRA rates measured at the North Sea sites were zero at  
230 4 of the 5 sites, but at one site the rate was equivalent to 17% of the denitrification rate. At the Wash  
231 site DNRA rates were equivalent to ~40% of the denitrification rate in June and September, 6% in  
232 October and 0% in May.

233 **4. Discussion**

234 The rates measured, and dominance of canonical denitrification, are very consistent with other data  
235 from coastal seas, while much higher rates have been measured in estuarine systems and lower rates



236 beyond the continental shelf (Trimmer and Engström, 2011 and references therein, Neubacher et al.,  
237 2011, Devol, 2015, Na et al., 2017, Deek et al., 2013). Indeed, these published studies demonstrate  
238 that there is quite a strong inverse global relationship between denitrification rates and depth and  
239 also that the proportion of anammox to denitrification increases with depth, over depth ranges from  
240 0-5000 m. A number of potential controlling variables for denitrification vary systematically with water  
241 depth. Several of these authors note these relationships parallel the decreasing oxygen consumption  
242 with depth and therefore it is argued that the supply of organic matter to the sediments, rather than  
243 the nitrate availability, controls the denitrification rate. Such a depth relationship is not directly  
244 obvious within the data set presented here, but this data set is small and spans a rather small depth  
245 range. The inverse relationship described by Trimmer and Engström (2011) includes very high  
246 denitrification rates within shallow estuarine systems, based on studies in several UK estuaries  
247 enriched in organic matter and nitrate (Trimmer et al., 2003, Trimmer et al., 2006, Dong et al., 2009).  
248 Similar high rates of denitrification have been reported from the Elbe estuary (Deek et al., 2013) and  
249 from permeable sediments in the German Bight (Marchant et al., 2016). Our data from the Wash  
250 suggests that in shallow intertidal areas in exposed bays, with lower nitrate and organic matter inputs,  
251 denitrification rates are lower than in confined estuaries, although canonical denitrification does still  
252 appear to dominate denitrification. The very low rates of denitrification seen in September in the  
253 Wash are associated with low water column nitrate and this may suggest that nitrate supply may play  
254 a role in regulating the rates of denitrification, although several factors change seasonally in such  
255 systems including the temperature and the supply and quality of organic matter as noted by other  
256 authors (Deek et al., 2013, Asmala et al., 2017, Brin et al., 2017, Deutsch et al., 2010).

257 A recent study (Kitidis et al. (2017) reported a detailed description of benthic nitrogen cycling in the  
258 Celtic Sea (water depths 100-150m) where they found anammox to dominate over canonical  
259 denitrification, although the overall denitrification rate was similar to our results for the North Sea.  
260 This observation together with another recent studies where anammox was not measurable at a site  
261 where it had previously been recorded (Trimmer et al., 2013), emphasises that we are still a long way  
262 from fully understanding the benthic nitrogen cycle in the shelf seas.

263 Recently Sharples et al. (2017) emphasised the global significance of denitrification for trapping fluvial  
264 nitrate within coastal waters and preventing its escape to the open ocean as previously discussed by  
265 Seitzinger et al. (2006). Sharples et al. (2017) also noted a systematic effect of the Coriolis force in  
266 increasing water residence time with increasing latitude which results in higher latitude shelves such  
267 as the North Sea being more effective at retaining nitrate because there is more time for  
268 denitrification to consume the fluvial nitrate, compared to low latitude tropical shelf systems.  
269 However, they also note that the impact of temperature on denitrification rate could offset this

270 gradient with shorter residence times in tropical regions with weaker Coriolis force compensated by  
271 higher denitrification rates due higher temperatures. The effects of temperature on denitrification  
272 rates have been investigated systematically recently (Canion et al., 2014) and it is clear that rates of  
273 denitrification do vary with temperature and that communities from different regions have different  
274 optimum temperatures demonstrating adaption to the *in situ* temperatures in the different regions.  
275 Similarly Brin et al (2017) have shown that denitrification rates increase from 4-25°C and then decline,  
276 with similar trends seen for both canonical denitrification and anammox. Within the data set  
277 presented here there is no obvious effect of temperature on rates even at the Wash site where  
278 seasonal cycling took place. By contrast, in offshore North Sea waters Trimmer et al (2009) did find a  
279 difference in rates in spring and autumn and Neubacher et al (2011) also found higher rates in summer  
280 and autumn than in winter and spring, although the temperature differences between the seasons  
281 was modest. Denitrification rates also depend on the supply and quality of organic matter and on the  
282 supply of nitrate (e.g. Deek et al., 2013, Deutsch et al., 2010, Asmala et al., 2017), and hence the overall  
283 significance of temperature on the retention of fluvial nitrate as discussed by Sharples et al. (2017)  
284 still requires resolution.

#### 285 *Implications of denitrification for coastal nutrient cycling*

286 The synthesis and scaling up of denitrification can provide useful insights into the role of denitrification  
287 in coastal nutrient cycling (e.g. Asmala et al., 2017, Deutsch et al., 2010, Devol, 2015). We therefore  
288 now consider the significance of the observed denitrification rates on fluvial nitrate transport and  
289 nitrogen cycling more generally in the North Sea. As noted earlier, the direct measurement of  
290 denitrification rates in sediments is a complex and time consuming task meaning that data available  
291 for scaling up are necessarily limited. Hence a comparison with alternative approaches based on  
292 models and budgets can serve to improve confidence in our estimates.

293 The Wash System. Jickells et al. (2014) estimated nutrient transport through the Wash system  
294 and the extent of nitrate trapping in the context of the loss of intertidal habitat to reclamation in the  
295 Wash system, and indeed more widely across the world. That analysis was based on earlier estimates  
296 of denitrification rates in the Wash and the associated Great Ouse estuary (Trimmer et al., 1998) using  
297 the acetylene block technique which yielded results that are substantially higher than the values  
298 reported here for the Mare Tail site. However, the Mare Tail site was selected to be representative of  
299 the large intertidal sand and mud banks in the Wash itself, and away from the immediate effects of  
300 the riverine nitrogen inputs from the Great Ouse where Trimmer et al sampled. Higher values of  
301 denitrification comparable to those reported by Trimmer et al. (1998) have been measured with the  
302 isotope technique in estuarine systems (Trimmer et al., 2003, Trimmer et al., 2006, Dong et al., 2009,

303 Deek et al., 2013). This may suggest that estuarine systems are particularly hot spots of denitrification,  
304 and hence the loss of intertidal area to reclamation in estuaries may have far reaching implications for  
305 coastal biogeochemistry (Jickells et al., 2016, Deek et al., 2013). If the Mare Tail results are  
306 representative of the Wash generally, the nitrate retention within this system will, however, be lower  
307 than suggested by Jickells et al. (2014).

308 The North Sea. The importance of denitrification on North Atlantic shelf systems and North Sea in  
309 particular has been emphasised in several studies (Seitzinger and Giblin, 1996, Hydes et al., 1999,  
310 Fennel et al., 2006). The overall average rate of denitrification from the data in Table 1 is 6.3 (median  
311 7.7)  $\mu\text{mol m}^{-2}\text{h}^{-1}$ . The average rates reported for the North Sea by Lohse et al. (Lohse et al., 1996) was  
312 9.8  $\mu\text{mol m}^{-2}\text{h}^{-1}$  and by Neubacher et al. (Neubacher et al., 2011) was 10  $\mu\text{mol m}^{-2}\text{h}^{-1}$ . Measurements  
313 on the Irish Sea shelf (4.8  $\mu\text{mol m}^{-2}\text{h}^{-1}$ ) were also similar (Trimmer and Nicholls, 2009) as were the  
314 results of Kitidis et al. (2017), although as noted earlier the latter authors found anammox to be  
315 dominant. These rates on the N W European shelf are similar to those reported for the Baltic (Deutsch  
316 et al., 2010, Asmala et al., 2017) and other shelf seas in the compilation by Trimmer and Engström  
317 (2011) and in more recent studies with Na et al. (2017) who report values of 9.2  $\mu\text{mol m}^{-2}\text{h}^{-1}$  in the  
318 east China Sea. Thus there is a general consensus for denitrification rates measured using the isotope  
319 pairing approach on temperate shelf seas fall in the range of 5-10  $\mu\text{mol m}^{-2}\text{h}^{-1}$ . These values can be  
320 compared to estimates derived by fundamentally different approaches. Seitzinger and Giblin (1996)  
321 estimated a denitrification rate for temperate shelves equivalent to 19  $\mu\text{mol m}^{-2}\text{h}^{-1}$ , and for the North  
322 Sea specifically of 25  $\mu\text{mol m}^{-2}\text{h}^{-1}$ , using a coupled nitrification/denitrification, sediment oxygen  
323 consumption and primary production model. Fennel (2006) developed a high resolution physical-  
324 biological model for the mid Atlantic bight region along with a similar approach to Seitzinger and  
325 Giblin, and from this estimated a denitrification rate equivalent to 46  $\mu\text{mol m}^{-2}\text{h}^{-1}$ . Hydes et al. (1999)  
326 used a completely different approach based on synthesis of nutrient and salinity data within the  
327 southern North Sea to estimate denitrification rate equivalent to 29  $\mu\text{mol m}^{-2}\text{h}^{-1}$ , although this  
328 estimate is quite sensitive to an estimated water residence time in the North Sea. Hydes et al. used a  
329 residence time of 1 year (Blaas et al., 2001, Prandle, 1984), and a longer residence time would result  
330 in a lower estimate of denitrification. Brion (2004) synthesised some of these estimates and suggested  
331 a North Sea denitrification rate equivalent to 23  $\mu\text{mol m}^{-2}\text{h}^{-1}$ . Thus estimates of denitrification derived  
332 from models and integration over large spatial scales (19-46  $\mu\text{mol m}^{-2}\text{h}^{-1}$ ) are similar, although higher,  
333 than the experimentally derived values based on incubations on sediment cores of 5-10  $\mu\text{mol m}^{-2}\text{h}^{-1}$ .  
334 This difference is relatively small and may simply reflect uncertainties in both approaches. However,  
335 assuming the budget/model calculations are correct, this may reflect a real difference and we would  
336 highlight three possible explanations. Firstly, as noted earlier the denitrification rates in estuaries may

337 be of the order of ten times higher than those for the more open shelf waters (see compilations of  
338 rates in Trimmer and Engström, 2011, Asmala et al., 2017). The area of estuaries is small (~2%),  
339 compared to the wider North Sea shelf system (McLusky, 2001) but the high rates of denitrification in  
340 estuaries could lead to an underestimate of North Sea shelf wide denitrification, when based on shelf  
341 sediment measurements alone. However, even denitrification rates in such estuaries that are 20  
342 times higher than in shelf sediments cannot quantitatively explain the whole difference between the  
343 sediment core measured and modelled rates. The second issue is that there may be some issues  
344 related to the assumptions behind the isotope pairing technique as discussed by Thamdrup (2012). A  
345 third possibility is that direct core water isotope techniques may underestimate total sedimentary  
346 denitrification. This could be because such measurements are based primarily on diffusive exchanges  
347 between sediments and the water column, and in sandy sediments advective exchanges may be  
348 important (Canion et al., 2014, Devol, 2015, Marchant et al., 2016, Asmala et al., 2017). Advection may  
349 serve to increase denitrification as has been shown in some simulations (Canion et al., 2014), but  
350 others argue that the effect may be small (Devol, 2015), possibly because the increased rate of supply  
351 of nitrate from the overlying waters may be offset by the increased oxygenation of the sediments.  
352 Diffusive measurements may also lead to underestimations of rates if bioturbation is an important  
353 process increasing the effective denitrification rate (Laverock et al., 2011, Eyre et al., 2011). Further  
354 work to understand the extent and significance of advective and bioturbation process on  
355 denitrification in sediments is clearly needed.

356

## 357 **5. Conclusions**

358 The measured rates of denitrification in the sampled North Sea sediments are in the range 5-10  $\mu\text{mol}$   
359  $\text{m}^{-2}\text{h}^{-1}$  and are dominated by canonical denitrification, consistent with other data from temperate shelf  
360 sea systems. These rates are rather lower than model and budget based estimates suggesting  
361 limitations in either the models/budgets approaches and/or in the directly measured rates, or their  
362 scaling up. Issues with the direct measurements could arise from assumptions inherent on the  
363 methods, the need to include a contribution of much higher denitrification rates in estuaries in overall  
364 budgets and/or issues associated with advective and bioturbation rather than just diffusional exchanges  
365 between sediments and the overlying water column.

366

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377

Table 1 Measured total denitrification rates and component canonical denitrification, anammox and DNRA rates, plus anammox potential along with sediment organic carbon, grain size and oxygen penetration depth (OPD) and water depth, bottom water temperature and nitrate concentrations. For sampling stations see Figure 1. Dw% is the percentage of total denitrification associated with diffusion of nitrate from the overlying water

Site	Denitrification	Canonical Denitrification	Anammox	DNRA	Dw%	Water Depth	Temperature	NO <sub>3</sub> <sup>-</sup>	OPD	Organic Carbon	Av Grain Size	Anammox Potential
	----- μmol m <sup>-2</sup> h <sup>-1</sup> -----					m	°C	μM	mm	Carbon%	Size μm	nmol cm <sup>-3</sup> h <sup>-1</sup>
North Sea 43	9.5	9.3	0.2	0	13	28	18	2.3	6	0.05	90	0.04
North Sea 68	7.7	7.7	0	1.3	12	60	13	2.0	7	0.08	141	0.16
North Sea 101	2.9	2.7	0.2	0	12	110	8	3.0	6	0.39	83	0.25
North Sea 127	7.8	7.8	0	0	19	116	9	6.0	7	0.48	76	0.73
North Sea 141	10.6	10.6	0	0	14	65	7	2.3	8	0.22	92	0.22
Wash May	6.9	6.9	0	0	21	0	12	-	3	0.12	165	0.06
Wash June	8.1	8.1	0	3.3	65	0	16	7.5	2	0.08	157	0.004
Wash Sept	0.4	0.4	0	0.2	100	0	20	0.7	3	0.08	141	0.02
Wash Oct	2.9	2.9	0	0.2	33	0	21	3.0	4	0.09	148	0.02

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