1 Niche differentiation of ammonia oxidising archaea (AOA) and bacteria (AOB)
2 in response to paper and pulp mill effluent

3

4

5 Abell, G.C.J.¹, Ross, D.J.², Keane, J.², Holmes, B.¹, Robert, S.S.¹, Keough,
6 M.J.³, Eyre, B.D.⁴ and Volkman, J.K.¹

7

8 ¹ CSIRO, Marine and Atmospheric Research and Wealth from Oceans,
9 National Research Flagship, Hobart, Tasmania, Australia

10 ² Institute for Marine and Antarctic Studies, Nubeena Crescent, Taroona,
11 Tasmania, Australia

12 ³Department of Zoology, University of Melbourne, Melbourne, Victoria 3010,
13 Australia

14 ⁴Centre for Coastal Biogeochemistry, Southern Cross University, Military
15 Road, Lismore, New South Wales, 2480, Australia

16

17 *Corresponding Author, Guy.Abell@csiro.au, CSIRO, Castray Esplanade,
18 Hobart 7000, Tasmania, Australia, Tel; +61 362325597, Fax; +61 362325485

19

20 Running Title: Effect of mill effluent on sediment nitrifiers.

21

22 **Summary**

23 Sediment organic loading has been shown to affect estuarine nitrification and
24 denitrification, resulting in changes to sediment biogeochemistry and nutrient
25 fluxes detrimental to estuarine health. This study examined the effects of
26 organic loading on nutrient fluxes and microbial communities in sediments
27 receiving effluent from a paper and pulp mill (PPM) by applying microcosm
28 studies and molecular microbial ecology techniques. Three sites near the
29 PPM outfall were compared to three control sites, one upstream and two
30 downstream of the outfall. The control sites showed coupled nitrification-
31 denitrification with minimal ammonia release from the sediment. In contrast,
32 the impacted sites were characterised by nitrate uptake and substantial
33 ammonia efflux from the sediments, consistent with a decoupling of
34 nitrification and denitrification. Analysis of gene diversity demonstrated that
35 the composition of nitrifier communities was not significantly different at the
36 impacted sites compared to the control sites, however analysis of gene
37 abundance indicated that whilst there was no difference in total bacteria, total
38 archaea or ammonia oxidising archaea (AOA) abundance between control
39 and impacted sites, there was a significant reduction in ammonia oxidising
40 bacteria (AOB) at impacted sites. The results of this study demonstrate an
41 effect of organic loading on estuarine sediment biogeochemistry and highlight
42 an apparent niche differentiation between AOA and AOB.

43

44

45 Introduction:

46 Anthropogenic impacts on the environment are of significant concern for the
47 health of our waterways with global levels of nitrogen deposition doubling in
48 recent times [1]. Likewise, the organic enrichment of estuarine sediment as a
49 consequence of increasing population density and industrialisation is also a
50 concern. The effect that increased loading of organic matter (OM) has on
51 biogeochemical cycling has been the subject of numerous studies [2-5].
52 Whilst these studies have demonstrated that changes to sediment
53 biogeochemistry are directly linked to OM inputs, the effects on the microbial
54 communities responsible for nitrification, a key step in the removal of nitrogen,
55 are poorly understood.

56
57 One of the primary effects of OM loading of sediments is an increase in
58 biological oxygen demand (BOD) and a concomitant decrease in the oxygen
59 penetration depth [6]. Decreased oxygen saturation has a significant effect on
60 the efficiency of sediment nitrification, an aerobic process [7,8] by limiting the
61 depth interval in which nitrification can occur. Denitrification, a critical next
62 step in the transformation of nitrogen from ammonia to gaseous forms is
63 dependent on anaerobic conditions and a supply of oxidised nitrogen species
64 usually supplied through sediment nitrification. When sediment nitrification is
65 inhibited the process of denitrification can continue only if oxidised nitrogen
66 species can be taken up from the water column, resulting in a decoupling of
67 the two processes. This shift to water column nitrate as a source for
68 denitrification when sediment nitrification is limited is widely reported [[9] and
69 references therein].

70
71 A previous study on the effects of dissolved oxygen (DO) concentration on
72 sediment biogeochemistry in a simulated microcosm system [10],
73 demonstrated that a reduction in bottom water DO resulted in a decoupling of
74 nitrification and denitrification and gave rise to increased ammonia (NH₄)
75 fluxes from the sediment as well as altered gene transcription in sediment
76 ammonia oxidiser communities. In that study the profile of expressed
77 ammonia monooxygenase (*amoA*) in bacterial communities changed with
78 decreasing DO, suggesting an adaptation to decreased DO conditions.

79

80 It has been suggested that bacterial nitrifiers are generalists that possess
81 varying degrees of tolerance for low oxygen with some members able to
82 withstand long periods of dormancy [11]. Factors other than DO concentration
83 have been demonstrated to affect nitrification rates in sediment. These
84 include, but are not limited to, ammonia and sulphide concentrations,
85 temperature, salinity and macrofaunal activity [12,11]. The inhibition of
86 sediment nitrification is implicated in the increased efflux of ammonia from
87 organically loaded sediments, increasing the risk of eutrophication events [2].
88 Studies of cultivated members of the ammonia oxidising archaea (AOA) have
89 demonstrated a high affinity for ammonia and a lower requirement for
90 dissolved oxygen [13]. This suggests that there may be a differential effect of
91 DO on bacterial and archaeal nitrifiers.

92

93 The Derwent estuary is a microtidal, stratified temperate estuary in southern
94 Tasmania, Australia, and has been the subject of previous studies of the role
95 of organic matter in sediment biogeochemistry and nitrogen cycling microbial
96 community structure [14,10,3,15]. The estuary is subject to a number of inputs
97 of organic matter including agriculture run-off, marine inflows, sewerage and
98 wastewater treatment plants, and a paper and pulp mill (PPM). Of these
99 previous studies, one examined the effect of PPM effluent on sediment
100 nitrogen cycling, through its addition to an intertidal sediment and subsequent
101 study over a period of 27 days, demonstrating a change in sediment nutrient
102 fluxes over the period [3]. A further study of the lower regions of the estuary
103 indicated the relationship between N-cycling microbial communities and
104 organic matter quality [14].

105

106 We hypothesised that the long-term effect of sediment organic loading
107 resulting from the PPM effluent would be reflected in the change in
108 biogeochemistry across the impacted sites and would be reflected in the
109 abundance and composition of the nitrifier and denitrifier communities.
110 Specifically, that an increase in respiration rates and changes to redox
111 conditions would select for nitrifier communities that are better suited to those
112 conditions.

113 *Materials and methods:*

114 The effects of a combined effluent stream (CES) from the PPM in the upper
115 Derwent estuary, Tasmania, Australia, on nutrient fluxes were examined in
116 two surveys conducted 4 months apart (April 2008 and August 2008). The
117 sites examined in this study are approximately 3-10 km upstream of the sites
118 described previously [14].

119

120 **Site description:**

121 The sampling region used in this study encompasses 3 sites within the
122 impacted zone of the PPM outfall (at 0 m, 250 m and 750 m downstream;
123 sites B, C and D respectively) and 3 sites outside the impacted zone (at 1200
124 m upstream, 3400 m and 8400 m downstream, sites A, E and F respectively),
125 Table 1. During each survey divers collected replicate sediment samples from
126 unvegetated soft sediment in the sub tidal zone. Samples were taken from
127 sub-sites within each site and consisted of 5 x 150 mm diameter intact cores
128 for nutrient flux and sediment geochemistry measurements (first and second
129 surveys) and 3 x 50 mm diameter sediment cores for DNA extraction (second
130 survey only). At the time of each sampling, measures of salinity, dissolved
131 oxygen (DO) and temperature were made using a HACH (HQ40d) dual-input
132 multi-parameter digital meter with optical dissolved oxygen (LDO101-15) and
133 conductivity probes (CDC401-15) (Hach Company, Colorado, USA).

134

135 **Sediment geochemical analysis:**

136 Sediment analysis was performed as described previously [15]. Briefly,
137 samples for C:N and stable isotope analysis were taken following nutrient flux
138 measurements and stored at -20 °C prior to analysis. For analysis of stable
139 isotopes and C:N, freeze-dried, homogenized, ground subsamples of oven-
140 dried (60 °C) sediment were weighed into silver capsules for $\delta^{13}\text{C}$ analysis
141 following acidification (1 M HCl) and into tin capsules for $\delta^{15}\text{N}$ analysis.
142 Sediment $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C and %N were determined using a Thermo Finnigan
143 Flash EA 112 interfaced *via* a Thermo ConFlo III with a Thermo Delta V Plus
144 IRMS [15]. Samples of acetanilide of known isotope composition were
145 analysed within each run to verify isotope values. Reproducibility for both
146 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was $\pm 0.2\%$.

147
148

149 **Nutrient flux measurement:**

150 Nutrient fluxes were calculated using 5 replicate microcosms from each site
151 as previously described [14]. Briefly, rates of nutrient exchange were
152 measured during a dark incubation on the second day following sampling.
153 Before the commencement of the flux measurements, the cores were flushed
154 with fresh site water and capped. Water samples (~250 ml) were collected
155 with syringes at the start and end of each of the incubations (~4 h). Filtered
156 (0.45 µm membrane) samples were taken for measurements of total dissolved
157 phosphorus (TDP), ammonia (NH₄), nitrite (NO₂), nitrate (NO₃), phosphate
158 (PO₄) and silicate (SiO₄). Nutrient samples were frozen in polypropylene
159 screw cap bottles until required for analysis.

160 Net flux rates were calculated in units of mmol m⁻² d⁻¹ representing change in
161 nutrient concentration over the time of the incubation. All nutrient analyses
162 were carried out colourimetrically using a Lachat Flow Injection Analyser.
163 Nitrite (NO₂) was determined using sulphanilamide, and oxidized nitrogen
164 (NO_x) was determined by cadmium reduction. Ammonia (NH₄⁺) was
165 determined using hypochlorite/phenolate, and DIN was determined as the
166 sum of NO_x and NH₄ [16].

167

168 **Microbial community analysis:**

169 DNA was extracted from ~0.5 g of sediment from the top 1 cm of each of the
170 sediment samples using a method described previously [17].

171

172 **Analysis of gene abundance:**

173 QPCR was performed as previously described [14]. Bacterial and archaeal
174 *amoA*, *nirS*, *nirK* and bacterial and archaeal 16S rRNA genes were quantified
175 from DNA extractions using a 7500 Real time PCR system (Applied
176 Biosystems, Carlsbad, USA). PCR reactions (15 µl) comprised 10 µg/µL BSA,
177 10 pmol of each primer, 7.5 µl of Q-PCR master mix (Applied Biosystems,
178 Carlsbad, USA) and 1 µL of template DNA (comprising ~10-20 ng of DNA).
179 Fluorescence acquisition was performed at the temperatures indicated for
180 each gene in Table S1, at which temperature all primer dimers had melted,

181 but specific products had not. The cycling conditions were 95 °C for 3 min,
182 followed by 40 cycles of 40 s at 95 °C, 30 s at the specific annealing
183 temperature followed by 1 min at 72 °C then 20 s at the appropriate
184 acquisition temperature (Table S1). Standards for qPCR were generated by
185 serial dilutions of known copies of PCR fragments of the respective functional
186 gene generated using M13 PCR from clones generated during the present
187 and previous studies. Samples were assayed as previously described [17] to
188 ensure that there was no significant effect of PCR inhibition on the qPCR
189 data.

190

191 Gene diversity analysis:

192 T-RFLP analysis of AOA *amoA* was performed as described previously [17]
193 using the *Taq-I* enzyme (New England Biolabs, Ipswich, USA) and FAM-
194 labelled forward primers described in Table S1. Profiles were analysed using
195 the GeneMapper 3.7 package (Applied Biosystems, Carlsbad, USA).
196 DGGE analysis of AOB *amoA* was performed as described previously [18]
197 using the primers *amoA*-1F and *amoA*-2R-Clamp (Table S1) and PCR
198 protocol described previously [19].

199

200 Gene sequence analysis:

201 Clone library construction, sequencing and analysis were performed as
202 previously described [10]. Sequences used in this study were deposited in the
203 NCBI database (www.ncbi.nlm.nih.gov) under accession numbers KC962857-
204 KC963011.

205

206 **Statistical analysis:**

207 For univariate analysis of the differences in nutrient fluxes, sediment physical
208 parameters and gene abundances, data were checked for normality and
209 homogeneity of variances using Q-Q and residual plots. Data not meeting the
210 assumptions of ANOVA were log-transformed (square root for gene
211 abundances and reciprocal for fluxes). Significant factors returned from the
212 two-way ANOVA were compared using Tukey's HSD *post hoc* tests. All

213 univariate tests (at $\alpha=0.05$) and graphing were done using the R programming
214 environment (R Project for statistical computing; www.r-project.org).
215 Multivariate analysis of variance between individual T-RFLP or DGGE profiles
216 was performed using the PERMANOVA+ package for the Primer 6 software
217 (PRIMER-E Ltd, Plymouth, UK). Relatedness of different gene profiles for
218 each sample was calculated using the Bray-Curtis similarity calculation on
219 standardised data [20,21].
220 The effect of site (6 levels) and outfall (2 levels) was tested using permutation-
221 based testing of multivariate analysis of variation (PERMANOVA) using
222 approaches described previously [22]. Default settings for PERMANOVA were
223 used (Type III sums of squares, 9999 permutations, permutations of residuals
224 under a reduced model). All tests were conducted at $p<0.05$. Pairwise
225 comparisons were conducted *post hoc* on factors found to be significant in the
226 'global' test.
227 Multi dimensional scaling (MDS) plots based on a Bray-Curtis similarity matrix
228 of T-RFLP profiles were generated using the vegan package in R (ver. 2.0-5)
229 [23]. Significant relationships between environmental and nutrient flux
230 variables and ordination were determined, based on 9999 permutations, using
231 the *envfit* function. Significant relationships between ordination and
232 environmental variables ($p<0.05$) were plotted as vectors, whereby the vector
233 indicates the direction of the gradient of the environmental variable and the
234 length indicates the correlation between the ordination and the environmental
235 variable.
236 Chao indices and rarefaction curves for each library were calculated based on
237 OTUs of 5% and 20% similarity for AOB and 5% or 15% similarity for AOA
238 [24,25], using the software mothur 1.19.3 [26] and the Unifrac Lineage-
239 Specific analysis was used to evaluate the difference between libraries of
240 *amoA* genes (0.05 branch length, minimum 3 descendants) [27].
241
242

243 *Results and discussion:*

244

245 During this study a survey of nutrient fluxes across different sites adjacent to
246 the PPM outfall highlighted a region of altered biogeochemistry associated
247 with inputs from the CES (Figure 1, top). This region of altered
248 biogeochemistry was characterised by an increase in ammonia efflux (from
249 sediment to water column) and corresponding nitrate and nitrite influx (from
250 water column to sediment) within the CES plume, which was observed during
251 a subsequent sampling (6 months later; Figure 1, bottom). This study was
252 aimed at determining if the altered biogeochemistry associated with increased
253 organic matter loads was reflected in total, nitrifying and denitrifying microbial
254 communities.

255

256 **Sediment nutrient fluxes, bottom water and sediment chemistry**

257 Nutrient fluxes (ammonia, nitrate + nitrite, phosphate, silicate), bottom water
258 chemistry (salinity, temperature, DO) and sediment chemistry (%C, %N and
259 stable isotope ratios of carbon [$\delta^{13}\text{C}$] and nitrogen [$\delta^{15}\text{N}$]) were assessed
260 during summer and winter 2008 (first and second surveys respectively).
261 Bottom water temperature varied between 15.2 and 16.1 °C during the first
262 survey and 6.2 and 9.4 °C during the second survey, whilst salinity was
263 constant (24) in the first survey and varied between 0 and 23.4 in the second
264 survey reflecting the large variance in flow rates and tide in the estuary from
265 day to day (Table 1). Bottom water dissolved oxygen was substantially lower
266 at sites within the plume during both surveys compared to the other sites, with
267 the exception of site B during the second survey (Table 1), however this
268 corresponded with a period of high flow in the river which is likely to have
269 limited the effect of oxygen drawdown at site B given its position directly
270 adjacent the outfall. The decreased bottom water DO at sites C and D is
271 consistent with an increase in BOD associated with the nutrient discharge
272 plume where the sediment was subject to increased loads of labile OM.
273 Increased oxygen consumption as a result of OM loading of sediment has
274 been shown in experimental systems previously [7,4]. The greatest impact
275 was seen at the two sites downstream of the plume (Sites C and D), where
276 the majority of the OM from the plume had settled.

277 The input of plume OM is reflected in the sediment chemistry analysis
 278 conducted on samples from the second survey (Figure S1) which
 279 demonstrated a significantly depleted $\delta^{15}\text{N}$ at the impacted sites compared to
 280 the control sites ($p < 0.01$) indicative of a change in the source of the organic
 281 matter in the sediment [28,29]. The $\delta^{15}\text{N} / \delta^{13}\text{C}$ signature within the impacted
 282 sites indicated that the sediment organic matter consisted mostly of terrestrial
 283 plant-derived OM and was consistent with the signature of the effluent POM
 284 (particulate organic matter) [15]. Control site signatures suggest a
 285 combination of terrestrial and marine OM inputs [30]. There was significant
 286 variation in the %N and %C between individual sites, however there was no
 287 significant difference between the impacted and control sites. Whilst there
 288 was a general trend of increasing $\delta^{13}\text{C}$ down the estuary there was no
 289 significant difference between sites.

290 During the initial survey there was a significant difference in NO_x flux between
 291 the impacted and control sites ($P < 0.01$) with all the control sites
 292 demonstrating a net efflux of nitrate and uptake or limited efflux of ammonia
 293 from the sediment suggesting highly efficient nitrification that exceeded
 294 denitrification (Figure 1, top panels). In contrast, the impacted sites all
 295 demonstrated an uptake of NO_x from the water column and an efflux of NH_4
 296 suggesting substantial mineralisation and limited nitrification decoupled from
 297 denitrification. This apparent inhibition of nitrification is consistent with
 298 previous studies that have demonstrated partial or total inhibition of
 299 nitrification with increased organic loading [7,5].

300

301 During the second nutrient flux survey (winter 2008, Figure 1, lower panels),
 302 the affected sites showed a substantial ammonia efflux whilst control sites
 303 showed an uptake or a slight efflux. There was a net efflux of NO_x at all of the
 304 control sites and a net uptake at each of the impacted sites (Figure 1, lower
 305 panels). As per the first survey, the efflux of NH_4 and uptake of NO_x within the
 306 impacted sites suggest a decoupling of nitrification and denitrification resulting
 307 from the increased BOD and subsequently decreased bottom water DO
 308 (Table 1). It is also possible that increased organic loading on the sediment,
 309 coupled with lower oxygen concentrations resulted in higher concentrations of
 310 sulfides in the sediment significantly inhibiting nitrification [12] and promoting

311 fermentation and dissimilatory nitrate reduction to ammonia (DNRA). Previous
312 studies of sediment nitrogen cycling have shown significant effects of
313 sediment hypoxia on nitrogen cycling in estuarine environments [10,31]
314 highlighting the role that increases in BOD can have on nutrient fluxes.

315 **Gene abundance**

316 Assessment of the abundance of different genes using real-time PCR (QPCR)
317 demonstrated no significant difference in the abundance of total bacteria or
318 archaea between surface sediment from impacted and control sites.

319 Abundance of marker genes for denitrification (*nirS* and *nirK*) varied between
320 individual sites but were not significantly different between the impacted and
321 control sites (Figure 2). The abundance of genes associated with denitrifiers

322 was consistent with a previous study of the lower regions of the Derwent
323 estuary [14] but were higher than the abundance measured in two previous
324 studies of estuarine sediment [17,33]. Assessment of the abundance of

325 bacterial (AOB) and archaeal (AOA) demonstrated ammonia oxidiser
326 abundance to be comparable to that of previous studies of estuarine sediment
327 [14,17,34]. Comparison of the control and impacted sites demonstrated a

328 significant decrease, of approximately 3 orders of magnitude, in the
329 abundance of AOB associated with the outfall, compared to the control sites,
330 whilst the AOA abundance was not significantly different (Figure 2). The

331 difference in gene abundance equates to an even greater difference in cell
332 numbers given the typical number of *amoA* copies per genome for the two
333 groups (AOB typically have 2-3 copies per genome whilst AOA have one;

334 [35,36]. The lower abundance of AOB in the heavily organically loaded sites
335 contrasts to a previous study of organically loaded sediments under fish farms
336 which demonstrated no reduction in the abundance of AOB [2], possibly
337 reflecting differences in the nature or magnitude of organic loading between
338 the studies.

339

340 The lack of a significant change in the abundance of total bacteria and
341 archaea or denitrifier communities suggests that heterotrophic microbial
342 abundance is not significantly affected by the organic loading or increased
343 BOD. Microbial communities in the impacted sites are likely metabolising

344 organic matter under anaerobic conditions as indicated by large effluxes of
345 phosphate and CO₂ (data not shown).

346

347 The increase in sediment respiration, subsequent BOD and decrease of AOB
348 abundance in the impacted sites is likely a response to the decrease in
349 sediment DO which is consistent with their aerobic physiology. The lack of a
350 corresponding decrease in the abundance of AOA in the same zone suggests
351 that these organisms are capable of persistence under reduced oxygen
352 conditions. A previous study of the effects of anoxia on sediment ammonia
353 oxidiser communities in laboratory manipulations of Derwent estuary
354 sediments demonstrated that decreased oxygen concentrations had no
355 significant effect on gene expression in AOA, whilst AOB showed significantly
356 reduced gene expression [10]. An adaptation to lower oxygen conditions may
357 be linked to the way in which AOA oxidise ammonia, with less oxygen
358 required per mole of NH₄ oxidised [37]. It is also possible that the organisms
359 represented by AOA *amoA* are inactive or not actively oxidising ammonia
360 under these conditions but rather are utilising organic compounds as an
361 energy source, as metagenomic and environmental studies indicate that
362 different AOA guilds may be capable of autotrophic, mixotrophic and even
363 heterotrophic lifestyles [24].

364 Whilst the possibility that other components of the effluent may explain the
365 difference in AOB and AOA abundance (toxicity) cannot be eliminated, the
366 effect is consistent with differences in the physiology of AOA and AOB, such
367 as lower half saturation constants for oxygen [38].

368

369 **Gene diversity**

370 The diversity of the different functional groups of microorganisms was
371 assessed using a combination of molecular methods including T-RFLP for
372 archaeal *amoA* and DGGE for bacterial *amoA*. Analysis was performed on all
373 replicate samples from each of the sites. Multi dimensional scaling (MDS,
374 Figure 3) and PERMANOVA analysis (Table S2) of both nitrifier groups
375 across the 6 sites demonstrated no overall difference between impacted and
376 control sites for archaeal or bacterial *amoA* ($p>0.05$, Figure 3), however there
377 was a strong site-selection effect on both bacterial and archaeal *amoA*

($p < 0.01$ and $p < 0.05$ respectively). The ordination of AOB profiles was significantly related to carbon and nitrogen abundance (%) in the sediment as well as $\delta^{15}\text{N}$ isotope ratio and C:N ($p < 0.05$), whilst AOA profiles were significantly related to $\delta^{15}\text{N}$ isotope ratio ($p < 0.05$). This suggests that the composition of both nitrifier communities at these sites are strongly related to the nature and composition of organic matter in the sediment consistent with a previous study of microbial populations in the estuary [14]. Whilst it is generally accepted that the AOA are inhibited by high ammonia concentrations as demonstrated by studies of isolates and environmental ecology [39,38], AOA isolates capable of growth under higher ammonia conditions (>15 mM) have been identified [40,41] and transcription of AOA *amoA* under high soil ammonia loads [42] also suggest that some of these organisms may be adapted to high ammonia loads. Furthermore, studies in soil environments indicated that the source of ammonia may play a role in the niche separation of AOA and AOB with AOB being stimulated by inorganic nitrogen and AOA favouring ammonia originating from mineralised organic nitrogen [43].

Given the potential limitations of both T-RFLP and DGGE for resolving the differences in community structure of *amoA*, 3 clone libraries of each gene were constructed from combined PCR reactions of each of the site replicate samples (corresponding to two control and one impacted site from the 2nd survey, sites A, C and E) to allow more detailed description of the diversity of AOA and AOB associated with these sites. AOA libraries comprised 30, 29 and 31 clones, whilst AOB libraries comprised 19, 23 and 26 clones from sites A, C and E respectively. Phylogenetic analysis of the sequences from this study demonstrated that the AOB clones clustered exclusively with other sequences from different estuarine environments [44,45], the majority of these sequences being from the 'uncultivated AOB I' cluster for which there is no physiological information (Figure 4). AOA clones clustered with sequences from a number of sources including soil, estuarine and bioreactor environments [46]. Approximately half of the AOA clones were associated with *Nitrosoarchaeum limnia*, a motile, low salinity associated ammonia

411 oxidising archaeon enriched from an estuarine environment [47], other clones
412 were distantly related to the genera *Nitrososphaera* (Figure 5). Whilst it
413 cannot be excluded that the ammonia oxidisers observed in sediment
414 samples from the affected sites were introduced from the plume, the majority
415 of sequences clustered mainly with estuarine derived clones and each of the
416 OTUs observed (T-RFLP peaks or DGGE bands) were common to all the
417 sediment samples (both control and affected) albeit with varying abundance,
418 this was also reflected in the clone library analysis (Figures 4 and 5).

419

420 *AmoA* sequences comprised 6 distinct lineages of AOB (Figure 4), and 9
421 distinct lineages of AOA (Figure 5). Rarefaction analysis of each of the
422 libraries demonstrated that the majority of the diversity was likely sampled by
423 each of the libraries. Interestingly the species richness, as indicated by
424 rarefaction, for AOB was highest in the impacted site (Figure 4), conversely
425 the lowest AOA species diversity was found in the impacted site library
426 (Figure 5). Chao richness estimation indicated that the total diversity of AOB
427 was 4, 13 and 8 phylotypes at 5% similarity for sites A, C and E and 2, 4 and
428 2 phylotypes at 20% similarity, whilst AOA diversity was 15, 9 and 13
429 phylotypes at 5% and 5, 8 and 8 phylotypes at 15% similarity. Unifrac analysis
430 of clone libraries demonstrated a significant difference in the composition of
431 both the AOB and AOA libraries between sites C and E ($p < 0.03$). However no
432 specific lineages were indicated as significantly associated with this
433 difference, unlike a previous study of the influence of fish farm organic loading
434 on AOB communities which identified a subgroup related to *Nitrosomonas*
435 that appeared to be selected for by organic loading [5].

436

437 The apparent decrease in oxygen, presumably the effect of increased
438 sediment BOD associated with the concomitant change in organic matter
439 loading in the impacted sites is likely to have affected the nitrifying community
440 to the extent that very little nitrification occurs within the sediment at these
441 sites. Assuming the AOB are responsible for the majority of nitrification in the
442 estuary, the large decrease in AOB abundance associated with this also
443 explains the apparent absence or substantial decrease in nitrification. In
444 contrast to the decrease in AOB abundance, the persistence of AOA in the

445 impacted sites suggests an adaptation to mixotrophic growth, as previously
446 suggested for *Nitrososphaera viennensis* [41]. In fact, their abundance
447 appears to be more closely related to distance down the estuary, suggesting
448 that the location of the sites, rather than the organic matter composition has a
449 greater role in determining their abundance and factors such as salinity
450 differences between sites may be important (Table 1).

451

452 It has been previously suggested that nitrifiers may exhibit varying degrees of
453 tolerance to low oxygen [11,10] and that phylotypes associated with the
454 impacted sites are likely more tolerant to low oxygen conditions. The
455 prolonged effect of decreased oxygen is likely to lead, ultimately, to a
456 significant decrease in the AOB population and thus nitrification potential in
457 the sediment. The lower requirement of AOA for oxygen, however, may
458 contribute to their persistence within the impacted sites. The most abundant
459 clones from the impacted site library were related to *Nitrosoarchaeum limnia*
460 which has demonstrated the ability to grow under oxygen concentrations as
461 low as 59 μM [48] highlighting a possible role of low oxygen tolerance in the
462 persistence of AOA in these sites. This ability of AOA to persist in low oxygen
463 environments is also evidenced by their abundance in anoxic zones, anoxic
464 marine sediments and soils [13].

465

466 Subsequent to this study, the paper and pulp mill operators have taken steps
467 to reduce the total suspended solid loads; since the completion of this study
468 total suspended solid loads have been reduced from 3-4 tonnes d^{-1} to around
469 0.5 tonne d^{-1} . with the intention of providing improvements in sediment
470 function in the vicinity of the outfall.

471

472 **Conclusions:**

473 The results of this study demonstrate differential effects of anthropogenic
474 organic matter loading on different groups of microbes. The impacted sites
475 examined during this study were significantly affected in terms of organic
476 matter composition and biogeochemistry, with an apparent decoupling of
477 nitrification and denitrification resulting in a substantial efflux of ammonia into
478 the estuary. Despite the influence of the outfall on biogeochemistry, there was

no corresponding effect on the abundance of total bacteria or archaea, or denitrifier populations. In contrast to this, AOB were significantly reduced in abundance reflecting the apparent inhibition of nitrification in the sediment. Surprisingly, however, the abundance of AOA was not affected by organic loading and appears to respond more strongly to other environmental stimuli. The ability of nitrifiers to withstand prolonged periods of dormancy [11] may explain their diversity in many estuarine sediments, however the results of this study indicate that for the AOB, prolonged high sediment OM loads, resulting in anoxia are likely to eventually reduce the abundance of AOB. This effect, however, was not seen for the AOA community raising the possibility that they are capable of persistence in high OM, low DO conditions due to either an ability to oxidise ammonia at low oxygen conditions or alternative metabolic strategies. This possibility contradicts the physiology of cultivated members of the AOA which are inhibited by high ammonia concentrations such as those expected to be experienced in the impacted sites in this study. In estuarine sediment, the ability to persist under conditions of high organic loading and subsequent low dissolved oxygen conditions may represent an important niche differentiation between the AOA and AOB.

Acknowledgements: This work was supported in part by an Australian Research Council Linkage grant (LP0770222) awarded to DJR, DBE and MJK. We would like to thank Andy Revil and Rebecca Esmay (CMAR) for sediment and stable isotope analysis. We would also like to thank Norske Skog Boyer for financial and in-kind assistance, and in particular, Des Richardson for reviewing the manuscript and providing technical advice on the mill operations and effluent characteristics.

505 **Tables:**

506 Table 1: Summary of bottom water characteristics across the 6 sites during
507 the first and second surveys (April 2008 and August 2008, respectively).

508

Site	Impacted / Control	Temperature (°C)		Salinity		Dissolved Oxygen (% sat)	
		1st	2nd	1st	2nd	1st	2nd
A	Control	15.6	6.2	24.3	0	38.2	93.2
B	Impacted	15.9	7.4	24.5	5.8	14.4	86.6
C	Impacted	16.1	9.4	24.4	23.4	14.0	32.5
D	Impacted	15.8	9.3	24.3	22.3	15.8	36.2
E	Control	15.7	7.8	25.4	13.2	32.9	80.7
F	Control	15.2	8.6	24.6	18.8	62.8	83.0

509

510 Table S1. PCR primers and PCR conditions used in this study. T_{Ann} denotes
511 the annealing temperature used and T_{Acq} denotes the temperature at which
512 fluorescence acquisition was performed during qPCR.

Primer	Target gene	Sequence (5'-3')	T_{Ann}/T_{Acq}	Reference
Arch amoA-1F	<i>amoA</i> AOA	STAATGGTCTGGCTTAGACG	56/76	[49]
Arch amoA-2R	<i>amoA</i> AOA	GCGGCCATCCATCTGTATGT	56/76	[49]
amoA-1F	<i>amoA</i> β -AOB	GGGGTTTCTACTGGTGGT	55/78	[50]
amoA-2R	<i>amoA</i> β -AOB	CCCCTCKGSAAGCCTTCTTC	55/78	[50]
amoA-2R-	<i>amoA</i> β -AOB	CGCCGCGCGCGGGCGGGCGGGG	55/-	[19]
Clamp		GCCCCCTCKGSAAGCCTTCTTC		
nirS1F	<i>nirS</i>	CCTAYTGCCGCCRCART	57/77	[51]
nirS6R	<i>nirS</i>	CGTTGAACCTTRCCGGT	57/77	[51]
F1aCu	<i>nirK</i>	ATCATGGTCTGCCGCG	57/77	[52]
R3Cu	<i>nirK</i>	TTGGTGTRGACTAGCTCCG	57/77	[52]
63f	Bacterial 16S rRNA	CAGGCCTAACACATGCAAGTC	55/76	[53]
1087r	Bacterial 16S rRNA	CTCGTTGCGGGACTTACCCC	55/76	[54]
Ar3f	Archaeal 16S rRNA	TTCCGGTTGATCCTGCCGGA	55/76	[55]
Ar927r	Archaeal 16S rRNA	CCCGCCAATTCCTTAAAGTTTC	55/76	[56]

513

514 Table S2. Results of PERMANOVA testing the effects of site and outfall
515 impact on the community composition (DGGE and T-RFLP profiles) of AOB
516 and AOA. CV denotes the square root of the component of variation [22], a
517 measure of the effect size in units of the community dissimilarities. Significant
518 effects ($p < 0.05$) are shown in bold.

		Site	Outfall
--	--	------	---------

Group	Residual	CV (√)	p	CV (√)	p
AOB	8.04	28.39	0.001	-6.91	0.501
AOA	38.22	15.47	0.024	12.6	0.207

519

520

521 **Figures:**

522

523

524 **Figure 1.** Box plots (n=5) representing benthic nutrient fluxes at each of the 6
525 sites spanning the mill outfall during the first (top) and second (bottom)
526 surveys. Significant differences between treatments are indicated with
527 different letters ($p < 0.01$), circles represent outliers (more than 1.5 x the upper
528 or lower quartile). Positive fluxes indicate a sediment to water column flux and
529 negative fluxes indicate a water column to sediment flux.

530

531 **Figure 2.** Bar plots demonstrating the mean (n=3) abundance of marker
532 genes for total bacteria and total Archaea (Upper panel), bacterial and
533 archaeal nitrifiers (mid panel) and *nirS* and *nirK* (lower panel) from samples
534 taken during the second survey. Error bars show standard deviation.
535 Abundances are expressed as copies per gram of dry sediment. Letters
536 indicate significant differences between sites ($p < 0.05$).

537

538 **Figure 3.** MDS analysis of T-RFLP (AOA) and DGGE (AOB) data,
539 demonstrating the differences in community composition between samples
540 taken during the second survey. Vectors indicate significant correlation
541 between sediment characteristics and ordination ($p < 0.01$) calculated using the
542 envfit routine in R (999 permutations). Characteristics are indicated at the end
543 of individual vectors, (sediment C:N, total N content [%N] and $\delta^{15}\text{N}$ isotope
544 ratio [N15]). Individual sites (bordered by dotted blue lines) are indicated on
545 each plot and the separation of impacted and control sites indicated on the
546 AOB plot by the dashed line. Stress values for MDS plots are 6 for AOB and
547 13 for AOA.

548

549 **Figure 4.** Phylogeny of partial bacterial *amoA* sequences from sites A, C and
550 E (Second survey, GenBank accession numbers: KC962944-KC963011) and
551 reference sequences from the database. Numbers next to collapsed clades
552 indicate the number of clones from site A (control), C (impacted) and E
553 (control), respectively highlighting the differences in sequences between the
554 sites. Bootstrap values greater than 75% are shown at the nodes.

555

556 **Figure 5.** Phylogeny of partial archaeal *amoA* sequences from sites A, C and
557 E (Second survey, GenBank accession numbers: KC962857-KC962943) and
558 reference sequences from the database. Numbers next to collapsed clades
559 indicate the number of clones from site A (control), C (impacted) and E
560 (control), respectively highlighting the differences in sequences between the
561 sites.. Letters indicate the site that individual sequences are from. Bootstrap
562 values greater than 75% are shown at the nodes.

563

564 **Figure S1.** Bar plot showing geochemical analysis of sediment samples from
565 each of the sites during the second survey. Letters indicate significant
566 differences between sites ($p < 0.05$).

567

568

569

570 **References:**

- 571
- 572 1. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW,
573 Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM,
574 Michaels AF, Porter JH, Townsend AR, Vöosmarty CJ (2004) Nitrogen
575 Cycles: Past, Present, and Future. *Biogeochemistry* 70 (2):153-226.
576 doi:10.1007/s10533-004-0370-0
- 577 2. Bissett A, Cook PLM, Macleod C, Bowman JP, Burke C (2009) Effects of
578 organic perturbation on marine sediment betaproteobacterial ammonia
579 oxidizers and on benthic nitrogen biogeochemistry. *Marine Ecology Progress*
580 *Series* 392:17-32. doi:10.3354/meps08244
- 581 3. Oakes JM, Eyre BD, Ross DJ (2011) Short-Term Enhancement and Long-
582 Term Suppression of Denitrification in Estuarine Sediments Receiving
583 Primary- and Secondary-Treated Paper and Pulp Mill Discharge.
584 *Environmental Science & Technology* 45 (8):3400-3406.
585 doi:10.1021/es103636d
- 586 4. Sloth NP, Blackburn, H., Hansen, L.S., Risgaard-Petersen, N., Lomstein,
587 B.A. (1995) Nitrogen cycling in sediments with different organic loading.
588 *Marine Ecology Progress Series* 116:163-170
- 589 5. McCaig AE, Phillips CJ, Stephen JR, Kowalchuk GA, Harvey SM, Herbert
590 RA, Embley TM, Prosser JI (1999) Nitrogen Cycling and Community Structure
591 of Proteobacterial β -Subgroup Ammonia-Oxidizing Bacteria within Polluted
592 Marine Fish Farm Sediments. *Applied and Environmental Microbiology* 65
593 (1):213-220
- 594 6. Cai W, Sayles, F.L. (1996) Oxygen penetration depths and fluxes in marine
595 sediments. *Marine Chemistry* 52:123-131
- 596 7. Caffrey JM, Sloth NP, Kaspar HF, Blackburn TH (1993) Effect of organic
597 loading on nitrification and denitrification in a marine sediment microcosm.
598 *FEMS Microbiology Ecology* 12 (3):159-167
- 599 8. Henriksen K, Kemp, W.M. (1986) Nitrification in estuarine and coastal marine
600 sediments: methods, patterns and regulating factors. In: Blackburn TH SJ (ed)
601 *Nitrogen Cycling in Coastal Marine Environments*. John Wiley and Sons, New
602 York, pp 207-250
- 603 9. Cornwell J, Kemp WM, Kana T (1999) Denitrification in coastal
604 ecosystems: methods, environmental controls, and ecosystem level controls,
605 a review. *Aquatic Ecology* 33 (1):41-54. doi:10.1023/a:1009921414151
- 606 10. Abell GCJ, Banks J, Ross DJ, Keane JP, Robert SS, Revill AT, Volkman
607 JK (2011) Effects of estuarine sediment hypoxia on nitrogen fluxes and
608 ammonia oxidizer gene transcription. *FEMS Microbiology Ecology* 75 (1):111-
609 122. doi:10.1111/j.1574-6941.2010.00988.x
- 610 11. Kowalchuk GA, Bodelier PLE, Heilig GHJ, Stephen JR, Laanbroek HJ
611 (1998) Community analysis of ammonia-oxidising bacteria, in relation to
612 oxygen availability in soils and root-oxygenated sediments, using PCR, DGGE
613 and oligonucleotide probe hybridisation. *FEMS Microbiology Ecology* 27
614 (4):339-350
- 615 12. Joye SB, Hollibaugh JT (1995) Influence of Sulfide Inhibition of
616 Nitrification on Nitrogen Regeneration in Sediments. *Science* 270 (5236):623-
617 625. doi:10.1126/science.270.5236.623
- 618 13. Park B-J, Park S-J, Yoon D-N, Schouten S, Sinninghe Damsté JS, Rhee
619 S-K (2010) Cultivation of Autotrophic Ammonia-Oxidizing Archaea from

620 Marine Sediments in Coculture with Sulfur-Oxidizing Bacteria. *Applied and*
 621 *Environmental Microbiology* 76 (22):7575-7587. doi:10.1128/aem.01478-10
 622 14. Abell GCJ, Ross, D.J., Keane, J.P., Oakes, J.M., Eyre, B.D., Robert, S.S.,
 623 Volkman, J.K. (2013) Nitrifying and denitrifying microbial communities and
 624 their relationship to nutrient fluxes and sediment geochemistry in the Derwent
 625 Estuary, Tasmania. *Aquatic Microbial Ecology* 70:63-75.
 626 doi:10.3354/ame01642
 627 15. Oakes JM, Eyre BD, Ross DJ, Turner SD (2010) Stable Isotopes Trace
 628 Estuarine Transformations of Carbon and Nitrogen from Primary- and
 629 Secondary-Treated Paper and Pulp Mill Effluent. *Environmental Science &*
 630 *Technology* 44 (19):7411-7417. doi:10.1021/es101789v
 631 16. Eyre BD, Ferguson, A.J.P (2005) Benthic metabolism and nitrogen cycling
 632 in a subtropical east Australian estuary (Brunswick): Temporal variability and
 633 controlling factors. *Limnol Oceanogr* 50 (1):81-96
 634 17. Abell GCJ, Revill AT, Smith C, Bissett AP, Volkman JK, Robert SS (2010)
 635 Archaeal ammonia oxidizers and nirS-type denitrifiers dominate sediment
 636 nitrifying and denitrifying populations in a subtropical macrotidal estuary.
 637 *ISME J* 4 (2):286-300
 638 18. Abell GCJ, and McOrist., A.L. (2007) Assessment of the diversity and
 639 stability of faecal bacteria from healthy adults using molecular methods.
 640 *Microbial Ecology in Health and Disease* 19:229-240
 641 19. Nicolaisen MH, Ramsing NB (2002) Denaturing gradient gel
 642 electrophoresis (DGGE) approaches to study the diversity of ammonia-
 643 oxidizing bacteria. *Journal of Microbiological Methods* 50 (2):189-203
 644 20. Kenkel NC, Orloci, L. (1986) Applying metric and nonmetric
 645 multidimensional scaling to some ecological
 646 studies: some new results. *Ecology* 67:919-928
 647 21. Minchin PR (1987) An evaluation of the relative robustness of techniques
 648 for ecological ordination. *Plant Ecology* 69:89-107
 649 22. Anderson MJ, Gorley, R.N., Clarke, K.R. (2008) PERMANOVA+ for
 650 PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth
 651 23. Oksanen J, Blanchet, G., Kindt, R., Legendre, P., O'Hara, R.G., Simpson,
 652 G.L., Solymos, P., Stevens, H.H., Wagner, H. (2012) Vegan: community
 653 ecology package.
 654 24. Pester M, Rattei T, Flechl S, Gröngöft A, Richter A, Overmann J,
 655 Reinhold-Hurek B, Loy A, Wagner M (2012) amoA-based consensus
 656 phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA
 657 genes from soils of four different geographic regions. *Environmental*
 658 *Microbiology* 14 (2):525-539. doi:10.1111/j.1462-2920.2011.02666.x
 659 25. Purkhold U, Pommerening-Röser A, Juretschko S, Schmid MC, Koops H-
 660 P, Wagner M (2000) Phylogeny of All Recognized Species of Ammonia
 661 Oxidizers Based on Comparative 16S rRNA and amoA Sequence Analysis:
 662 Implications for Molecular Diversity Surveys. *Applied and Environmental*
 663 *Microbiology* 66 (12):5368-5382. doi:10.1128/aem.66.12.5368-5382.2000
 664 26. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB,
 665 Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B,
 666 Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: Open-
 667 Source, Platform-Independent, Community-Supported Software for Describing
 668 and Comparing Microbial Communities. *Applied and Environmental*
 669 *Microbiology* 75 (23):7537-7541. doi:10.1128/aem.01541-09

670 27. Lozupone C, Knight R (2005) UniFrac: a New Phylogenetic Method for
 671 Comparing Microbial Communities. *Applied and Environmental Microbiology*
 672 71 (12):8228-8235. doi:10.1128/aem.71.12.8228-8235.2005
 673 28. Dover CLV, Grassle JF, Fry B, Garritt RH, Starczak VR (1992) Stable
 674 isotope evidence for entry of sewage-derived organic material into a deep-sea
 675 food web. *Nature* 360 (6400):153-156
 676 29. Robinson D (2001) $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle. *Trends in*
 677 *Ecology & Evolution* 16 (3):153-162. doi:[http://dx.doi.org/10.1016/S0169-](http://dx.doi.org/10.1016/S0169-5347(00)02098-X)
 678 [5347\(00\)02098-X](http://dx.doi.org/10.1016/S0169-5347(00)02098-X)
 679 30. Peterson BJ (1999) Stable isotopes as tracers of organic matter input and
 680 transfer in benthic food webs: A review. *Acta Oecologica* 20 (4):479-487.
 681 doi:[http://dx.doi.org/10.1016/S1146-609X\(99\)00120-4](http://dx.doi.org/10.1016/S1146-609X(99)00120-4)
 682 31. McCarthy M, McNeal K, Morse J, Gardner W (2008) Bottom-water
 683 Hypoxia Effects on Sediment–Water Interface Nitrogen Transformations in a
 684 Seasonally Hypoxic, Shallow Bay (Corpus Christi Bay, TX, USA). *Estuaries*
 685 *and Coasts* 31 (3):521-531
 686 32. Cloern JE (2001) Our evolving conceptual model of the coastal
 687 eutrophication problem. *Marine Ecology-Progress Series* 210:223-253
 688 33. Mosier AC, Francis CA (2010) Denitrifier abundance and activity across
 689 the San Francisco Bay estuary. *Environmental Microbiology Reports* 2
 690 (5):667-676. doi:10.1111/j.1758-2229.2010.00156.x
 691 34. Mosier A, C. , Francis CA (2008) Relative abundance and diversity of
 692 ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary.
 693 *Environmental Microbiology* 10 (11):3002-3016
 694 35. Nicol GW, Leininger, S., Schleper, C. (2011) Distribution and Activity of
 695 Ammonia-Oxidizing Archaea in Natural Environments. In: Bess B. Ward DJA,
 696 and Martin G. Klotz (ed) *Nitrification*. ASM Press, Washington, pp 157-178
 697 36. Santoro AE, Casciotti KL, Francis CA (2010) Activity, abundance and
 698 diversity of nitrifying archaea and bacteria in the central California Current.
 699 *Environmental Microbiology* 12 (7):1989-2006. doi:10.1111/j.1462-
 700 2920.2010.02205.x
 701 37. Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, Arp DJ,
 702 Brochier-Armanet C, Chain PSG, Chan PP, Gollabgir A, Hemp J, H^ogler M,
 703 Karr EA, Könneke M, Shin M, Lawton TJ, Lowe T, Martens-Habbena W,
 704 Sayavedra-Soto LA, Lang D, Sievert SM, Rosenzweig AC, Manning G, Stahl
 705 DA (2010) *Nitrosopumilus maritimus* genome reveals unique mechanisms for
 706 nitrification and autotrophy in globally distributed marine crenarchaea.
 707 *Proceedings of the National Academy of Sciences* 107 (19):8818-8823.
 708 doi:[10.1073/pnas.0913533107](http://dx.doi.org/10.1073/pnas.0913533107)
 709 38. Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA
 710 (2009) Ammonia oxidation kinetics determine niche separation of nitrifying
 711 Archaea and Bacteria. *Nature* 461 (7266):976-979
 712 39. Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims
 713 H, Wagner M (2008) A moderately thermophilic ammonia-oxidizing
 714 crenarchaeote from a hot spring. *Proceedings of the National Academy of*
 715 *Sciences* 105 (6):2134-2139. doi:10.1073/pnas.0708857105
 716 40. Lehtovirta-Morley LE, Stoecker K, Vilcinskas A, Prosser JI, Nicol GW
 717 (2011) Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying
 718 acid soil. *Proceedings of the National Academy of Sciences* 108 (38):15892-
 719 15897. doi:[10.1073/pnas.1107196108](http://dx.doi.org/10.1073/pnas.1107196108)

- 720 41. Tourna M, Stieglmeier M, Spang A, Könneke M, Schintlmeister A, Urich T,
721 Engel M, Schlöter M, Wagner M, Richter A, Schleper C (2011) *Nitrososphaera*
722 *viennensis*, an ammonia oxidizing archaeon from soil. *Proceedings of the*
723 *National Academy of Sciences* 108 (20):8420-8425.
724 doi:[10.1073/pnas.1013488108](https://doi.org/10.1073/pnas.1013488108)
725 42. Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk H-P, Schleper C
726 (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a
727 role of uncultivated mesophilic crenarchaeota in nitrogen cycling.
728 *Environmental Microbiology* 7 (12):1985-1995. doi:[10.1111/j.1462-](https://doi.org/10.1111/j.1462-2920.2005.00906.x)
729 [2920.2005.00906.x](https://doi.org/10.1111/j.1462-2920.2005.00906.x)
730 43. Schleper C, Nicol GW, Robert KP (2010) Ammonia-Oxidising Archaea, Æi
731 Physiology, Ecology and Evolution. In: *Advances in Microbial Physiology*, vol
732 Volume 57. Academic Press, pp 1-41. doi:[http://dx.doi.org/10.1016/B978-0-](http://dx.doi.org/10.1016/B978-0-12-381045-8.00001-1)
733 [12-381045-8.00001-1](http://dx.doi.org/10.1016/B978-0-12-381045-8.00001-1)
734 44. Dang H, Li J, Chen R, Wang L, Guo L, Zhang Z, Klotz MG (2010)
735 Diversity, Abundance, and Spatial Distribution of Sediment Ammonia-
736 Oxidizing Betaproteobacteria in Response to Environmental Gradients and
737 Coastal Eutrophication in Jiaozhou Bay, China. *Applied and Environmental*
738 *Microbiology* 76 (14):4691-4702. doi:[10.1128/aem.02563-09](https://doi.org/10.1128/aem.02563-09)
739 45. O'Mullan GD, Ward BB (2005) Relationship of Temporal and Spatial
740 Variabilities of Ammonia-Oxidizing Bacteria to Nitrification Rates in Monterey
741 Bay, California. *Applied and Environmental Microbiology* 71 (2):697-705.
742 doi:[10.1128/aem.71.2.697-705.2005](https://doi.org/10.1128/aem.71.2.697-705.2005)
743 46. Bernhard AE, Landry ZC, Blevins A, de la Torre JR, Giblin AE, Stahl DA
744 (2010) Abundance of Ammonia-Oxidizing Archaea and Bacteria along an
745 Estuarine Salinity Gradient in Relation to Potential Nitrification Rates. *Applied*
746 *and Environmental Microbiology* 76 (4):1285-1289. doi:[10.1128/aem.02018-](https://doi.org/10.1128/aem.02018-09)
747 [09](https://doi.org/10.1128/aem.02018-09)
748 47. Blainey PC, Mosier AC, Potanina A, Francis CA, Quake SR (2011)
749 Genome of a Low-Salinity Ammonia-Oxidizing Archaeon Determined by
750 Single-Cell and Metagenomic Analysis. *PLoS ONE* 6 (2):e16626
751 48. Mosier A, Lund M, Francis C (2012) Ecophysiology of an Ammonia-
752 Oxidizing Archaeon Adapted to Low-Salinity Habitats. *Microbial Ecology* 64
753 (4):955-963. doi:[10.1007/s00248-012-0075-1](https://doi.org/10.1007/s00248-012-0075-1)
754 49. Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005)
755 Ubiquity and diversity of ammonia-oxidizing archaea in water columns and
756 sediments of the ocean. *Proceedings of the National Academy of Sciences of*
757 *the United States of America* 102 (41):14683-14688
758 50. Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia
759 monooxygenase structural gene *amoA* as a functional marker: molecular fine-
760 scale analysis of natural ammonia-oxidizing populations. *Appl Environ*
761 *Microbiol* 63 (12):4704-4712
762 51. Braker G, Fesefeldt A, Witzel K-P (1998) Development of PCR Primer
763 Systems for Amplification of Nitrite Reductase Genes (*nirK* and *nirS*) To
764 Detect Denitrifying Bacteria in Environmental Samples. *Appl Environ Microbiol*
765 64 (10):3769-3775
766 52. Hallin S, Lindgren P-E (1999) PCR Detection of Genes Encoding Nitrite
767 Reductase in Denitrifying Bacteria. *Appl Environ Microbiol* 65 (4):1652-1657
768 53. Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade
769 WG (1998) Design and Evaluation of Useful Bacterium-Specific PCR Primers

770 That Amplify Genes Coding for Bacterial 16S rRNA. Appl Environ Microbiol 64
771 (2):795-799
772 54. Hauben L, Vauterin L, Swings J, Moore ERB (1997) Comparison of 16S
773 Ribosomal DNA Sequences of All Xanthomonas Species. Int J Syst Bacteriol
774 47 (2):328-335. doi:10.1099/00207713-47-2-328
775 55. Giovannoni SJ, DeLong EF, Olsen GJ, Pace NR (1988) Phylogenetic
776 group-specific oligodeoxynucleotide probes for identification of single
777 microbial cells. J Bacteriol 170 (2):720-726
778 56. Jurgens G, Lindstrom K, Saano A (1997) Novel group within the kingdom
779 Crenarchaeota from boreal forest soil. Appl Environ Microbiol 63 (2):803-805
780
781

NOx

NH4

NOx

NH4

Mean Species Richness

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Abell, GCJ;Ross, DJ;Keane, J;Holmes, BH;Robert, SS;Keough, MJ;Eyre, BD;Volkman, JK

Title:

Niche Differentiation of Ammonia-Oxidising Archaea (AOA) and Bacteria (AOB) in Response to Paper and Pulp Mill Effluent

Date:

2014-05-01

Citation:

Abell, G. C. J., Ross, D. J., Keane, J., Holmes, B. H., Robert, S. S., Keough, M. J., Eyre, B. D. & Volkman, J. K. (2014). Niche Differentiation of Ammonia-Oxidising Archaea (AOA) and Bacteria (AOB) in Response to Paper and Pulp Mill Effluent. MICROBIAL ECOLOGY, 67 (4), pp.758-768. <https://doi.org/10.1007/s00248-014-0376-7>.

Persistent Link:

<http://hdl.handle.net/11343/282866>