Referee #2 Florian Moeller, received at 10 Jul 2013. We thank Florian Moeller for the comments.

General comments: In general, there are aspects of this study that are highly interesting and can contribute meaningfully to nitrification research in the form of nitrification rates, the co-correlation of relevant environmental parameters, and the relative distribution and potential activity of AOAs and β-AOBs, particularly in estuarine environments. However, the authors neglect most of the interesting discussions pertaining to this set of results as well as previous relevant studies, in pursuit of a set of alternative discussion arcs that are, in my opinion, tenuously supported by the data. Although the idea of reactive Fe or Mn participating as alternative electron acceptors for ammonia oxidation is highly intriguing, certainly plausible in this sort of environment given the right conditions, and deserving of further research, by ignoring more logical conclusions, some inherent biases in their methods (which the whole field suffers from), and previous high profile studies, the authors veer off the main crux of their research topic. However, by re-focusing on the meat of their biological data and 'fleshing' out these details, the authors can hopefully regain the narrative and provide us with more logical and meaningful conclusions.

Reply:
We deeply appreciate this comment. Our perspective is mainly biogeochemical rather than microbial; we focused on the spatial pattern of NR in turbid river plume of the largest river in China and potential factors that regulate NR and tried to explore the hidden oxidant beside oxygen by stoichiometric calculation. To avoid distracting, only a small part of microbiological data including the distribution of bacterial and archaeal *amo*A of the same cruise is applied to support our hypothesis. The complete dataset regarding the microbial story will be presented by our co-author Dr. Zhang Yao. The manuscript will be going to the special issue "The impact of anthropogenic perturbations on open ocean carbon transformation, export and sequestration" in Biogeosciences.

General comments: Specifically, the focus of this study should be concentrated on the results pertaining to the relative distribution and activity of AOAs and _-AOBs throughout this estuarinesystem, and how certain prominent estuarine features, such as salinity gradients and estuarine turbidity maxima (ETMs), affect these components. Certainly, by demonstrating a clear difference between particulate and planktonic nitrification rates and thequantity of ammonia-oxidizing microorganisms (AOMs) between these fractions in an estuarine setting, the authors have neatly confirmed and re-affirmed that ETMs, and the particles associated with them, can be hot-beds of nitrification activity. The method of partitioning between the

particulate and planktonic fractions in both of these analyses is also not commonly done and a strong point of the data set. And yet, the authors cite only two studies from freshwater river systems in their discussion of the correlationsbetween TSM and nitrification, when a rich amount of research on nitrification in estuaries is much more relevant. For example, high nitrification activity is commonly associated with intermediate salinities (Berounsky and Nixon, 1993) and ETMs (Owens,1986), although there are also cases where increasing salinities decrease nitrification rates (Rysgaard et al., 1999; Brion et al., 2000, Cébron et al., 2003). Phytoplankton have also been hypothesized to lyse upon contact with saline water and release POC (Lara-Lara et al. 1990). In the case of a quantitative AOM physical affiliation with particles, again, this study demonstrates what has heretofore only been sparsely observed in analogous systems (Wuchter et al., 2006; Woebken et al., 2007 (in an OMZ so it serves as a contrast); Galand et al., 2008).

Reply:

This comment is well taken. We expands paragraphs in P8696, L21~L28 to include all references reviewer suggested. After that we add one more paragraph to discuss the correlation between salinity and the distribution of nitrifying activity. The two paragraphs are shown below.

"The positive correlation between TSM and nitrification had been addressed in freshwater systems (Xia et al., 2009; Wang et al., 2010) and in relative turbid region in estuaries, where nitrification activities were found to be higher (Berounsky et al., 1993, Helder and Devries, 1983), especially in the zone of estuarine turbidity maxima (Owens, 1986). Moreover， similar correlation can be found in OMZ (Wuchter et al., 2006; Woebken et al., 2007, Galand et al., 2008) though the POM concentration were much less.

The particulate fractions of *amo*A abundance and AOR_b results supported that ammonia oxidizer was mainly particulate associated. To our knowledge, such distinctive correlations between AOR_b and TSM (Fig. 4b) observed along salinity gradient in river plume were firstly reported and the slope of the linear regression for river mouth was 1/40 that of the inner plume. Since the PON content (%) on TSM (can be derived from Fig. 2h and 2j) was significantly higher for the inner plume than the river mouth (p = 0.0046, unpaired t-test), we suspected that enriched PON can provide ammonia more efficiently. The variation in regression slopes in one single estuary was likely controlled by specific surface area, which further determines the amount of organics and bacteria, being driven by dynamic energy to sustain particle suspension.

Besides, salinity is also an important factor controlling the distribution of nitrifying activity and nitrifiers. Our AOR_b peaked at intermediate salinity(S=29) and decreased

seaward to undetectable range. This pattern is similar to that reported in many estuaries (Beronsky and Nixon, 1993; Pakulski et al. 1995; Dai et al., 2008; Brion et al., 2000; Cébron et al.,2003). One possibility of high nitrifying activity at intermediate salinity is the lysis of phytoplankton, which may cause the release of organic nitrogen, while it contacts the saline water (Lara-Lara et al. 1990). Meanwhile, gradually elevated salinity seaward may also decrease the ammonium absorption capacity of surface sediments as well as suspended particles (Rasgaard et al., 1999) that made the suspended particles becomes less favorable environment for nitrification. Accordingly, both β-AOB and AOA no longer preferred to be associated with large particles (>3μm) in the outer plume where salinity is higher and water is clearer."

General comments: Salinity, ostensibly also seems to have an effect on the relative distribution and abundances of AOAs and _-AOBs, and so it would be nice to see what the correlations here are in comparison to previous reports (Mosier and Francis, 2008; Bernhard et al., 2010). In particular, it would be nice to see some discussions regarding these results as they pertain to potential differences between sediment and water column parameters with a potential focus on the varying substrate affinities of AOAs and _-AOBs (Martens-Habbena, 2009). Since it is not a very large dataset (although still passable) it may help to add some more sites for some more qPCR analyses of AOMs, provided the samples for DNA isolation were taken for more than the sites listed. By the looks of it, more NRb measurements were made than qPCR analyses.

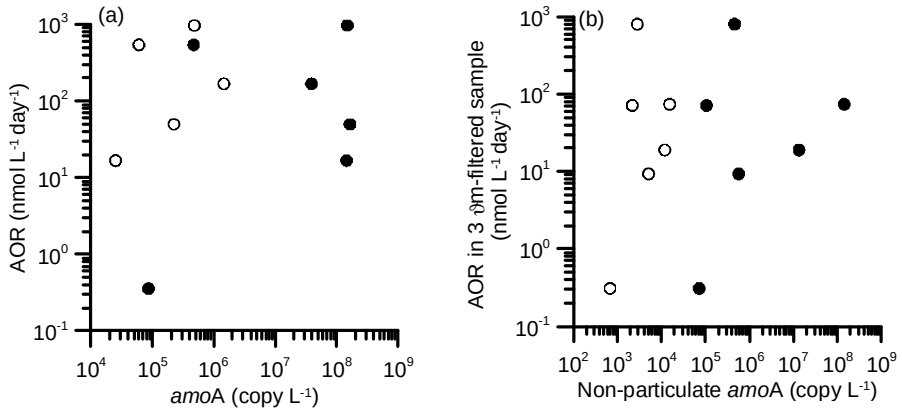
Reply:
As replied earlier, more data and deeper discussions of AOAs and AOBs, DNA analysis including qPCR and microbial diversity analysis along Changjiang plume from two seasons will be presented by Dr. Zhang Yao.

General comments: In a similar vein, if light transmissivity was measured by the CTD in this study, the authors could further explore the relationship they postulated to exist between light and nitrification, in particular by looking at the specific nitrification rate (the daily rate of nitrification divided by the corresponding ammonium concentration). Of course, all of these correlations between environmental parameters and the biological data (qPCR copy numbers and nitrification rates) should be first analyzed using multivariate statistical tests, such as canonical correspondence analysis or correlation matrices, such that co-correlating complications can be avoided and dominant factors can be teased out for further discussion and analysis. This was a major pitfall the authors fell into and it led to the postulation of weakly supported conclusions.

Reply:

Thanks for this suggestion. The plot of light intensity vs. specific nitrification activity (nitrification rate divided by the corresponding ammonium concentration) should be useful to explain photoinhibition effect. Unfortunately, the light transmissivity was not measured in this cruise. And the turbidity sensor of CTD was malfunctioned that we were unable to compute from it.

The correlation matrixes among three regions of the plume (shown below) were made to replace the original Table 2. The qPCR result was not included in since no significant correlation was found toward the genetic abundance because of insufficient data number (n=6) . The correlations between the activity and gene abundance in large (>3 μm) and small (0.22~3 μm) particle fraction, respectively, were plotted as supplemental Fig. 1 (also shown below), though the correlation is not significant.



Supplemental Fig. 1. The correlation between ammonia oxidation rate(AOR) andthe archaeal(closed symbol) and β-proteobacterial(open symbol) *amo*A abundance in (a) bulk samples and (b) large particle excluded(0.22~3 μm) samples.

Table 2-1. The correlation matrix of field surveyed data in river mouth of Changjiang River plume.

	Temp.	Sal.	NH_4^+	NO_3^-	NO_2^-	AOR	DO	TSM	CR	POC	PON	HCl-Al	HCl-Fe	HCl-Mn	DON
Unit	^oC		μmol L^{-1}	μmol L^{-1}	μmol L^{-1}	nmol L^{-1} day^{-1}	μmol Kg^{-1}	mg L^{-1}	μmol L^{-1} day^{-1}	μg L^{-1}	μg L^{-1}	g L^{-1}	mg L^{-1}	ng L^{-1}	μmol L^{-1}
N	10	10	10	10	10	10	10	10	2	10	10	10	10	10	6
Temp.	1.00														
Sal.	-0.75*	1.00													
$[NH_4^+]$			1.00												
$[NO_3^-]$	0.98**	-0.69*		1.00											
$[NO_2^-]$		-0.85*			1.00										
AOR			0.90*			1.00									
DO	0.67*	-0.65*		0.64*			1.00								
TSM						0.85**		1.00							
CR									1.00						
POC						0.85**		0.99**		1.00					
PON						0.85**		0.99**		1.00**	1.00				
HCl-Al			0.66*		-0.71*	0.90**		0.96**		0.95**	0.96**	1			
HCl-Fe			0.66*		-0.67*	0.89**		0.98**		0.98**	0.98**	0.994**	1		
HCl-Mn						0.84**		1.00**		0.99**	0.99**	0.964**	0.986**	1	
DON		-0.86*			0.88*					-0.86*	-0.86*	-0.839*	-0.834*		1

n is sample number. The others are the Pearson's correlation coefficient of two by two parameters.

* indicates the $p < 0.05$ and ** indicates $p < 0.01$.

Table 2-2. The correlation matrix of field surveyed data in inner plume of Changjiang River plume.

	Temp.	Sal.	$[NH_4^+]$	$[NO_3^-]$	$[NO_2^-]$	AOR	DO	TSM	CR	POC	PON	HCl-Al	HCl-Fe	HCl-Mn	DON
	^oC		μmol L^{-1}	μmol L^{-1}	μmol L^{-1}	nmol L^{-1} day^{-1}	μmol Kg^{-1}	mg L^{-1}	μmol L^{-1} day^{-1}	μg L^{-1}	μg L^{-1}	g L^{-1}	mg L^{-1}	ng L^{-1}	μmol L^{-1}
N	22	22	22	22	22	21	22	22	19	20	20	22	22	22	14
Temp.	1.00														
Sal.	-0.89*	1.00													
NH_4^+			1.00												
NO_3^-	0.73*	-0.91*		1.00											
NO_2^-					1.00										
AOR			0.57*			1.00									
DO	0.59*	-0.46*					1.00								
TSM		-0.69*	0.56*	0.72*		0.72**		1.00							
CR									1.00						
POC	0.47*	-0.60*		0.65*		0.52*		0.81**		1.00					
PON	0.46*	-0.59*		0.64*		0.51*		0.80**		1.00**	1.00				
HCl-Al		-0.48*		0.43*		0.50*		0.56**				1.00			
HCl-Fe		-0.65*	0.58*	0.67*		0.72**		0.97**		0.74**	0.73**	0.67**	1.00		
HCl-Mn		-0.66*	0.57*	0.70*		0.73**	-0.08*	0.99**		0.79**	0.78**	0.63**	0.99**	1.00	
DON					0.595*		0.70**								1.00

n is sample number. The others are the Pearson's correlation coefficient of two by two parameters.

* indicates the $p < 0.05$ and ** indicates $p < 0.01$.

Table 2-3. The correlation matrix of field surveyed data in outer plume of Changjiang River plume.

	Temp.	Sal.	NH_4^+	NO_3^-	NO_2^-	AOR	DO	TSM	CR	POC	PON	HCl-Al	HCl-Fe	HCl-Mn	DON
Unit	^oC		μmol L^{-1}	μmol L^{-1}	μmol L^{-1}	nmol L^{-1} day^{-1}	μmol Kg^{-1}	mg L^{-1}	μmol L^{-1} day^{-1}	μg L^{-1}	μg L^{-1}	g L^{-1}	mg L^{-1}	ng L^{-1}	μmol L^{-1}
N	11	11	11	11	11	8	11	11	6	11	11	11	11	11	10
Temp.	1.00														
Sal.	-0.74*	1.00													
NH_4^+			1.00												
NO_3^-	-0.65*			1.00											
NO_2^-					1.00										
AOR	-0.74*					1.00									
DO	0.86*	-0.92*					1.00								
TSM								1.00							
CR						-0.98*		0.93**	1.00						
POC		-0.85*					0.77**			1.00					
PON		-0.83*					0.72*			1.00**	1.00				
HCl-Al												1.00			
HCl-Fe	-0.83*				-0.71*		-0.63*					0.79**	1.00		
HCl-Mn	-0.86*	0.61*			-0.71*		-0.68*					0.73*	0.99**	1.00	
DON	0.68*	-0.91*	0.73*				0.75*			0.76*	0.75*				1.00

n is sample number. The others are the Pearson's correlation coefficient of two by two parameters.

* indicates the $p < 0.05$ and ** indicates $p < 0.01$.

General comments: In their postulation of reactive Fe and Mn as potential and likely roles as alternative electron acceptors for ammonia oxidation or nitrification (a distinction which needed to be elaborated) in the seasonal periods of hypoxia/anoxia in this system, the authors ignored some fundamentally known pieces of information. Firstly, in the absence of oxygen or in anoxic micro-niches on particles, NO3- and NO2- are much more energetically favorable electron acceptors than Fe or Mn, and are commonly used by heterotrophic denitrifiers in breaking down organics. In addition, the process of anaerobic ammonium oxidation has been known for quite some time now and has been found in many diverse habitats, even associated with particles. This process is carried out by bacterial members within the family Planctomycetaceae and involves the oxidation of NH4+ by NO2- to produce N2 gas. By only measuring AOMs and hypothesizing that reactive Fe and Mn are involved as alternative electron acceptors, it can be easy for readers to mistakenly conflate that the authors are proposing a process involving AOMs, that if it were to occur, most likely would involve completely different and uncharacterized organisms and mechanisms.

Reply:
We agreed with reviewer that denitrification or anammox may occur in the summer hypoxia region. In that case, nitrate or nitrite may become oxidant for heterotrophic degradation of organic matter. However, in this cruise the water column was oxygenated due to the typhoon disturbance, thus, the environmental condition does not facilitate denitrification or anammox. Actually, we indeed measured the potential denitrification and anammox activity for water column in this cruise by isotope pairing technique (IPT) (Hsu and Kao, 2013). The rates of N_2O or N_2 production from ammonium or nitrate are less than 1% of the ammonia oxidation rate (production of nitrite plus nitrate from ammonium). The ignorable denitrification and anammox activity suggested nitrite and nitrate were not important oxidant in our study area (even in the anoxic micro-niches). Nevertheless, we added several sentences to describe the situation.

General comments: Second, at no point in any of the incubations would the O2 concentrations have been depleted by the measured CR rates, since the maximum CR rates (10.79 _M d-1) were about 5 times lower than the lowest oxygen concentrations (58 _M). Although the authors do point out that the O2 consumption from nitrification would commonly exceed the amount of O2 needed by other heterotrophs for the breakdown of organics, this assumption can be violated by a few factors: 1) as mentioned by Referee 1, the CR rate may be underestimated; 2) relatedly, since the nitrification rates were conducted in the dark, AOMs are relieved from light inhibition thereby potentially inflating in situ rates; 3) AOAs are known for their extremely high substrate affinity for NH4+ and O2, that they may

even be able to outcompete heterotrophs for both substrates thereby forcing the heterotrophs to use alternative electron acceptors such as NO3- and NO2- ; 4) this scenario could then fit with anammox bacteria operating in anoxic micro-niches on particles; 5) if a significant proportion of ammonium does not come from immediate or adjacent organics; 6) since the nitrification rates combine both the production of 15NO2- and 15NO3- and ammonia oxidation only uses 1.5 moles of O2 per mole of ammonia oxidized, by not separating the rates into two processes, one can slightly overestimate the O2 consumption from nitrification (although to be fair, ammonia-oxidation is the rate-limiting step and most nitrite produced should be eventually oxidized).

Reply:
Reviewer listed some possible reasons against our hypothesis. First is the underestimation of CR. As replied to the Referee 1, we thought our method is commonly used in various environmental studies. The second point is AOR probably overestimate under dark incubation. We thought it was possible that the natural daily AOR might lower than we measured because sunlight may inhibit the activity during day time. Since both CR and AOR are incubated in the same condition (dark and temperature controlled by circulated surface seawater), the hypothesis we proposed will be still true in our incubation experiment. The third and forth point lied on the AOA might outcompete the heterotrophs for the substrate ammonia and oxygen then the heterotrophs might utilize nitrate or nitrite as main electron acceptor. This means significant amount of denitrification or anammox activity should be observed. However, the denitrification or anammox potential activity measured by IPT experiment was insignificant. To clarify questions raised by reviewer, we added one more paragraph to discuss the potential denitrification/anammoox activity data. The fifth point is allochthonous ammonia may enhance the ratio between ammonia oxidation associated oxygen demand (AOOD) and CR. We agreed with this point and have explained it in the original manuscript P.8697 L25-26. The sixth point suggested incomplete nitrification may reduce the oxidant demand. Since both referees made this suggestion, we added a conservative estimate by assuming nitrite is the end product. Accordingly, oxygen demand for ammonia oxidation is 3/4 of the original one. In this case, the AOOD in CR ranged from 0.1~252(%). However, 12 values among all were still higher than the Redfield model estimation (17.4%) showing no influence on our story.

"According to oxidation sequence, Fe/Mn reduction would occur only when the redox potential were lower than that to facilitate nitrate or nitrite reduction. Thus the heterotroph may utilize nitrate or nitrite rather than oxygen as electron acceptor to break down organic matter. This means a coupled reaction of denitrification, anammox and organic matter degradation, a common phenomena occurred in

sediment. However, the potential denitrification/anammox activity (< 1.6 nmol L^{-1} d^{-1}) in water column measured by isotope pairing technique (Hsu and Kao, 2013) was (Hsu et al., unpublished data) much lower when comparing with the oxygen consumption. Therefore, the utilization of nitrate and nitrite as oxidant were ignorable."

General comments: Finally, a more interesting analysis would be to calculate the cell-specific ammonia-oxidation rates for the AOAs and _-AOBs, respectively, from the derived qPCR numbers and nitrification rates, by assuming the typical amount of amoA copy numbers per cell for each type of organism (Norton, et al. 2002), and comparing them to the cell-specific rates found in cultured representatives (e.g. Könneke et al. 2005; Prosser, 1989). One can then theoretically determine how much either the assayed AOAs or _-AOBs are capable of contributing to the measured nitrification rates. Depending on how close these numbers are to each other, a discussion could then focus on the inherent biases in the DNA extractions, primer coverage, and the nitrification rates (e.g. 15NH4+ tracer rates vs. 15NO3- isotope dilution rates vs. rates derived from bulk changes in NO2-/NO3-). Only at this point would it be more appropriate to invoke other potential actors – that is if the rates and AOMs do not significantly match up, even by accounting for biases – such as anammox, heterotrophic nitrifiers, and even Fe/Mn mediated processes. Again, one could at least go back to the existing DNA extracts and assay them for the anammox marker gene nirS, although these numbers should not be correlated to the nitrification rates, since anammox bacteria would have converted labeled 15NH4+ or 15NO2- into N2 gas.

Reply:
Thanks for this interesting idea. We had tried to calculate the potential activity according to *amo*A abundance and previously reported potential activity in pure or enriched culture of AOA and AOB. We used the average copy number of *amo*A in AOA (1 copy/cell, Hallam et al., 2006) and AOB (2.5 copy/cell, Norton et al., 2002) to calculate the cell number of AOA and AOB. Then we multiplied it with the cell-specific potential activity in pure or enriched culture of AOA (9.26 fmol $cell^{-1}$ d^{-1}, Konneke et al., 2005) and AOB (744 fmol $cell^{-1}$ d^{-1}, Prosser, 1989) to calculate the potential activity. The estimated result was listed in the table below showing both AOA and AOB were capable to contribute significant proportion of activity except for subsurface of Sta. Y3 where AOB can only contribute up to 2% of the measured AOR. Other group such as γ-proteobacterial or heterotrophic ammonia oxidizer might also participate the ammonia oxidation at the subsurface of Sta. Y3 since our primers only target the archaeal and β-proteobacterial *amo*A. However, we still cannot identify which group of ammonia oxidizer was dominant contributor for AOR through this estimation. We preferred not to include this table into discussion since too many factors may

bias the estimation such as primer coverage, rate determination method and DNA extraction though this idea is interesting. Not to mention the concentration of ammonium used in pure or enriched culture for cell specific potential activity estimation was 0.5~5 mM which is 2-orders magnitude higher than the abundance in natural environment. We appreciated that to apply RNA abundance of *amo*A could be more effective than DNA in this estimation. However, RNA data are rarely reported in both laboratory culture and field study

Besides, anammox and denitrification activity did not bias this estimation because the potential rate we measured were ignorable.

Location	Station	Depth	TSM	O_2 saturation	Ammonia oxidation rate		β-proteobacterial *amo* A			Archaeal *amo* A		
					Bulk	Filtered*	Part.(>3μm)	Part. (0.22-3μm)	Potential activity	Part.(>3μm)	Part. (0.22-3μm)	Potential activity
		(m)	(mg L^{-1})		(nmol L^{-1} day^{-1})	(nmol L^{-1} day^{-1})	(copy L^{-1})	(copy L^{-1})	(nmol L^{-1} day^{-1})	(copy L^{-1})	(copy L^{-1})	(nmol L^{-1} day^{-1})
River mouth	Y0	7	261.0	80.4%	168.23 ± 0.02	18.87 ± 0.04	1.44×10^{6} ± 4.01×10^{5} (99%)	1.20×10^{4} ± 1.03×10^{3} (1%)	4.33×10^{2} ± 1.20×10^{2}	2.56×10^{7} ± 6.40×10^{6} (66%)	1.35×10^{7} ± 1.61×10^{7} (34%)	3.62×10^{2} ± 6.08×10^{1}
		3	170.2	80.3%	49.97 ± 0.02	9.29 ± 0.01	2.19×10^{5} ± 8.16×10^{4} (98%)	5.13×10^{3} ± 6.69×10^{2} (2%)	6.67×10^{1} ± 2.45×10^{1}	1.65×10^{8} ± 2.54×10^{6} (100%)	5.73×10^{5} ± 2.45×10^{4} (0%)	1.53×10^{3} ± 2.37×10^{1}
Inner plume	Y3	21	111.1	56.9%	818.59 ± 0.36	22.40 ± 2.15	—	—	—	—	—	—
		10	41.1	64.2%	578.64 ± 0.25	28.81 ± 0.25	—	—	—	—	—	—
		3	4.6	100.8%	543.05 ± 0.19	798.01 ± 0.34	5.64×10^{4} ± 6.26×10^{3} (95%)	2.86×10^{3} ± 2.78×10^{2} (5%)	1.78×10^{1} ± 1.94×10^{0}	6.38×10^{3} ± 1.79×10^{3} (1%)	4.62×10^{5} ± 7.96×10^{3} (99%)	4.34×10^{0} ± 9.02×10^{-2}
Inner plume	2Y3	20	48.1	53.0%	973.25 ± 0.73	71.15 ± 0.05	4.79×10^{5} ± 3.00×10^{4} (100%)	2.20×10^{3} ± 7.65×10^{2} (0%)	1.43×10^{2} ± 9.14×10^{0}	1.50×10^{8} ± 3.40×10^{6} (100%)	1.10×10^{5} ± 2.80×10^{4} (0%)	1.39×10^{3} ± 3.14×10^{1}
		10	22.1	61.8%	408.28 ± 0.37	215.09 ± 0.02	—	—	—	—	—	—
		3	9.2	82.5%	283.50 ± 0.11	152.97 ± 0.02	—	—	—	—	—	—
Outer plume	Y5	46	4.5	59.6%	16.75 ± 0.01	73.60 ± 0.01	9.55×10^{3} ± 2.04×10^{3} (38%)	1.55×10^{4} ± 7.29×10^{2} (62%)	7.44×10^{0} ± 8.23×10^{-1}	2.70×10^{6} ± 2.60×10^{5} (2%)	1.40×10^{8} ± 2.60×10^{6} (98%)	1.35×10^{3} ± 2.66×10^{1}
		30	3.0	60.3%	32.8	44.6	—	—	—	—	—	—
		20	3.0	77.6%	BDL	7.8	—	—	—	—	—	—
		10	3.8	92.7%	2.5	2.5	—	—	—	—	—	—
		3	10.5	119.2%	BDL	BDL	BDL	6.87×10^{2} ± 7.36×10^{0}	2.04×10^{-1} ± 2.19×10^{-3}	1.40×10^{4} ± 2.80×10^{3} (16%)	7.20×10^{4} ± 3.30×10^{3} (84%)	7.97×10^{-1} ± 5.64×10^{-2}

Minor comments and technical notes:
It may be more helpful to express the amoA copy number concentrations in ml-1 notation and using orders of 10 (e.g. 7.6 _ 0.5 x 104 copies ml-1) so that comparisons with previous studies are easier

Reply:
We have corrected it in Table 1.

Minor comments and technical notes:
Ammonia is oxidized by AOMs, not ammonium.
Would it be possible to quickly re-calculate the nitrification rates based on the equation found in Ward and O'Mullan (2005) and also found in the supplemental methods of Beman et al. (2011)? This is just to ensure your rates agree between different calculation methods and should not be too complicated, unless you are missing crucial pieces of information.

Reply:
Our calculation method, in fact, is identical to that by Beman et al. (2011) but we used different description The equation in Beman et al. (2011) is listed below.

$$^{15}R_{ox} = \frac{\left(n_t - n_{o_{NO_x^-}}\right) \times \left[NO_3^- + NO_2^-\right]}{\left(n_{NH_4^+} - n_{o_{NH_4^+}}\right) \times t},$$

where n_t is the at% ^{15}N in the NO_3^- + NO_2^- pool measured at time t, n_{oNO-x} is the measured at% ^{15}N of unlabeled NO_3^-+NO_2^-, n_{NH4+} is the at% of ^{15}N in NH_4^+ pool at right after the enrichment, n_{oNH4} is background at% 15N of NH_4^+, and [NO_3^- + NO_2^-] is the concentration of the NOx^- pool. The ((n_t-n_{0NOx-})×[NO_3^- + NO_2^-]/t) indicates single end point estimation of the changing rate of ^{15}N in NO_x^- pool through time t, however, to ensure the accuracy we used the regression slope ($\frac{d[^{15}NO_x^-]}{dt}$) derived from a 4-point in time-course to represent the same thing. And the (n_{NH4+} - n_{0NH4+}) indicates the ^{15}N content difference between the samples before and after we added the ^{15}N tracer, simply said, it is the final content of ^{15}N tracer we added into the samples. In our equation we presented the same by multiplying the reciprocal but Beman et al. (2011) put the amount of ^{15}N tracer content in denominator. By the way, Ward and O'Mullan (2005) did not show their equation, however, their calculation should be the same judging from the text.

Minor comments and technical notes:
In lines 9-11, paragraph 1 of page 8695, is it possible to clarify what is meant by

'unraveled factors'.

Reply:
We can hardly find a good reason why the nitrification activity in British Columbia fjord was low (< 0.319 μmol L^{-1} d^{-1}) even ammonium concentration was high (0-5μM) (Grundle and Juniper, 2011). The possible reasons might be the competition with primary producer (high primary productivity reported in Grundle et al., 2009), the presence of inhibitory substance such as toxic heavy metal or antibiotics, or anammox or coupled nitrification-denitrification activity since hypoxia had occurred. Photo-inhibition was excluded by them since the light intensity did not reach the threshold of photoinhibition. In this revision, we also changed "unraveled" to "non-measured".

Minor comments and technical notes:
Finally, the difference in slopes in Figure 4b (13 vs. 0.33) is a difference of around 40-fold, instead of 5-fold, right?

Reply:
Thanks for the reviewer. We will correct it.

According to reply above, we put additional reference below into our revision.

1. Beman, J. M., C. E. Chow, A. L. King, Y. Y. Feng, J. A. Fuhrman, A. Andersson, N. R. Bates, B. N. Popp, and D. A. Hutchins (2011), Global declines in oceanic nitrification rates as a consequence of ocean acidification, *P Natl Acad Sci USA*, *108*(1), 208-213.
2. Berounsky, V. M., and S. W. Nixon (1993), Rates of Nitrification Along an Estuarine Gradient in Narragansett Bay, *Estuaries*, *16*(4), 718-730.
3. Brion, N., G. Billen, L. Guezennec, and A. Ficht (2000), Distribution of nitrifying activity in the Seine River (France) from Paris to the estuary, *Estuaries*, *23*(5), 669-682.
4. Cebron, A., T. Berthe, and J. Garnier (2003), Nitrification and nitrifying bacteria in the lower Seine River and estuary (France), *Applied and environmental microbiology*, *69*(12), 7091-7100.
5. Dai, M., L. Wang, X. Guo, W. Zhai, Q. Li, B. He, and S. J. Kao (2008), Nitrification and inorganic nitrogen distribution in a large perturbed river/estuarine system: the Pearl River Estuary, China, *Biogeosciences*, *5*(5), 1227-1244.
6. Galand, P. E., C. Lovejoy, J. Pouliot, and W. F. Vincent (2008), Heterogeneous archaeal communities in the particle-rich environment of an arctic shelf

ecosystem, *J Marine Syst*, *74*(3-4), 774-782.

7. Grundle, D. S., D. A. Timothy, and D. E. Varela (2009), Variations of phytoplankton productivity and biomass over an annual cycle in Saanich Inlet, a British Columbia fjord, *Cont Shelf Res*, *29*(19), 2257-2269.
8. Grundle, D. S., and S. K. Juniper (2011), Nitrification from the lower euphotic zone to the sub-oxic waters of a highly productive British Columbia fjord, *Mar Chem*, *126*(1-4), 173-181.
9. Hallam, S. J., T. J. Mincer, C. Schleper, C. M. Preston, K. Roberts, P. M. Richardson, and E. F. DeLong (2006), Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota, *Plos Biol*, *4*(4), 520-536.
10. Helder, W., and R. T. P. Devries (1983), Estuarine Nitrite Maxima and Nitrifying Bacteria (Ems-Dollard Estuary), *Neth J Sea Res*, *17*(1), 1-18.
11. Hsu, T. C. and S. J. Kao (2013) Technical Note: Simultaneous measurement of sedimentary N2 and N2O production and new 15N isotope pairing technique, *Biogeoscience discussion*, *10,* 6861-6898
12. Konneke, M., A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury, and D. A. Stahl (2005), Isolation of an autotrophic ammonia-oxidizing marine archaeon, *Nature*, *437*(7058), 543-546.
13. Lara-Lara, J. R., B. E. Frey, and L. F. Small (1990), Primary Production in the Columbia River Estuary I. Spatial and Temporal Variability of Properties!, *Pacific Science*, *44*(1), 17-37.
14. Norton, J. M., J. J. Alzerreca, Y. Suwa, and M. G. Klotz (2002), Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria, *Arch Microbiol*, *177*(2), 139-149.
15. Owens, N. J. P.: Estuarine Nitrification - a Naturally-Occurring Fluidized-Bed Reaction, Estuar Coast Shelf S, 22, 31-44, Doi 10.1016/0272-7714(86)90022-3, 1986.
16. Pakulski, J. D., R. Benner, R. Amon, B. Eadie, and T. Whitledge (1995), Community Metabolism and Nutrient Cycling in the Mississippi River Plume - Evidence for Intense Nitrification at Intermediate Salinities, *Mar Ecol-Prog Ser*, *117*(1-3), 207-218.
17. Prosser, J. I. (1989), Autotrophic Nitrification in Bacteria, *Adv Microb Physiol*, *30*, 125-181.
18. Rysgaard, S., P. Thastum, T. Dalsgaard, P. B. Christensen, and N. P. Sloth (1999), Effects of salinity on NH4+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments, *Estuaries*, *22*(1), 21-30.
19. Wang, H. Y., Z. Y. Shen, X. J. Guo, J. F. Niu, and B. Kang (2010), Ammonia

adsorption and nitritation in sediments derived from the Three Gorges Reservoir, China, *Environ Earth Sci*, *60*(8), 1653-1660.

20. Woebken, D., B. M. Fuchs, M. M. M. Kuypers, and R. Amann (2007), Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system, *Applied and environmental microbiology*, *73*(14), 4648-4657.
21. Wuchter, C., et al. (2006), Archaeal nitrification in the ocean, *P Natl Acad Sci USA*, *103*(33), 12317-12322.
22. Xia, X. H., Z. F. Yang, and X. Q. Zhang (2009), Effect of Suspended-Sediment Concentration on Nitrification in River Water: Importance of Suspended Sediment-Water Interface, *Environ Sci Technol*, *43*(10), 3681-3687.