Anonymous Referee #1', reicieved at 10 Jul 2013. We thank Anonymous Referee for their comments.

General comments: The Aim of this study according to tittle is " Nitrification and its oxygen consumption along a turbid river plume". The article present many data (DOC, DO , DON, CR, DIN, POC, PON, NOD, ammonium, nitrate, nitrite, Mn, Fe, TSM, salinity), AmoA quantification, nitrifying rate. In addition of the description of the peculiar river plume, the interesting data of this study is the presence and activity of nitrifyers on particles.

However, reading the article, it is difficult to keep in mind the aim of this study (Nitrification and its oxygen consumption along a turbid river plume) because of the multiplicity of results (DOC, DO , DON, CR, DIN, POC, PON, NOD, ammonium, nitrate, nitrite, Mn, Fe, TSM) , that serve more to describe the river plume than to bring information about nitrification and its oxygen consumption and the reader is a little lost in the reading. The multiplicity of acronyms does not help reader. It would be probably better to present only relevant results identified by multiparametric statistic tests than the important list of two by two parameter correlations. The manuscript should better focus on the subject. I have reservations about the accuracy of the measurement of the community respiration and on nitrification rate.

Response:

We have simplified the part for plume description from p.8695 Line16 to p.8696 Line 20. As suggested by reviewer, we made two by two parameter correlation matrixes for the three regions of the plume separately and a combined among all (shown below) to replace the original Table 2. More explanations for the correlation of geochemical data were added. However, we would like to keep Figures 4a and 4b to emphasize the main factors influencing the ammonia oxidation rate (AOR).

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	°C		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
	°C		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	°C		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 $p < 0.01$.

Table 2-4. The correlation matrix of field surveyed data among all regions 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	°C		$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$nmol\ L^{-1}\ day^{-1}$	$\mu mol\ Kg^{-1}$	$mg\ L^{-1}$	$\mu mol\ L^{-1}\ day^{-1}$	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$	$g\ L^{-1}$	$mg\ L^{-1}$	$ng\ L^{-1}$	$\mu mol\ L^{-1}$
n	11	11	11	11	11	8	11	11	6	11	11	11	11	11	10
Temp.	1														
Sal.	-0.84*	1													
NH_4^+			1												
NO_3^-	-0.79*	-0.96*		1											
NO_2^-					1										
AOR	-0.74*		0.34*			1									
DO	0.58*	-0.33*			-0.36*		1								
TSM	0.54*	-0.52*		0.68*	-0.31*			1							
CR			0.51*				0.46**		1						
POC	0.55*	-0.52*		0.69*	-0.32*			0.99**		1					
PON	0.55*	-0.52*		0.69*	-0.32*			0.99**		1.00**	1				
HCl-Al	0.58*	-0.56*		0.72*	-0.34*			0.97**		0.97**	0.97**	1			
HCl-Fe	0.58*	-0.55*		0.72*	-0.33*			0.99**		0.98**	0.98**	0.79**	1		
HCl-Mn	0.55*	-0.53*		0.69*	-0.31*			1.00**		0.99**	0.99**	0.73*	0.99**	1	
DON	0.73*	-0.91*		0.79*											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 $p < 0.01$.

General comments:Indeed, nitrification is composed of two independent steps, each of one is performedby a specific community. In aerobiosis the first one is Ammoniac oxidizing bacteria orArchaea and oxidize ammoniac (NH3) into nitrite (NO2-) (NH3 + 3/2O2 à NO2- + H2O +H+), This community is analyzed usually through AmoA gene (and that is done in thisstudy). In some circumstances, ammonia oxidizing prokaryotes produce N2O as byproduct (when oxygen is limiting, probably because some strain possess denitrificationgene) The second step is performed by nitrite oxidizing bacteria (NO2- + 1/2 O2 àNO3-) that are followed through norA or nrxA gene. Ammonia and Nitrite oxidizingcommunities are phylogenetically and physiologically different. This latter community was not analyzed in this study. Authors should better describe the process they are studying.

Response:

This comment is well taken. We clarified our data in describing the processes ammonia oxidation and nitrification. Additional text describing the two steps of nitrification was amended as below (p.8687 Line10). The "nitrification rate" through entire articles had been corrected to "ammonia oxidation rate (AOR)" also.

"Nitrification is composed by two steps, ammonia oxidation and nitrite oxidation. Ammonium oxidation is carried out by ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB), while nitrite oxidation is executed by nitrite oxidizing bacteria (NOB). Ammonia oxidation requires three fourth of oxidants demand in nitrification and produce nitrous oxide, a greenhouse gas, as a byproduct."

General comments: The main results of this study imply nitrification rate measurement. To do so, author have overflow water in bottle without head space, and incubated for up to 24h. I am concern about the oxygen concentration in flask during this 24h incubation , since nitrification is performed only in aerobiosis and oxygen might be exhausted during time. So if oxygen concentration is too low, nitrification rate would be inaccurate. Furthermore, nitrification rate in this study correspond to the sum of ammoniac oxidizing rate and nitrite oxidizing rate since the sum of 15NO3 and 15NO2 is used in the calculation, whereas only community corresponding to the first step (AmoA) were analyzed. Author should take consideration about this fact. . For community respiration, despite the fact that this process correspond to the main topic of this study, this rate seem to be done by the decrement of oxygen after 24h, and not by a kinetic, so this rate could be underestimated if it has been measured only with two points. This point is crucial since Dissolved oxygen might be low in this area. As several abiotic processes could consume oxygen (oxidation of Mn2+ or Fe2+ for example) , CR rate should be also corrected with abiotic value.

Response:

Reviewer will be correct if no typhoon disturbance had occurred; however, we did not observed hypoxic bottom water this studied period. For all our incubationss, oxygen had never reach hypoxia (25% saturation) according to the end-point DO we observed for CR calculation. The lowest dissolved oxygen content after 24 hour incubation would be 55.7 μmol L^{-1} (bottom depth of Sta.2Y9a, original DO: 58.0 μmol L^{-1}, CR: 2.3μmol $L^{-1}d^{-1}$). Thus the measurement of ammonia oxidation rate (AOR) should not be interfered by low oxygen content during incubation. We appreciate that the kinetic measurement of DO or 15N-nitrate and 15N-nitrite can provide more reliable rate estimation though the rates might change hour by hour.

Our duplicated single-point measurement of 24-hour incubation for CR explained the net oxygen consumption for one whole day. This 24-hour incubation was often-used in coastal ocean, estuaries and lakes (McCarthy et al., 2013;Murrell and Lehrter, 2011;Berman et al., 2004;Smith and Kemp, 2003;Nguyen et al., 2012;Murrell et al., 2013;Chen et al., 2006;Chen et al., 2009). The CR measured by this method can be up to 31 μmol $L^{-1}d^{-1}$(Chen et al., 2006). This method for dissolved oxygen is listed in the protocol for the Joint Global Ocean Flux Study (JGOF) (Bender, 1996) and the precision is good ($< \pm 0.1\%$).

As for abiotic alteration, we checked the unpublished results (by Huang Yongming at XMU) of the distribution of dissolved Fe^{2+} and Fe^{3+} in subsurface water along Changjiang River plume in 2009 August. The highest dissolved Fe^{2+} is 0.2 μmol L^{-1} and the oxygen consumption by its chemical oxidation can be ignorable in our incubation experiment.

General comments:I found some discrepancy in the manuscript concerning degradation of the organic matter. P8694, l5, it is say that aerobic degradation of the organic matter was the major source of ammonium which may fuel nitrification. Latter (p8697l, 15) author used eq 1, that correspond to mineralization of organic matter by redfield model, but the product is nitrate not ammonium. So it seem that mineralization of organic matter can not fuel nitrification since ammonium is not formed. However, eq 2 same page, author still affirm that the product of eq 1 substrate of eq 2 are connected. I do not understand also how the author can calculated the % of oxygen consume theoretically by nitrification according to the equation. Since the product of eq1(NO3) is not the substrate (NH3)of equation 2. Redfield value are widely used in the manuscript. This parameter is still useful in deep ocean or away from coast, however deviations from the canonical Redfield Ratio have been observed for many areas, and this plume strongly influence by human activity can be also concerned. This fact weakens the conclusion of authors.

Response:

This comment is well taken. We separated the Equation 1 in P8697 into two steps as below (Equation 1). The first one is to degradation of organic matter to CO_2, PO_4^{3-} and NH_4^+, the second one is the oxidation of the NH_4^+ to NO_3^-. And the original Equation 2 in P.8697 has been separate into two equations representing ammonia oxidation(Equation 2) and nitrite oxidation(Equation 3).

$$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)_1 + 138O_2 \rightarrow 106CO_2 + 16NH_4^+ + PO_4^{3-} + 106H_2O + 19H^+ + 32O_2$$
$$\rightarrow 106CO_2 + 16NO_3^- + PO_4^{3-} + 122H_2O + 19H^+ \quad (1)$$

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H_2O + H^+ \quad (2)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (3)$$
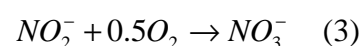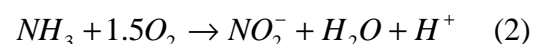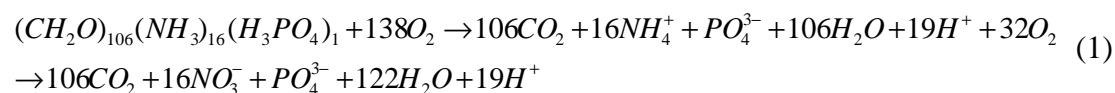
General comments: The conclusion of the author about the possible role of ammonium oxidation implying MnOx or FeOx, seem to be overestimated since, the 317 % of oxygen consummation calculated for nitrification correspond only to one point of the data set and all the other are lower or just above 100% of CR. Furthermore, the CR rate is probably underestimated as outline before. In addition, the author stipulate that all ammonium is converted to nitrate that imply that consumption of 2 O2 by NH3 whereas only 1.5 oxygen is necessary if ammonium is converted to nitrite.

Response:

Thanks for reviewer's suggestion. Now we gave 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 ammonia oxidation associated oxygen demand (AOOD) in CR ranged from 0.2~252(%). However, 12 values among all were still higher than the Redfield model estimation (17.4%) showing no influence on our story.

General comments:Further more, author suggest another reaction implying NO2- and MnO2 to form Mn2+ (eq 4) , I do not understand why this reaction would take place and why the author have chosen this reaction instead of other putative suggested by Hulth et al 1999 (4MnO2 + NH4+ + 6H+ -> 4Mn2+ + NO3- + 5H2O) for example

Response:

The equation the reviewers suggested should be possible. In the original manuscript, we calculated if the oxidant requirement of nitrite oxidation can be compensate only by Mn^{4+} without the participation of oxygen in Table 3 based on original equation 4. The result showed reactive Mn were not sufficient to support nitrite oxidation nor the whole nitrification

as the reviewer suggested in our incubation experiment. However, we will change the calculation to just focus on the ammonia oxidation. Since ammonia oxidation requires 3-fold amount of oxidant than nitrite oxidation, reactive Mn is still not sufficient to support the ammonia oxidation. The Mn mediated (as oxidant) ammonia oxidation could contribute only less than 16% based on our calculation.

General comments: Fe and Mn Oxyde are particulate so it is logic to find them associated with particle.The nitrification is mainly associated to particle, but this is maybe casual, due to otherfactor and not necessary due to the presence of these metal oxide.

Response:

Thanks the reviewer raises this question. We think the nitrification was mainly associated to particle was not casual. The reasons are ammonium is concentrated on particles (Wang et al., 2010) and remineralization of PON also provides ammonium for ammonia oxidation. The refined Table 1 in the following shows that the high percentage of particle ($>3\mu m$) associated nitrifiers and AOR always occurred under low oxygen saturation which may indicate high heterotrophic activity. When oxygen saturation was high in outer plume and subsurface of Sta. Y3, maybe the large particle ($>3\mu m$) was composed by more alive primary producers which did not provide but compete for ammonium. And elevated salinity may also reduce the ammonium absorption capacity of suspended particles(Rysgaard et al., 1999). Then the nitrifier may prefer to get rid of particles and be planktonic.

We are also surprised to the significant positive correlation between reactive Fe/Mn and AOR. One possibility is the reactive Fe/Mn attracts the nitrifiers that the reviewer disagree. Another one is the nitrification occurred on particles alters the forms of Fe/Mn when utilizing them as alternative oxidant. We can not verify these hypotheses now. Investigating the speciation changes of particulate Fe/Mn during incubation may clarify them. And that will be our future direction.

Table 1. The particulate associated archaeal and β-proteobacterial *amo*A copies and the nitrification rate in bulk versus filtered water for three stations along the Changjiang River plume

Location	Station	Depth	TSM	O$_2$ saturation	Ammonia oxidation rate		β-proteobacterial *amo*A		Archaeal *amo*A	
					Bulk	Filtered*	Part.(>3μm)	Part. (0.22-3 μm)	Part.(>3μm)	Part. (0.22-3 μm)
		(m)	(mg L^{-1})		(nmol L^{-1} day^{-1})	(nmol L^{-1} day^{-1})	(copy L^{-1})	(copy L^{-1})	(copy L^{-1})	(copy L^{-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%)	2.56×10^7 ± 6.40×10^6 (66%)	1.35×10^7 ± 1.61×10^7 (34%)
		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%)	1.65×10^8 ± 2.54×10^6 (100%)	5.73×10^5 ± 2.45×10^4 (0%)
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%)	6.38×10^3 ± 1.79×10^3 (1%)	4.62×10^5 ± 7.96×10^3 (99%)
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%)	1.50×10^8 ± 3.40×10^6 (100%)	1.10×10^5 ± 2.80×10^4 (0%)
		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%)	2.70×10^6 ± 2.60×10^5 (2%)	1.40×10^8 ± 2.60×10^6 (98%)
		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	1.40×10^4 ± 2.80×10^3 (16%)	7.20×10^4 ± 3.30×10^3 (84%)

Nitrification rate was presented as mean ± standard deviation. BDL: below detection limit.

* Filtered: particles larger than 3 μm were removed in the incubation for nitrification rate measurement.

Technical corrections : Please check that all acronyms are defined, for example I am not sure that DON was defined.

Response:
Thanks for the notification of acronyms. We will add the definition of DON in abstract.

Technical corrections : P8693 line 4, sentence refer to Fig2i for Al, Fe Mn pattern whereas this figure concern only active Fe. Line 5, is % correspond to w/w or w/v can you precise.

Response:
We will add the figures of the distribution of Al and Mn in Fig.2. The percentage in P8694 line5 is w/w. We will add (w/w) after the %.

Technical corrections : P8693 line 3. I am concern about the linear correlation found Fig4d, I do not seem that it is valid since there is a cloud containing many data and very few data are outside

Response:
We checked the linear correlation in Fig 4d again and it is significant for combining all data along the plume (n=32, R^2=0.2589, *p*=0.0362).

Technical corrections : P8696 line 24-25, I do not understand the sentence.

Response:
We have changed the sentence as the following.
"However, distinctive correlations between AOR_b and TSM(Fig. 4b) observed along the salinity gradient of one river plume were firstly reported."

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

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2. Berman, T., Parparov, A., and Yacobi, Y. Z.: Planktonic community production and respiration and the impact of bacteria on carbon cycling in the photic zone of Lake Kinneret, Aquat Microb Ecol, 34, 43-55, Doi 10.3354/Ame034043, 2004.

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10. Rysgaard, S., Thastum, P., Dalsgaard, T., Christensen, P. B., and Sloth, N. P.: Effects of salinity on NH4+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments, Estuaries, 22, 21-30, Doi 10.2307/1352923, 1999.

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12. Wang, H. Y., Shen, Z. Y., Guo, X. J., Niu, J. F., and Kang, B.: Ammonia adsorption and nitrition in sediments derived from the Three Gorges Reservoir, China, Environ Earth Sci, 60, 1653-1660, DOI 10.1007/s12665-009-0299-7, 2010.