

Supporting Information for Publication

Figure S1 Schematic diagram of vertical flow constructed wetland units.

Figure S2 Rarefaction analysis of anammox bacterial communities in activated sludge and CW-C.

Table S1 Primer set used in this study for amplifying anammox bacteria

Table S2 The layout of OTU number and related anammox sequences accession numbers in the tree

Figure S1

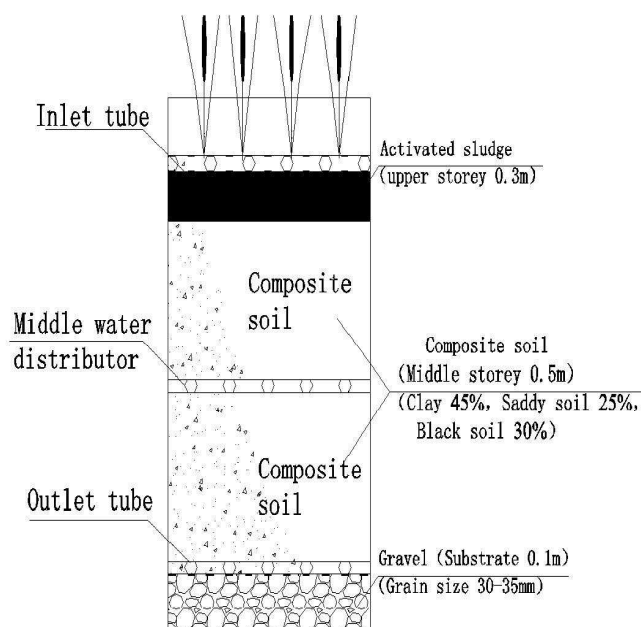


Figure S2

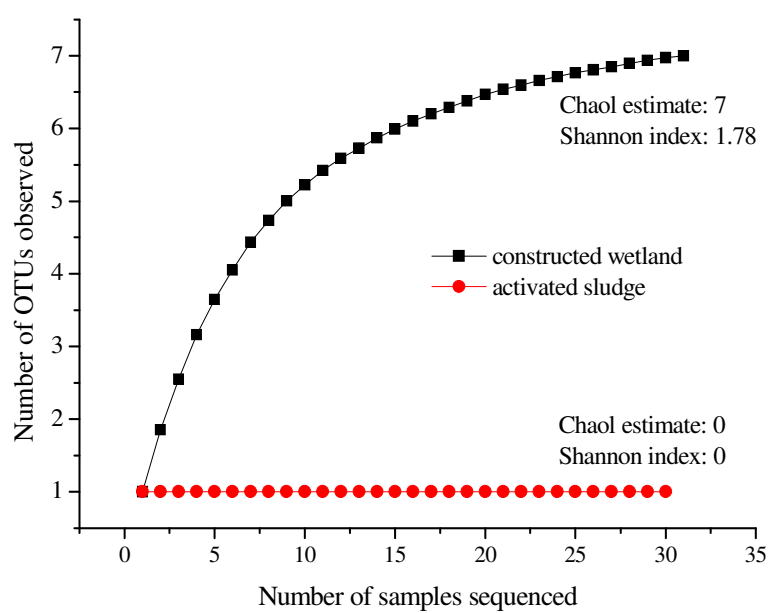


Table S1 Primer set used in this study for amplifying anammox bacteria

Primer	Sequence (5'-3')	Specificity	Position	References
PLA46f	GACTTGCATGCCTAATCC	Planctomycetes	46	28
630r	CAKAAAGGAGGTGATCC	Bacteria	1529	29
Amx368f	TTCGCAATGCCCCGAAAGG	Anammox 16S	368–385	30
Amx820r	AAAACCCCTCTACTTAGTGCCC	Anammox 16S	820–841	

Table S2 The layout of OTU number and related anammox sequences accession numbers in the tree

OTU	Sequence number	Sequence ID	Accession number(s)	The close match anammox bacteria
OTU 0#	30	as2-1, as2-2, as2-3, as2-4, as2-5, as2-11, as2-12, as2-13, as2-14, as2-15, as2-31, as2-32, as2-33, as2-34, as2-35, as2-41, as2-26, as2-36, as2-46, as2-56, as2-38, as2-37, as2-39, as2-98, as2-97, as2-47, as2-27, as2-48, as2-86, as2-87	JF346172-JF346201	<i>Candidatus</i> 'Brocadia fulgida'
OTU 1#	4	cw-19-11,cw-19-1,cw-19-2,cw-19-21	JF346225, JF346215, JF346216, JF346226	<i>Candidatus</i> 'Jettenia asiatica'
OTU 2#	2	cw-23-1,cw-23-11	JF346221, JF346231	<i>Candidatus</i> 'Jettenia asiatica'
OTU 3#	3	cw-4-2,cw-4-3,cw-8-1	JF346206, JF346207, JF346208	<i>Candidatus</i> 'Anammoxoglobus propionicus'
OTU 4#	11	cw-11-1,cw-11-11,cw-23-3,cw-23-31,cw-19-31,cw-19-3,cw-9-2,cw-2-1,cw-11-3,cw-11-31,cw-8-2	JF346213, JF346223, JF346222, JF346232, JF346227, JF346217, JF346211, JF346202, JF346214, JF346224, JF346209	<i>Candidatus</i> 'Anammoxoglobus propionicus'
OTU 5#	2	cw-21-2,cw-21-21	JF346219, JF346229	<i>Candidatus</i> 'Brocadia fulgida'
OTU 6#	2	cw-2-2,cw-2-3	JF346203, JF346204	<i>Candidatus</i> 'Brocadia anammoxidans'
OTU 7#	7	cw-4-1,cw-8-3,cw-21-1,cw-21-11,cw-9-3,cw-21-3, cw-21-31	JF346205, JF346210, JF346218, JF346228, JF346212, JF346220, JF346230	<i>Candidatus</i> 'Brocadia anammoxidans'

Overview of experimental set up

Step 1: Constructing the wetlands

Two similar 1 m³ CW tanks built in April 2008

Step 2: Time is zero controls and adding nitrifying/anammox sludge to CW-C

The sludge containing nitrifying and anammox bacteria was added to CW-C in March 2009
Before the incubation

- The PCR with CW-A and CW-C soil were done (No anammox was amplified).
- The biodiversity, abundance and activity of anammox bacteria of the sludge were investigated
- The potential nitrification rate (PNR) of CW-A and CW-C were investigated

Step 3: five month incubation

After the incubation the ¹⁵N tests were done on Sep 2nd, 2009 in CW-C and CW A
furthermore in CW C anammox diversity and PNR were determined

- After the incubation, the (q)PCR with CW-C soil was done to investigate the anammox biodiversity and abundance.
- The isotopic ¹⁵N test were done in CW A and CW C
- The potential nitrification rate of CW-C was investigated after the incubation

Step 4: effect on N₂O emission

The effect of anammox on reducing N₂O flux was done on April 20th 2010
CW A and CW C were operated under the same temperature, influent loading, retention time, sampling time to compare the N₂O flux in CW-A (no anammox) and CW-C (with anammox bacteria and activity).

