Applied Microbiology and Biotechnology (2018) 102:5859–5871
https://doi.org/10.1007/s00253-018-9078-2

MINI-REVIEW

Practical applications of PCR primers in detection of anammox bacteria effectively from different types of samples

Zhichao Zhou[1] · Qiaoyan Wei[2,3] · Yuchun Yang[1] · Meng Li[2] · Ji-Dong Gu[1,4]

Received: 26 February 2018 / Revised: 5 May 2018 / Accepted: 7 May 2018 / Published online: 25 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract
Research on anammox (anaerobic ammonium oxidizing) bacteria is important due to their biogeochemical and industrial application significance since the first discovery made over two decades ago. By coupling NH_4^+ and NO_2^- biochemically to form N_2 gas, anammox bacteria contribute significantly to global marine and terrestrial nitrogen balance (responsible for 50, 9~40, and 4~37% of the nitrogen loss for marine, lakes, and paddy soil) and are also useful in energy-conserving nitrogen removal in wastewater treatment. PCR-based detection and quantification of anammox bacteria are an easy, essential, and widely accessible technique used ubiquitously for studying them in many environmental niches. In this article, we make a summary on practical applications of 16S rRNA and functional gene PCR primers, including hydrazine dehydrogenase (Hzo), nitrite reductase (NirS), hydrazine synthase (Hzs), and cytochrome *c* biogenesis proteins (Ccs) in detection of them. PCR primer performances in both practical applications and tests in silico are also presented for comparison. For detecting general and specific anammox bacterial groups, selection of appropriate PCR primers for different environmental samples and practical application guidance on choice of appropriate primer pairs for different purposes are also offered. This article provides practical information on selection and application of PCR technique in detection of anammox bacteria from the diverse environments to further promote convenient applications of this technique in research and other purposes.

Keywords Anammox bacteria · PCR · 16S rRNA gene primer · Functional gene primer · Hydrazine synthase · Hydrazine dehydrogenase

Zhichao Zhou and Qiaoyan Wei contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00253-018-9078-2) contains supplementary material, which is available to authorized users.

✉ Meng Li
limeng848@szu.edu.cn

✉ Ji-Dong Gu
jdgu@hku.hk

[1] Laboratory of Environmental Microbiology and Toxicology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, Hong Kong, People's Republic of China

[2] Institute for Advanced Study, Shenzhen University, Shenzhen 518060, People's Republic of China

[3] Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, People's Republic of China

[4] State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, SAR, People's Republic of China

Introduction

The first evidence of the anaerobic ammonium oxidizing (anammox) process by bacteria from a denitrifying fluidized bed reactor was confirmed in a laboratory bioreactor of the Netherlands (Mulder et al. 1995). By harnessing the sequencing batch reactor with efficient biomass retaining capability, the physiological characteristics of the anammox process and the possible biochemical reaction mechanism of anammox bacteria are delineated and proposed, in which ammonium is oxidized to hydrazine first by the electron acceptor hydroxylamine; hydroxylamine is originated from nitrite reduction, and hydrazine was finally oxidized to dinitrogen gas, N_2 (Jetten et al. 1998). Anammox biochemical process in marine ecosystems was estimated to be responsible for 30~50% marine nitrogen loss (Arrigo 2005; Devol 2003). In addition, comparing to canonical denitrification processes, the application of anammox process contributes to less production of N_2O and NO, the gaseous nitrogen intermediates which causes severe greenhouse effects (Jetten et al. 1997). Partial denitrification

coupled with anammox is a common technical approach in industrial systems to effectively treat wastewater containing high ammonium and low biochemical oxygen demand (BOD) to decrease the operational cost and produce less greenhouse gases (Den Camp et al. 2006; Jetten et al. 2002). The ecological and industrial significance make anammox process and the micro-organisms to become one of the hot topics in environmental engineering and microbial ecology recently (Jetten et al. 2001, 2005; Kartal et al. 2011b).

Anammox bacteria, deeply branched within *Planctomycetales*, are responsible for anammox biochemical reactions for the nitrogen transformation (Strous et al. 1999). They occur in anoxic niches, including saline conditions, marine and ocean sediments, marine water column; freshwater wetland, paddy soils, wastewater treatment plants (WWTPs), reactors, groundwater, freshwater, and freshwater sediments and mixed-saline conditions, estuary, and mangrove sediments (Hu et al. 2011; Humbert et al. 2010; Li and Gu 2016a; Oshiki et al. 2016; Sonthiphand et al. 2014). There are additional reports on anammox bacterial populations in extreme and special environments, such as hydrothermal vents, hypersaline sulfidic basins, petroleum reservoirs, marine sponge, fish and polychaete guts (Borin et al. 2013; Byrne et al. 2009; Chan and Gu 2016; Li et al. 2010a; Li and Gu 2016b; Mohamed et al. 2010). Studies on anammox bacteria genome, biology and biochemistry, diversity, and application rely on better practical applications of detecting them from natural and laboratory environments. Currently, no agreement is available on a common gene marker or a set of PCR primer for detecting them in various samples for a comparison of the results available.

The isotope-pairing technique could help the quantitative measurement on anammox activity rates (Risgaard-Petersen et al. 2003; Ward et al. 2009). Molecular probes of fluorescent in situ hybridization (FISH) and catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH) specific for selective genera have been developed since the beginning of discovery of new anammox bacteria species (Li and Gu 2011; Schmid et al. 2000). Anammox bacteria specific core ladderane lipids and intact ladderane glycerophospholipids of living cells can serve as the bioindicators for general and viable abundance of anammox bacteria (Damsté et al. 2002; Jaeschke et al. 2009; Sinninghe Damsté et al. 2005), but the complicated procedures and sophisticated instruments prevent many from using them practically. PCR primer pairs for qualitative and quantitative detection of anammox bacteria have been developed and applied widely in majority of the studies for distribution and diversity patterns, community composition, and quantification of the metabolic activity by reverse transcription PCR (RT-PCR) (Li and Gu 2011). PCR primers of 16S rRNA gene are the most used in various researches and can offer phylogeny details, while primers targeting functional genes have high specificity and can reflect functional activity (Li and Gu 2011). Up to now, there are four categories of functional gene-based PCR primer pairs developed, targeting central anammox activity enzymes, including hydrazine dehydrogenase (Hzo), nitrite reductase (NirS), hydrazine synthase (Hzs), and cytochrome *c* biogenesis proteins (Ccs) (Harhangi et al. 2012; Hirsch et al. 2011; Li et al. 2010b, 2011b; Schmid et al. 2008; Wang et al. 2012c; Zhou et al. 2017).

Detection of anammox bacteria in various samples has not been successful as expected, even though the PCR technique has been available for a long time. Since PCR primers were designed in silico and, as a result, their efficiencies and specificities depend on the biomass, sample characteristics and interfering factors for PCR reaction to take place effectively. Many practical applications of the reported PCR primers on sensitive detection of anammox bacteria do not offer acceptable performance unfortunately (Han et al. 2017; Li and Gu 2011; Zhou et al. 2017). Large and comprehensive database of rRNA gene, such as RDP, Greengenes, and SILVA, could serve as a suitable platform to evaluate primer performance in silico (Cole et al. 2005; DeSantis et al. 2006; Quast et al. 2013). Previously published reviews only provide primer details instead of practical information of the primer performance. In this review, an updated summary was made on several aspects: (i) the current diversity and ecology of *Scalindua* and non-*Scalindua* genera of anammox bacteria, (ii) evaluation of primer performance both in silico and in practical tests, and (iii) information of PCR performance outcome of general and specific anammox bacteria groups and on different sample types. This mini-review aims to offer PCR information of practical applications and guidance on selection of appropriate PCR primer pairs for different purposes.

Diversity of anammox bacteria

The first identified anammox bacteria were given the provisional name *Candidatus* Brocadia anammoxidans (Strous et al. 1999). Up to now, there are five genera of anammox bacteria including at least 22 *Candidatus* species (Fig. 1). The *Brocadia* includes *Ca*. Brocadia anammoxidans, *Ca*. Brocadia fulgida, *Ca*. Brocadia sinica, *Ca*. Brocadia caroliniensis, *Ca*. Brocadia sapporoensis, and *Ca*. Brocadia brasiliensis (Araujo et al. 2011; Kartal et al. 2008; Narita et al. 2017; Oshiki et al. 2011; Rothrock et al. 2011; Strous et al. 1999). All *Brocadia* species were enriched in anammox bioreactors (Sonthiphand et al. 2014). *Ca*. Kuenenia stuttgartiensis is the only identified species in *Kuenenia* so far (Schmid et al. 2000). *Ca*. Anammoxoglobus propionicus is the only identified species in *Anammoxoglobus*, a propionate oxidizing species out-competing other anammox bacteria and denitrifiers in an anammox bioreactor in the presence of propionate (Kartal et al. 2007). *Ca*. Jettenia asiatica, *Ca*. Jettenia caeni and *Ca*. Jettenia moscovienalis are three identified species in the genus *Jettenia*, enriched in a granular

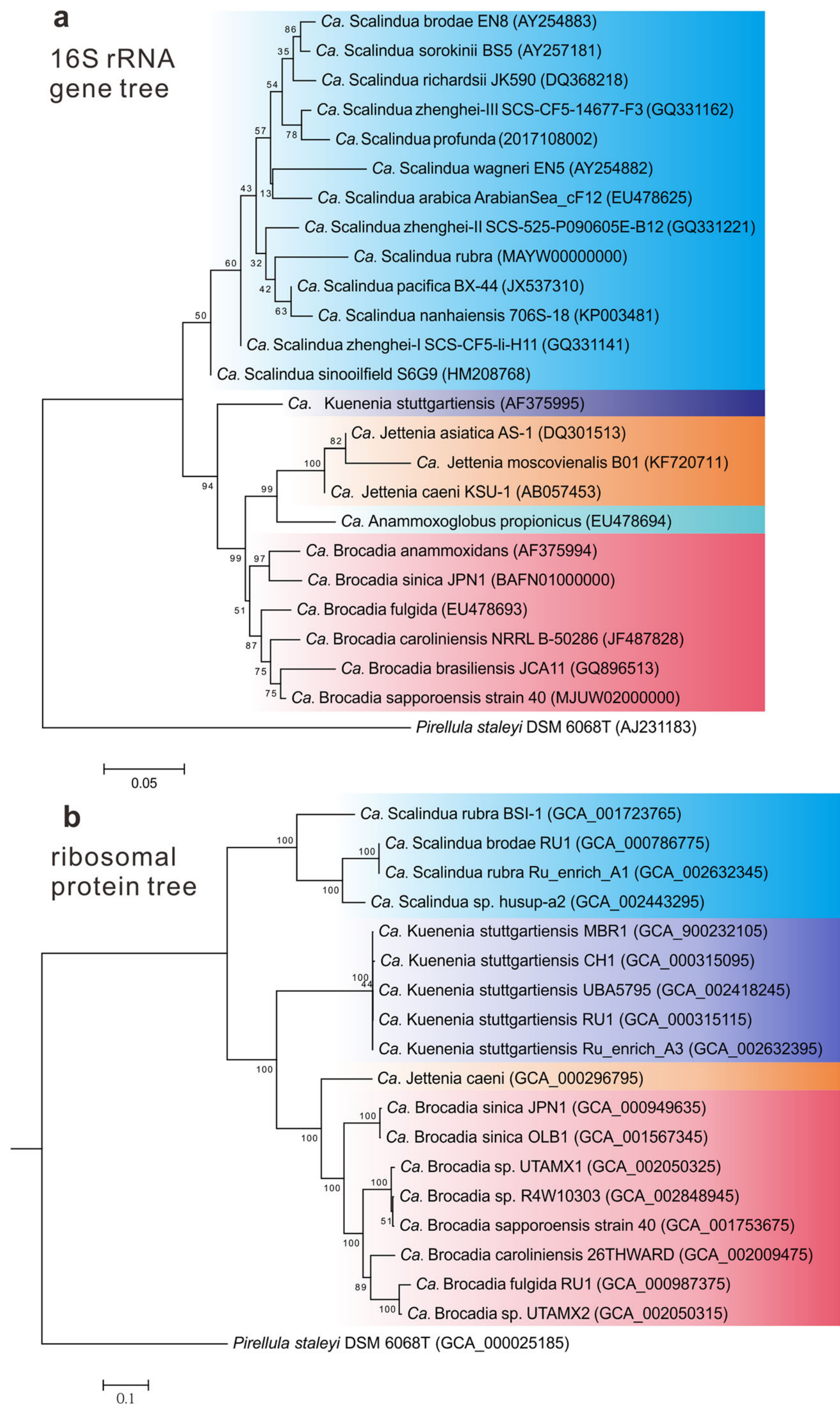


Fig. 1 Phylogenetic tree of 16S rRNA gene of currently defined anammox species (**a**) and phylogenetic tree of ribosomal protein (12 ribosomal protein alignment) of currently available anammox bacteria genomes. Jukes-Cantor method, gamma distribution of site variation, pairwisely aligning for each sequence pair, and 1000 time bootstrap testing were applied to make the neighbor-joining tree (**a**). RAxML-HPC v.8 (implemented in CIPRES) with "-m PROTGAMMALG -n 1000" was applied to make the RAxML tree (**b**)

sludge anaerobic anammox reactor from river sediment as inoculum, an active sludge incubated membrane bioreactor (MBR), and an active sludge treating municipal wastewater, respectively (Ali et al. 2015; Nikolaev et al. 2015; Quan et al. 2008).

Up to now, there are 11 established species in *Scalindua*, namely *Ca*. S. sorokinii, *Ca*. S. richardsii, *Ca*. S. brodae, *Ca. S. wagneri*, *Ca. S. arabica*, *Ca. S. pacifica*, *Ca. S. profunda*, *Ca*. S. sinooilfield, *Ca*. S. naihaiensis, *Ca. S. rubra*, and *Ca*. S. zhenghei (Dang et al. 2013; Fuchsman et al. 2012; Hong et al. 2011; Kuypers et al. 2003; Li et al. 2010a; Schmid et al. 2003; Speth et al. 2017; van de Vossenberg et al. 2008; Woebken et al. 2008; Zhou et al. 2017). However, these tentatively named species could not cover the complete diversity of uncultured *Scalindua* and regional specific distribution pattern of *Scalindua* sp. clades, suggesting that more undiscovered endemic groups are still available (Oshiki et al. 2016). *Scalindua* spp. have a considerably large micro-diversity (Schmid et al.

2007; Woebken et al. 2008). Recent studies suggest that regional specific sub-clusters are evident, while at the same time, for large clades, such as *Scalindua arabica* clade and *Scalindua sorokinii/brodae* clade, some species have a globally wide occurrence (Hong et al. 2011; Oshiki et al. 2016; Woebken et al. 2008). Until now, there is still no pure anammox bacterial culture achieved, even though anammox bacterial species could be enriched to nearly pure culture (~ 90% biomass) allowing investigation on their physiological properties possible (Ali et al. 2015; Kartal et al. 2007; Oshiki et al. 2016; Park et al. 2010).

Ecology and environmental influence

Scalindua in marine ecosystem

As for *Scalindua* spp., many studies showed that they are mainly distributed in marine sediments or water column with a large micro-diversity within the genus (Dang et al. 2013; Schmid et al. 2007; Woebken et al. 2008). However, some non-*Scalindua* populations are commonly detected in the coastal and estuarine ecosystems and they might be exogenous inputs from adjacent terrestrial river runoff or anthropogenic discharges, thus could serve as bioindicators for anthropogenic/terrestrial input and pollution (Han and Gu 2015). Meanwhile, change of anammox bacteria community composition from sediments of riparian locations, coastal wetlands to marginal sea further showed that such a distribution pattern of diversity was negatively correlated with inorganic nitrogen species from pollution (Dale et al. 2009; Dang et al. 2010; Han and Gu 2015; Li et al. 2011a, c, 2013). Anammox bacterial activities play an important ecological role in nitrogen loss in marine deep sediments (20~80% N loss), continental slope, and estuarine and coastal sea ecosystems (less than 20% N loss); meanwhile, within the anoxic marine water column, anammox process is considered the major contributor to the nitrogen production (Dalsgaard et al. 2005; Jensen et al. 2011; Kuypers et al. 2005; Lam et al. 2009; Thamdrup et al. 2006).

Environmental influence on marine anammox bacteria

Dissimilatory nitrate reduction to ammonium (DNRA) could couple with anammox process by fueling anammox activity with reduced NH_4^+, while, denitrification in oligotrophic and electron donors limited conditions reduces NO_3^- to NO_2^-, and the latter nitrogen compound is also the direct substrate of anammox bacteria (Hu et al. 2011; Jensen et al. 2011). Aerobic ammonia oxidizers in a marine oxygen-limited zone could oxidize NH_4^+ to NO_2^- to further deplete oxygen available to create a suitable condition for anammox activity (Lam et al. 2007; Woebken et al. 2007). Both denitrification and availability of ammonium in the marine water column depend on availability of organic matters, thus local availability of organic matters and their mineralization rates are the critical influencing factors on anammox processes (Hu et al. 2011; Lam et al. 2009). In marine sediments and water column, factors favoring anammox existence and activity include low availability of oxygen, low temperature, and stable environmental conditions (Dalsgaard et al. 2005, 2014; Jensen et al. 2008; Oshiki et al. 2016; Russ et al. 2013).

Non-*Scalindua* in terrestrial ecosystem

In terrestrial ecosystem, anammox process is also an important pathway for nitrogen loss, such as fertilized paddy soil (4–37% N loss), lake riparian zones (< 10% N loss), water column of freshwater lake (~ 13% N loss), contaminated groundwater (18–36% N loss), and constructed wetland (24% N loss) (Erler et al. 2008; Moore et al. 2011; Schubert et al. 2006; Zhu et al. 2011, 2013). Most of the previously investigated sites of freshwater ecosystems were dominated by *Brocadia* and *Kuenenia* species (Moore et al. 2011; Zhu et al. 2013). While, in a paddy field, surface soil layers contained *Kuenenia*, *Anammoxoglobus*, and *Jettenia* but deep soil was dominated by *Brocadia* (Zhu et al. 2011). However, there are still several specific reports of *Scalindua* appearing or dominating in pristine environments, including freshwater Lake Tanganyika water column (Schubert et al. 2006), wetland (Lee et al. 2016), subsurface oil reservoirs (Li et al. 2010a), and rice paddy in northeast China (Wang and Gu 2013). These indicate a more diverse distribution pattern of anammox bacteria in freshwater column and sediments, resulting from the heterogeneous micro-niches in terrestrial ecosystems favoring the growth of different anammox bacterial groups. The versatile metabolism of *Brocadia* and *Kuenenia* on utilizing various electron acceptors and donors also facilitated their adaptation to specific environmental conditions even with limited substrates available for anammox metabolism to take place (Strous et al. 2006).

Salinity is a major factor correlated with the global anammox bacteria distribution pattern and is also the key driving force determining the niche specificity of non-*Scalindua* and *Scalindua* (Sonthiphand et al. 2013). Community composition differences between natural and engineered ecosystems could be due to the selection pressure imposed by long-term perturbation to select superior competitors to become dominant (Sonthiphand et al. 2013). Freshwater sediments have the highest diverse anammox bacterial population while marine water column the lowest. In terms of the co-occurrence, *Scalindua* taxa tend to occur alone in pristine condition, but *Brocadia* taxa could be found to coexist with all other anammox bacterial genera (Sonthiphand et al. 2013).

PCR primers of 16S rRNA and functional genes

PCR primers of 16S rRNA gene are most frequently used in previous and current studies (Table 1). According to the information summary (Table S1), specific primer pairs to detect *Scalindua* or non-*Scalindua* are compatible between in silico and practical tests, though there are still some exceptions. Amx368F-Amx820R is recommended for retrieving non-*Scalindua*, and Amx368F-BS20R for *Scalindua*, both with good performance in silico test (Fig. 2). Amx368F-Amx820R has poor in silico coverage (18%) for *Scalindua*; however, many researches of both marine and terrestrial environments suggest that it can also amplify *Scalindua* sequences from marine origin samples free of mismatches in the 5' end and obtain similar community as Amx368F-BS20R from both marine and terrestrial environments (Amano et al. 2007; Humbert et al. 2010; Yoshinaga et al. 2011).

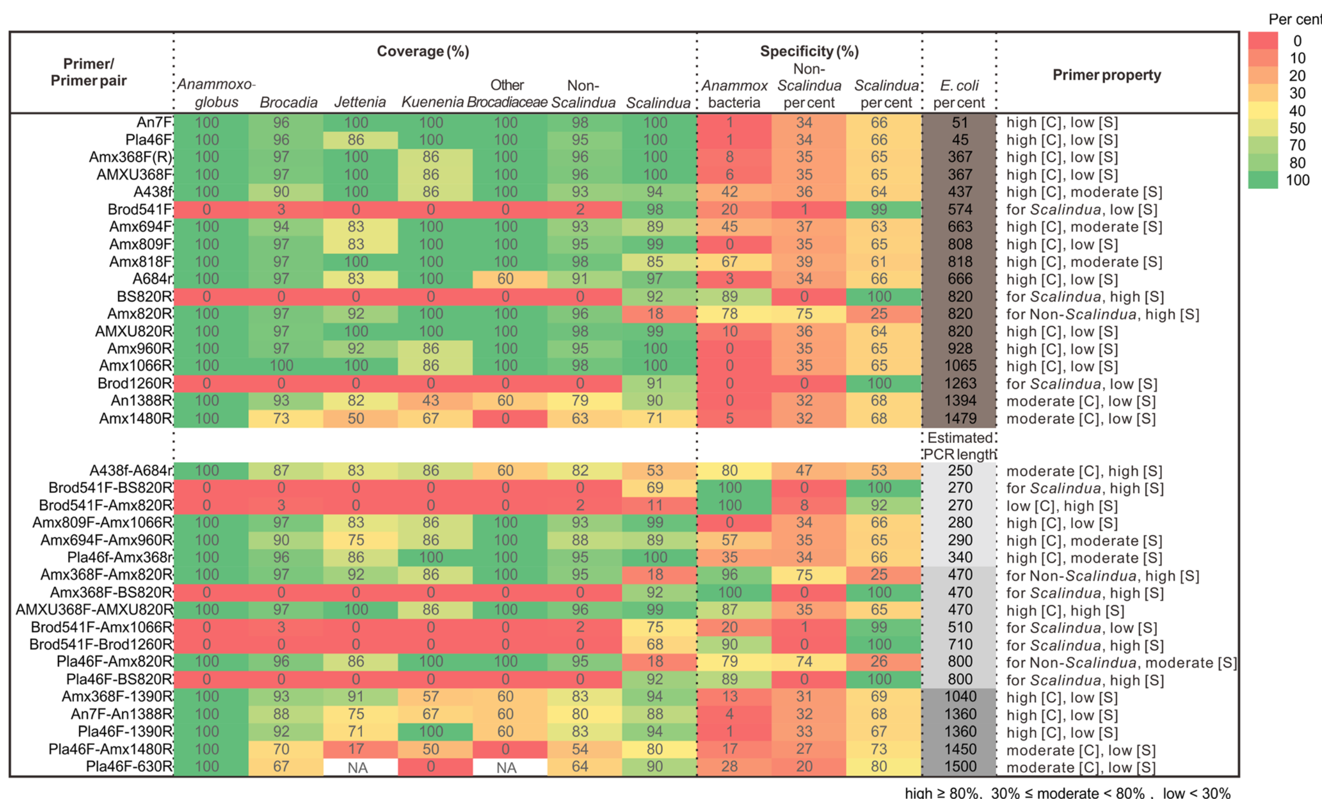
Functional proteins, including cluster 1 of hydrazine oxidoreductase (Hzo) of anammox bacteria, converting N_2H_4 to N_2, nitrite reductase (NirS), catalyzing NO_2^- to NO, hydrazine synthase (Hzs), converting NH_4^+ and NO to N_2H_4, and cytochrome *c* biogenesis proteins (Ccs), facilitating heme transport into anammoxosome to maintain its reducing state, reflect phylogeny and biochemistry function well (Harhangi et al. 2012; Li et al. 2011b; Schmid et al. 2008; Zhou et al. 2017). Some of them acquired good genera coverage and specificity on general anammox bacteria and selective genera (Table 2 and Table S2). Because of the divergence of protein biochemistry and coding sequence between the anammox bacteria and the others, use of functional gene primers could facilitate detecting specific anammox bacteria and measuring

Table 1 PCR primers of 16S rRNA gene for clone library and qPCR of anammox bacteria from various samples

Primer	5′ - 3’	*E. coli* position	Annealing T. (°C)	Reference
An7F	GGCATGCAAGTCGAACGAGG	51	60.4	Penton et al. (2006)
Pla46F[1]	GGATTAGGCATGCAAGTC	45	52.8	Neef et al. (1998)
Amx368F(R)	TTCGCAATGCCCGAAAGG	367	58.1	Schmid et al. (2003)
AMXU368F	TTCGCAATGCCCGMAAGG	367	59.5	Amano et al. (2011)
A438f[1]	GTCRGGAGTTADGAAATG	437	49.3	Humbert et al. (2012)
Brod541F[1]	GAGCACGTAGGTGGGTTTGT	574	59.2	Penton et al. (2006)
Amx694F[1]	GGGGAGAGTGGAACTTCGG	663	58.5	Ni et al. (2010)
Amx809F[1]	GCCGTAAACGATGGGCACT	808	59.7	Tsushima et al. (2007)
Amx818F[1]	ATGGGCACTMRGTAGAGGGGTTT	818	62.1	Tsushima et al. (2007)
A684r[1]	ACCAGAAGTTCCACTCTC	666	53	Humbert et al. (2012)
BS820R	TAATTCCCTCTACTTAGTGCCC	820	56	Kuypers et al. (2003)
Amx820R[1]	AAAACCCCTCTACTTAGTGCCC	820	58.8	Schmid et al. (2000)
AMXU820R	CCCTCTACYKAGTGCCC	820	55	Amano et al. (2011)
Amx960R[1]	GCTCCACCGCTTGTGCGAGC	928	64.8	Ni et al. (2010)
Amx1066R[1]	AACGTCTCACGACACGAGCTG	1065	61.1	Tsushima et al. (2007)
Brod1260R	GGATTCGCTTCACCTCTCGG	1263	59.1	Penton et al. (2006)
An1388R	GCTTGACGGGCGGTGTG	1394	60.4	Penton et al. (2006)
Amx1480R	TACGACTTAGTCCTCCTCAC	1479	54.8	Amano et al. (2007)
For Taqman qPCR				
AMX-808-F	ARCYGTAAACGATGGGCACTAA	807	59.2	Hamersley et al. (2007)
AMX-1040-R	CAGCCATGCAACACCTGTRATA	1039	59	Hamersley et al. (2007)
AMX-931 (probe)	TCGCACAAGCGGTGGAGCATGTGGCTTA	930	–	Hamersley et al. (2007)
Reserve universal primer				
630R (also as 1545R)[1]	CAKAAAGGAGGTGATCC	1528	49.5	Juretschko et al. (1998)
1390R	GACGGGCGGTGTGTACAA	1389	59.1	Kjeldsen et al. (2009)
1037R (23S rRNA)	CGACAAGGAATTTCGCTAC	–	53.5	Ludwig et al. (1992)

Alternative names with “PLA,” “AMX,” “F/R,” or “f/r” are also seen in references, we adopt the most commonly used names. 1390R and 1392R are the same primer. Taqman qPCR primer pair and probe are not suitable for SYBR green qPCR (or similar methods). Taqman qPCR primers and probe are integrated and should be used together to achieve high specificity. Annealing temperature is calculated using OligoAnalyzer 3.1 (http://sg.idtdna.com/calc/analyzer) with default settings of oligo, cation, and dNTP concentration in regular PCR (use T_m—5 °C). AMXU368Fis modified from Amx368F to increase degeneracy and coverage; AMXU820R is modified from BS820R and Amx820R to increase degeneracy and coverage

[1] Reported to be used as qPCR primers



Primer/Primer pair	Coverage (%) *Anammoxoglobus*	*Brocadia*	*Jettenia*	*Kuenenia*	Other *Brocadiaceae*	Non-*Scalindua*	*Scalindua*	Specificity (%) *Anammox* bacteria	Non-*Scalindua* per cent	*Scalindua* per cent	*E. coli* per cent	Primer property
An7F	100	96	100	100	100	98	100	1	34	66	51	high [C], low [S]
Pla46F	100	96	86	100	100	95	100	1	34	66	45	high [C], low [S]
Amx368F(R)	100	97	100	86	100	96	100	8	35	65	367	high [C], low [S]
AMXU368F	100	97	100	86	100	96	100	6	35	65	367	high [C], low [S]
A438f	100	90	100	86	100	93	94	42	36	64	437	high [C], moderate [S]
Brod541F	0	3	0	0	0	2	98	20	1	99	574	for *Scalindua*, low [S]
Amx694F	100	94	83	100	100	93	89	45	37	63	663	high [C], moderate [S]
Amx809F	100	97	83	100	100	95	99	0	35	65	808	high [C], low [S]
Amx818F	100	97	100	100	100	98	85	67	39	61	818	high [C], moderate [S]
A684r	100	97	83	100	60	91	97	3	34	66	666	high [C], low [S]
BS820R	0	0	0	0	0	0	92	89	0	100	820	for *Scalindua*, high [S]
Amx820R	100	97	92	100	100	96	18	78	75	25	820	for Non-*Scalindua*, high [S]
AMXU820R	100	97	100	100	100	98	99	10	36	64	820	high [C], low [S]
Amx960R	100	97	92	86	100	95	100	0	35	65	928	high [C], low [S]
Amx1066R	100	100	100	86	100	98	100	0	35	65	1065	high [C], low [S]
Brod1260R	0	0	0	0	0	0	91	0	0	100	1263	for *Scalindua*, low [S]
An1388R	100	93	82	43	60	79	90	0	32	68	1394	moderate [C], low [S]
Amx1480R	100	73	50	67	0	63	71	5	32	68	1479	moderate [C], low [S]

Primer/Primer pair	*Anammoxoglobus*	*Brocadia*	*Jettenia*	*Kuenenia*	Other *Brocadiaceae*	Non-*Scalindua*	*Scalindua*	*Anammox* bacteria	Non-*Scalindua* per cent	*Scalindua* per cent	Estimated PCR length	Primer property
A438f-A684r	100	87	83	86	60	82	53	80	47	53	250	moderate [C], high [S]
Brod541F-BS820R	0	0	0	0	0	0	69	100	0	100	270	for *Scalindua*, high [S]
Brod541F-Amx820R	0	3	0	0	0	2	11	100	8	92	270	low [C], high [S]
Amx809F-Amx1066R	100	97	83	86	100	93	99	0	34	66	280	high [C], low [S]
Amx694F-Amx960R	100	90	75	86	100	88	89	57	35	65	290	high [C], moderate [S]
Pla46f-Amx368r	100	96	86	100	100	95	100	35	34	66	340	high [C], moderate [S]
Amx368F-Amx820R	100	97	92	86	100	95	18	96	75	25	470	for Non-*Scalindua*, high [S]
Amx368F-BS820R	0	0	0	0	0	0	92	100	0	100	470	for *Scalindua*, high [S]
AMXU368F-AMXU820R	100	97	100	86	100	96	99	87	35	65	470	high [C], high [S]
Brod541F-Amx1066R	0	3	0	0	0	2	75	20	1	99	510	for *Scalindua*, low [S]
Brod541F-Brod1260R	0	0	0	0	0	0	68	90	0	100	710	for *Scalindua*, high [S]
Pla46F-Amx820R	100	96	86	100	100	95	18	79	74	26	800	for Non-*Scalindua*, moderate [S]
Pla46F-BS820R	0	0	0	0	0	0	92	89	0	100	800	for *Scalindua*, high [S]
Amx368F-1390R	100	93	91	57	60	83	94	13	31	69	1040	high [C], low [S]
An7F-An1388R	100	88	75	67	60	80	88	4	32	68	1360	high [C], low [S]
Pla46F-1390R	100	92	71	100	60	83	94	1	33	67	1360	high [C], low [S]
Pla46F-Amx1480R	100	70	17	50	0	54	80	17	27	73	1450	moderate [C], low [S]
Pla46F-630R	100	67	NA	0	NA	64	90	28	20	80	1500	moderate [C], low [S]

Fig. 2 Properties of 16S rRNA gene primer and primer pairs on different genera of anammox bacteria deduced by in silico primer evaluation. SILVA SSU132 RefNR as database, 2 maximum number mismatches were used in TestProbe 3.0; and SILVA SSU132 RefNR as database, 2 maximum number mismatches and 1 site of 0-mismatch zone at 3' end were used in TestPrime 1.0. (https://www.arb-silva.de/search). A Perl script was used to calculate primer coverage and specificity for anammox bacterial groups. Primer pairs are the most frequently reported ones in references. "Other *Brocadiaceae*" stands for *Brocadiaceae* group which is not clustered in any of the defined genera of *Anammoxoglobus*, *Brocadia*, *Jettenia*, and *Kuenenia*

corresponding functional activity by reverse transcription (RT)-PCR (Li and Gu 2011; Zhou et al. 2017).

As mentioned previously, primers with mismatches in silico could still offer acceptable PCR results (high specificity); however, it will potentially cause (i) bias of results, reflecting unreal community or (ii) offering non-specific results or (iii) reduced primer coverage. Primer sets of 16S rRNA gene with good performance both in silico and in practical tests are highly recommended for amplifying non-*Scalindua*, *Scalindua*, and whole anammox bacteria and the relevant information is summarized in Table 3. Both coverage and specificity are taken into consideration. Nested PCRs are encouraged to increase specificity of complex environmental samples, e.g., soil and sediments with high organic matter, and those with very small anammox bacteria proportion (< 1%) in the total microbial biomass, though it sacrifices coverage (Kartal et al. 2011a).

PCR primers for different genera

Scalindua species generally appear in marine ecosystem, intertidal or closed niches without human disturbance, e.g., estuary sediment, groundwater, and wetland ecosystems, though some exceptions are possible in terrestrial ecosystems, while non-*Scalindua* species are completely the reverse. Primer pairs targeting all anammox bacteria and specifically *Scalindua*, non-*Scalindua*, *Kuenenia*, and *Jettenia* are all summarized, and some of them suitable for both qPCR and PCR for clone library (Table 3). Due to the high divergence of 16S rRNA gene among different genera of anammox bacteria (< 87.1% identity), higher coverage leads to higher primer degeneracy (Junier et al. 2010; Li and Gu 2011). However, high primer degeneracy will probably lead to non-specific amplification from complex sample matrix. A pre-assessment of microbial community will offer a better choice of proper specific primers and improve the PCR outcome. The practical performance of specific primers on different genera also serves as a checklist for researchers to pick PCR primers based on similar situations (Tables S1 and S2).

PCR primers for different samples

For saline environmental category, e.g., marine sediments, marine water column, PCR primers for all anammox bacteria and for specific *Scalindua* usually have good performance (Tables S1 and S2). These environments tend to be of less

Table 2 PCR primer pairs based on functional genes of anammox bacteria for clone library and qPCR

Primer	5′ - 3’	Product size	Annealing T. (°C)	Reference
hzocl1F1	TGYAAGACYTGYCAYTGG	470	54.3	Schmid et al. (2008)
hzocl1R2	ACTCCAGATRTGCTGACC		54.4	
hzocl1F2	TGYAAGACYTGYCAYTGGG	470	56.9	Schmid et al. (2008)
hzocl1R2	ACTCCAGATRTGCTGACC		54.4	
Ana-hzo1F	TGTGCATGGTCAATTGAAAG	1030	54.4	Quan et al. (2008)
Ana-hzo2R	ACCTCTTCWGCAGGTGCAT		58.6	
Hzo_LV2F[1]	GGTCATCACAACGTRCAGAG	300	56.4	Pitcher et al. (2011)
hzo_LV1R[1]	ACTCCARACGTGCTCACC		57.7	
hzoF1	TGTGCATGGTCAATTGAAAG	1000	54.4	Li et al. (2010b)
hzoR1	CAACCTCTTCWGCAGGTGCATG		60.4	
hzoAB1F	GAAGCNAAGGCNGTAGAAATTATCAC	1550	58.8	Hirsch et al. (2011)
hzoAB1R	CTCTTCNGCAGGTGCATGATG		59.1	
hzoAB4F	TTGARTGTGCATGGTCTAWTGAAAG	600	58	Hirsch et al. (2011)
hzoAB4R	GCTGACCTGACCARTCAGG		57.9	
Scnir372F	TGTAGCCAGCATTGTAGCGT	470	59	Lam et al. (2009)
Scnir845R	TCAAGCCAGACCCATTTGCT		59.4	
AnnirS379F	TCTATCGTTGCATCGCATTT	440	55.4	Li et al. (2011b)
AnnirS821R	GGATGGGTCTTGATAAACA		51.9	
hzsA_526F	TAYTTTGAAGGDGACTGG	1330	50.6	Harhangi et al. (2012)
hzsA_1857R	AAABGGYGAATCATARTGGC		55.1	
hzsA_382F	GGYGGDTGYCAGATATGGG	2000	57.6	Harhangi et al. (2012)
hzsA_2390R	ATRTTRTCCCAYTGYGCHCC		58.1	
hzsA_1597F[1]	WTYGGKTATCARTATGTAG	260	47.6	Harhangi et al. (2012)
hzsA_1857R[1]	AAABGGYGAATCATARTGGC		55.1	
hzsA_757F_Sca	AGTTCNAAYTWTGACCC	1070	48.5	Harhangi et al. (2012)
hzsA_1829R_Sca	CTGAACCACCARTTGTA		49.7	
hzsA_1600F_Sca[1]	GGKTATCARTATGTAGAAG	230	46.8	Harhangi et al. (2012)
hzsA_1829R_Sca[1]	CTGAACCACCARTTGTA		49.7	
HSBeta396F[1]	ARGGHTGGGGHAGYTGGAAG	340	60.3	Wang et al. (2012a)
HSBeta742R[1]	GTYCCHACRTCATGVGTCTG		57.2	
hzsB364f	TGYGCVAGYTGYCAYTAYGARAG	450	61.2	Zhou et al. (2017)
hzsB790r	CCSGTYCCHACRTCATGVGTCTG		63.1	
hzsB364f[1]	TGYGCVAGYTGYCAYTAYGARAG	300	61.2	Zhou et al. (2017)
hzsB640r[1]	CTGAAHGGACTYCCBGTRAAYTC		58.6	
hzsC496f_An	ACRGGRAGGATGGAGAAG	440	55.1	Zhou et al. (2017)
hzsC496f_Sc	ACTGCAATGAACAGTAAG		49.9	
hzsC862r	TAHGGATTNCCRTCRTARTTRTT		54.9	
hzsC745f[1]	CCRAAGAACTGGYTDCCKGTDTG	140	60.6	Zhou et al. (2017)
hzsC862r[1]	TAHGGATTNCCRTCRTARTTRTT		54.9	
ccsA376f	CCSGCRYTRMARAGYAAYTGGMTG	310	62.6	Zhou et al. (2017)
ccsA668r	AAAGACCABRTYTCYTTDGGAT		55.9	
ccsB581f	TNGARAARMATSMDCCRAAAT	1030	54.6	Zhou et al. (2017)
ccsB1589r	GCWARRTTRTTRTCDKKATACCA		54.5	
ccsB1116f	AYAATCCWGCYGTWMWVGTDGA	490	57.6	Zhou et al. (2017)
ccsB1589r	GCWARRTTRTTRTCDKKATACCA		54.5	

Annealing temperature is calculated in the same way as Table 1

[1] Reported to be used as qPCR primers

Table 3 Summary of PCR primer pairs of 16S rRNA and functional genes of anammox bacteria for practically detecting anammox bacteria with good performance and proper use

Target	Primer pair	In practical	In silico	For qPCR	Product size (bp)	Product size range	For use	Reference
16S rRNA	A438f-A684r	[Amx], moderate-high [S]	moderate [C], high [S]	[Y]	250	Short	qPCR for [Amx]	Humbert et al. (2012)
	Amx368F-Amx820R	[Non-Sca], high [S]	[Non-Scan], high [S]	[Y]	470	Medium	qPCR and clone library for [Non-Scan]	Schmid et al. (2000, 2003)
	Amx368F-BS820R	[Sca], high [S]	[Sca], high [S]	[Y]	470	Medium	qPCR and clone library for [Sca]	Kuypers et al. (2003); Schmid et al. (2003)
	AMXU368F-AMXU820R	[Amx], low-high [S]	[Amx], high [S]	[Y]	470	Medium	qPCR and clone library for [Amx]	Amano et al. (2011)
	Pla46F-BS820R	[Sca], low-high [S]	[Sca], high [S]	[N]	800	Long	1st round nest PCR for [Sca]	Kuypers et al. (2003); Neef et al. (1998)
	Amx368F-1390R	[Sca], low [S]	high [C], low [S]	[N]	1040	Long	1st round nest PCR for [Amx]	Kjeldsen et al. (2009); Schmid et al. (2003)
	An7F-An1388R	[Amx], low-moderate [S]	high [C], low [S]	[N]	1360	Long	1st round nest PCR for [Amx]	Penton et al. (2006)
	Pla46F-1390R	[Non-Sca], high [S]	high [C], low [S]	[N]	1360	Long	1st round nest PCR for [Amx]	Kjeldsen et al. (2009); Neef et al. (1998)
hzoA	hzocl1F1-hzocl1R2	[Amx], high [S]	–	[Y]	470	Medium	qPCR and clone library for [Amx]	Schmid et al. (2008)
	Hzo_LV2F-Hzo_LV1R	[Sca], good [qPCR]	–	[Y]	300	Short	qPCR for *Scalindua*	Pitcher et al. (2011)
	Ana-hzo1F-Ana-hzo2R	[Kue] & [Jet], high [S]	–	[N]	1030	Long	clone library for [Kuen] & [Jett]	Quan et al. (2008)
nirS	Scnir372F-Scnir845R	[Sca], high [S]	–	[Y]	470	Medium	qPCR and clone library for [Scan]	Lam et al. (2009)
	AnnirS379F-AnnirS821R	[Kue], high [S]	–	[Y]	440	Medium	qPCR and clone library for [Non-Scan]	Li et al. (2011b)
hzsA	hzsA_526F-hzsA_1857R	[Amx], high [S]	–	[N]	1330	Long	clone library for [Amx]	Harhangi et al. (2012)
	hzsA_382F-hzsA_2390R	[Amx]	–	[N]	2000	Long	1st round nest PCR for [Amx]	Harhangi et al. (2012)
	hzsA_1597F-hzsA_1857R	[Amx], high [S]	–	[Y]	260	Short	qPCR and clone library for [Amx]	Harhangi et al. (2012)
	hzsA_757F_Sca-hzsA_1829R_Sca	[Sca]	–	[N]	1070	Long	clone library for [Sca]	Harhangi et al. (2012)
	hzsA_1600F_Sca-hzsA_1829R_Sca	[Sca]	–	[Y]	230	Short	qPCR for [Sca]	Harhangi et al. (2012)
hzsB	hzsB364f-hzsB790r	[Amx], high [S]	–	[Y]	450	Medium	qPCR and clone library for [Amx]	Zhou et al. (2017)
	hzsB364f-hzsB640r	[Amx]	–	[Y]	300	Short	qPCR for [Amx]	Zhou et al. (2017)
	HSBeta396F-HSBeta742R	[Amx]	–	[Y]	340	Short	qPCR for [Amx]	Wang et al. (2012a)
hzsC	hzsC496f_An-hzsC862r	[Non-Sca]	–	[Y]	440	Medium	qPCR and clone library for [Non-Sca]	Zhou et al. (2017)
	hzsC496f_Sc-hzsC862r	[Sca]	–	[Y]	440	Medium	qPCR and clone library for [Sca]	Zhou et al. (2017)
	hzsC496f_An&Sc 4:1- hzsC862r	[Amx]	–	[Y]	440	Medium	qPCR and clone library for [Amx]	Zhou et al. (2017)
	hzsC745f-hzsC862r	[Amx]	–	[Y]	140	Short	qPCR and clone library for [Amx]	Zhou et al. (2017)
ccsA	ccsA376f-ccsA668r	[Amx]	–	[Y]	310	Short	qPCR and clone library for [Amx]	Zhou et al. (2017))
ccsB	ccsB1116f-ccsB1589r	[Amx]	–	[N]	490	Medium	clone library for [Amx]	Zhou et al. (2017)
	ccsB581f-ccsB1589r	[Amx]	–	[N]	1030	Long	1st round nest PCR for [Amx]s	Zhou et al. (2017)

Product size range: short <400 bp, 400 bp ≤ medium ≤800 bp, long >800 bp. The practical information is further summarized from Table S1 and Table S2

[S] specificity, *[Y]* suitable for qPCR, *[N]* unsuitable for qPCR, *[Amx]* for anammox bacteria, *[Sca]* for *Scalindua*, *[Non-Sca]* for non-*Scalindua*, *[Kue]* for *Kuenenia*, *[Jet]* for *Jettenia*

diverse community and less PCR inhibitory chemicals, such as humid acids (Schrader et al. 2012). Many researches adopted 16S rRNA and functional genes to quantify *Scalindua* with good performance on study of biogeochemistry processes and activities (Bale et al. 2014; Lam et al. 2007; Pitcher et al. 2011).

For non-saline environment category, including a wide range of soils, WWTPs, reactors, groundwater, freshwater, and freshwater sediments, and intertidal or brackish environmental category, estuary and mangrove sediments, selection of primers shall consider (i) interests on function or phylogeny, (ii) quantification or qualification, and (iii) pre-assessment of bacterial community as complex or simple, low, or high anammox biomass and marine or terrestrial origin, to achieve better outcomes (Tables S1 and S2). For example, WWTPs and reactors are long-time operated to enrich anammox biomass, thus, less diverse community and simple environmental background will lead to less non-specific PCR amplification, and most of the suggested primer pairs could offer good outcome (Han et al. 2017; Han and Gu 2015). For samples with potential complex background, such as soil, estuary and mangrove sediments, primers with high degeneracy rate could potentially cause over-estimation of anammox bacterial community and yield more non-specific PCR products. Nested PCR procedures are suggested to retrieve anammox bacteria from these environments to improve specificity. Additionally, functional gene primers, specifically targeting functional proteins, could also enhance specificity and avoid over-estimation (Table 3).

Summary and future perspectives

Anammox bacteria are attracting increasingly attention in both research and applications on nitrogen cycle and transformation. The first discovery and applications of anammox bacteria not only fill the missing gap of microbial participants on nitrogen cycle but also improve industrial implementation of nitrogen removal in WWTPs comparing to conventional technologies (Meng et al. 2017). DNA-based molecular detection and quantification methods for anammox bacteria specifically have been applied in many studies in environmental engineering, microbial ecology, and biogeochemistry, and PCR is a basic tool and accessible widely for studying community diversity, abundance, and activity. The evaluation and a summary of performance of 16S rRNA and functional gene PCR primers in practical and in in silico tests in this article serve as a practical guide for researchers to select PCR primers according to specific samples type and/or the group of anammox bacteria of interest for detection and quantification. The effective implementation of PCR primers and PCR techniques will facilitate successful execution on detection of anammox bacteria and therefore contribution to further information on their ecology, diversity, and functions in different ecosystems.

As the rapid development of high throughput sequencing in the recent years, sequencing millions of PCR amplicons is practically feasible and even more suitable for comparing community structures among different samples (Caporaso et al. 2012). To include more phylogenetic information into community structure results by sequence analysis, it will be better to use 16S rRNA gene-based primers to study anammox bacteria, comparing to functional genes. Meanwhile, it will also be easier for users to adapt to currently most frequently used pipelines and softwares, such as QIIME and mothur, to get sequence analyzed (Caporaso et al. 2010; Schloss et al. 2009). The 16S-tag is generated from merging two pair-end reads (PE reads) according to the most currently adopted Illumina platform (Wang et al. 2012b). Since the read length of mainstream version of PE reads is usually 150 bp, the merged 16S-tag will be less than 300 bp. The 16S rRNA gene primer, A438f-A684r, with the targeted PCR product length of 250 bp, is suitable for this purpose with good coverage and specificity.

A general guideline on the selection of most appropriate PCR primers for different situations could be achieved from currently ten major environmental types and such information can serve as basic background information. Since more and more research reports on the practical applications of PCR primers in different types of environmental samples are becoming available, a more detailed, up-to-date, and comprehensive summary of the information on PCR primer performance and PCR outcome is necessary to the research community, especially the new ones into this research field because different choices can be made easily and the results may be biased without in-depth knowledge of the information available comprehensively.

Funding This study was funded by the Key Project of Department of Education of Guangdong Provice (No. 2017KZDXM071) (ML), the Science and Technology Innovation Committee Shenzhen City (No. JCYJ20170818091727570) (ML); General Research Fund (Grant No. 701913) (J-DG); and Theme-based Research Scheme Project (Grant No. T21-711/16-R) (J-DG).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ali M, Oshiki M, Awata T, Isobe K, Kimura Z, Yoshikawa H, Hira D, Kindaichi T, Satoh H, Fujii T (2015) Physiological characterization

of anaerobic ammonium oxidizing bacterium '*Candidatus* Jettenia caeni'. Environ Microbiol 17(6):2172–2189

Amano T, Yoshinaga I, Okada K, Yamagishi T, Ueda S, Obuchi A, Sako Y, Suwa Y (2007) Detection of anammox activity and diversity of anammox bacteria-related 16S rRNA genes in coastal marine sediment in Japan. Microbes Environ 22(3):232–242. https://doi.org/10.1264/Jsme2.22.232

Amano T, Yoshinaga I, Yamagishi T, Van Thuoc C, Ueda S, Kato K, Sako Y, Suwa Y (2011) Contribution of anammox bacteria to benthic nitrogen cycling in a mangrove forest and shrimp ponds, Haiphong, Vietnam. Microbes Environ 26(1):1–6. https://doi.org/10.1264/jsme2.ME10150

Araujo J, Campos A, Correa M, Silva E, Matte M, Matte G, Von Sperling M, Chernicharo C (2011) Anammox bacteria enrichment and characterization from municipal activated sludge. Water Sci Technol 64(7):1428–1434. https://doi.org/10.2166/wst.2011.632

Arrigo KR (2005) Marine microorganisms and global nutrient cycles. Nature 437(7057):349–355

Bale NJ, Villanueva L, Fan H, Stal LJ, Hopmans EC, Schouten S, Sinninghe Damsté JS (2014) Occurrence and activity of anammox bacteria in surface sediments of the southern North Sea. FEMS Microbiol Ecol 89(1):99–110. https://doi.org/10.1111/1574-6941.12338

Borin S, Mapelli F, Rolli E, Song B, Tobias C, Schmid MC, De Lange GJ, Reichart GJ, Schouten S, Jetten M (2013) Anammox bacterial populations in deep marine hypersaline gradient systems. Extremophiles 17(2):289–299. https://doi.org/10.1007/s00792-013-0516-x

Byrne N, Strous M, Crepeau V, Kartal B, Birrien J-L, Schmid M, Lesongeur F, Schouten S, Jaeschke A, Jetten M, Prieur D, Godfroy A (2009) Presence and activity of anaerobic ammonium-oxidizing bacteria at deep-sea hydrothermal vents. ISME J 3(1):117–123. https://doi.org/10.1038/ismej.2008.72

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6(8):1621–1624. https://doi.org/10.1038/ismej.2012.8

Chan HW, Gu J-D (2016) Anammox bacteria detected in fish intestinal tract systems. Appl Environ Biotechnol 1:13–18. https://doi.org/10.18063/AEB.2016.01.010

Cole JR, Chai B, Farris RJ, Wang Q, Kulam S, McGarrell DM, Garrity GM, Tiedje JM (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res 33(suppl 1):D294–D296

Dale OR, Tobias CR, Song B (2009) Biogeographical distribution of diverse anaerobic ammonium oxidizing (anammox) bacteria in Cape Fear River Estuary. Environ Microbiol 11(5):1194–1207

Dalsgaard T, Stewart FJ, Thamdrup B, De Brabandere L, Revsbech NP, Ulloa O, Canfield DE, DeLong EF (2014) Oxygen at nanomolar levels reversibly suppresses process rates and gene expression in anammox and denitrification in the oxygen minimum zone off northern Chile. MBio 5(6):e01966–e01914

Dalsgaard T, Thamdrup B, Canfield DE (2005) Anaerobic ammonium oxidation (anammox) in the marine environment. Res Microbiol 156(4):457–464. https://doi.org/10.1016/j.resmic.2005.01.011

Damsté JSS, Strous M, Rijpstra WIC, Hopmans EC, Geenevasen JA, van Duin AC, van Niftrik LA, Jetten MS (2002) Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature 419(6908):708–712

Dang H, Chen R, Wang L, Guo L, Chen P, Tang Z, Tian F, Li S, Klotz MG (2010) Environmental factors shape sediment anammox bacterial communities in hypernutrified Jiaozhou Bay, China. Appl Environ Microbiol 76(21):7036–7047

Dang H, Zhou H, Zhang Z, Yu Z, Hua E, Liu X, Jiao N (2013) Molecular detection of *Candidatus* Scalindua pacifica and environmental responses of sediment anammox bacterial Community in the Bohai Sea, China. PLoS One 8(4):e61330

Den Camp HO, Kartal B, Guven D, Van Niftrik L, Haaijer S, Van Der Star W, Van De Pas-Schoonen K, Cabezas A, Ying Z, Schmid M (2006) Global impact and application of the anaerobic ammonium-oxidizing (anammox) bacteria. Biochem Soc Trans 34(1):174–178

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72(7):5069–5072

Devol AH (2003) Nitrogen cycle: solution to a marine mystery. Nature 422(6932):575–576

Erler DV, Eyre BD, Davison L (2008) The contribution of anammox and denitrification to sediment N_2 production in a surface flow constructed wetland. Environ Sci Technol 42(24):9144–9150

Fuchsman CA, Staley JT, Oakley BB, Kirkpatrick JB, Murray JW (2012) Free-living and aggregate-associated *Planctomycetes* in the Black Sea. FEMS Microbiol Ecol 80(2):402–416

Hamersley MR, Lavik G, Woebken D, Rattray JE, Lam P, Hopmans EC, Damsté JSS, Krüger S, Graco M, Gutiérrez D (2007) Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. Limnol Oceanogr 52(3):923–933. https://doi.org/10.4319/lo.2007.52.3.0923

Han P, Gu J-D (2015) Further analysis of anammox bacterial community structures along an anthropogenic nitrogen-input gradient from the riparian sediments of the Pearl River Delta to the deep-ocean sediments of the South China Sea. Geomicrobiol J 32:789–798. https://doi.org/10.1080/01490451.2014.1001502

Han P, Klümper U, Wong A, Li M, Lin J-G, Quan Z, Denecke M, Gu J-D (2017) Assessment of molecular detection of anaerobic ammonium-oxidizing (anammox) bacteria in different environmental samples using PCR primers based on 16S rRNA and functional genes. Appl Microbiol Biotechnol 101(20):7689–7702. https://doi.org/10.1007/s00253-017-8502-3

Harhangi HR, Le Roy M, van Alen T, B-l H, Groen J, Kartal B, Tringe SG, Quan Z-X, Jetten MS, den Camp HJO (2012) Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. Appl Environ Microbiol 78(3):752–758

Hirsch MD, Long ZT, Song B (2011) Anammox bacterial diversity in various aquatic ecosystems based on the detection of hydrazine oxidase genes (*hzoA*/*hzoB*). Microb Ecol 61(2):264–276. https://doi.org/10.1007/s00248-010-9743-1

Hong Y-G, Li M, Cao H, Gu J-D (2011) Residence of habitat-specific anammox bacteria in the deep-sea subsurface sediments of the South China Sea: analyses of marker gene abundance with physical chemical parameters. Microb Ecol 62(1):36–47

Hu B, Shen L, Xu X, Zheng P (2011) Anaerobic ammonium oxidation (anammox) in different natural ecosystems. Biochem Soc Trans 39(6):1811–1816

Humbert S, Tarnawski S, Fromin N, Mallet M-P, Aragno M, Zopfi J (2010) Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. ISME J 4(3):450–454. https://doi.org/10.1038/ismej.2009.125

Humbert S, Zopfi J, Tarnawski SE (2012) Abundance of anammox bacteria in different wetland soils. Environ Microbiol Rep 4(5):484–490. https://doi.org/10.1111/j.1758-2229.2012.00347.x

Jaeschke A, Rooks C, Trimmer M, Nicholls JC, Hopmans EC, Schouten S, Damsté JSS (2009) Comparison of ladderane phospholipid and core lipids as indicators for anaerobic ammonium oxidation (anammox) in marine sediments. Geochim Cosmochim Acta 73(7):2077–2088

Jensen MM, Kuypers MM, Gaute L, Thamdrup B (2008) Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea. Limnol Oceanogr 53(1):23–36

Jensen MM, Lam P, Revsbech NP, Nagel B, Gaye B, Jetten MS, Kuypers MM (2011) Intensive nitrogen loss over the Omani Shelf due to anammox coupled with dissimilatory nitrite reduction to ammonium. ISME J 5(10):1660–1670

Jetten M, Cirpus I, Kartal B, van Niftrik L, Van De Pas-Schoonen K, Sliekers O, Haaijer S, Van der Star W, Schmid M, van de Vossenberg J (2005) 1994–2004: 10 years of research on the anaerobic oxidation of ammonium. Biochem Soc Trans 33(1):119–123

Jetten MS, Logemann S, Muyzer G, Robertson LA, de Vries S, van Loosdrecht MC, Kuenen JG (1997) Novel principles in the microbial conversion of nitrogen compounds. Antonie Van Leeuwenhoek 71(1–2):75–93

Jetten MS, Schmid M, Schmidt I, Wubben M, Van Dongen U, Abma W, Sliekers O, Revsbech NP, Beaumont HJ, Ottosen L (2002) Improved nitrogen removal by application of new nitrogen-cycle bacteria. Rev Environ Sci Biotechnol 1(1):51–63

Jetten MS, Wagner M, Fuerst J, van Loosdrecht M, Kuenen G, Strous M (2001) Microbiology and application of the anaerobic ammonium oxidation ('anammox') process. Curr Opin Biotechnol 12(3):283–288

Jetten MSM, Strous M, van de Pas-Schoonen KT, Schalk J, van Dongen U, van de Graaf AA, Logemann S, Muyzer G, van Loosdrecht MCM, Kuenen JG (1998) The anaerobic oxidation of ammonium. FEMS Microbiol Rev 22(5):421–437. https://doi.org/10.1016/s0168-6445(98)00023-0

Junier P, Molina V, Dorador C, Hadas O, Kim O-S, Junier T, Witzel K-P, Imhoff JF (2010) Phylogenetic and functional marker genes to study ammonia-oxidizing microorganisms (AOM) in the environment. Appl Microbiol Biotechnol 85(3):425–440

Juretschko S, Timmermann G, Schmid M, Schleifer KH, Pommerening-Roser A, Koops HP, Wagner M (1998) Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus* mobilis and *Nitrospira*-like bacteria as dominant populations. Appl Environ Microbiol 64(8):3042–3051

Kartal B, Geerts W, Jetten MS (2011a) Cultivation, detection, and ecophysiology of anaerobic ammonium-oxidizing bacteria. Methods Enzymol 486(Part A):89–108

Kartal B, Maalcke WJ, de Almeida NM, Cirpus I, Gloerich J, Geerts W, den Camp HJO, Harhangi HR, Janssen-Megens EM, Francoijs K-J (2011b) Molecular mechanism of anaerobic ammonium oxidation. Nature 479(7371):127–130

Kartal B, Rattray J, van Niftrik LA, van de Vossenberg J, Schmid MC, Webb RI, Schouten S, Fuerst JA, Damsté JS, Jetten MS (2007) *Candidatus* "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol 30(1):39–49

Kartal B, Van Niftrik L, Rattray J, Van De Vossenberg JL, Schmid MC, Damsté JS, Jetten MS, Strous M (2008) *Candidatus* 'Brocadia fulgida': an autofluorescent anaerobic ammonium oxidizing bacterium. FEMS Microbiol Ecol 63(1):46–55

Kjeldsen KU, Tang L, Jørgensen MG, Ingvorsen K (2009) Enumeration and identification of dominant types of sulfate-reducing bacteria in pulp from a paper-recycling plant: a multiphasic approach. FEMS Microbiol Ecol 69(3):481–494

Kuypers MM, Sliekers AO, Lavik G, Schmid M, Jørgensen BB, Kuenen JG, Damsté JSS, Strous M, Jetten MS (2003) Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422(6932):608–611

Kuypers MMM, Lavik G, Woebken D, Schmid M, Fuchs BM, Amann R, Jorgensen BB, Jetten MSM (2005) Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc Natl Acad Sci U S A 102(18):6478–6483. https://doi.org/10.1073/pnas.0502088102

Lam P, Jensen MM, Lavik G, McGinnis DF, Müller B, Schubert CJ, Amann R, Thamdrup B, Kuypers MM (2007) Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proc Natl Acad Sci U S A 104(17):7104–7109

Lam P, Lavik G, Jensen MM, van de Vossenberg J, Schmid M, Woebken D, Gutiérrez D, Amann R, Jetten MS, Kuypers MM (2009) Revising the nitrogen cycle in the Peruvian oxygen minimum zone. Proc Natl Acad Sci U S A 106(12):4752–4757

Lee K-H, Wang Y-F, Wang Y, Gu J-D, Jiao JJ (2016) Abundance and diversity of aerobic/anaerobic ammonia/ammonium-oxidizing microorganisms in an ammonium-rich aquitard in the Pearl River Delta of South China. Microb Ecol 1–11. https://doi.org/10.1007/s00248-016-0815-8

Li H, Chen S, Mu B-Z, Gu J-D (2010a) Molecular detection of anaerobic ammonium-oxidizing (anammox) bacteria in high-temperature petroleum reservoirs. Microb Ecol 60(4):771–783. https://doi.org/10.1007/s00248-010-9733-3

Li M, Cao H, Hong Y-G, Gu J-D (2011a) Seasonal dynamics of anammox bacteria in estuarial sediment of the Mai Po Nature Reserve revealed by analyzing the 16S rRNA and hydrazine oxidoreductase (*hzo*) genes. Microbes Environ 26(1):15–22. https://doi.org/10.1264/jsme2.ME10131

Li M, Cao H, Hong Y, Gu J-D (2013) Using the variation of anammox bacteria community structures as a bio-indicator for anthropogenic/terrestrial nitrogen inputs in the Pearl River Delta (PRD). Appl Microbiol Biotechnol 97(22):9875–9883

Li M, Ford T, Li X, Gu J-D (2011b) Cytochrome cd_1-containing nitrite reductase encoding gene *nirS* as a new functional biomarker for detection of anaerobic ammonium oxidizing (anammox) bacteria. Environ Sci Technol 45(8):3547–3553

Li M, Gu J-D (2011) Advances in methods for detection of anaerobic ammonium oxidizing (anammox) bacteria. Appl Microbiol Biotechnol 90(4):1241–1252

Li M, Gu J-D (2016a) The diversity and distribution of anammox bacteria in the marine aquaculture zones. Appl Microbiol Biotechnol 100:8943-8953. https://doi.org/10.1007/s00253-016-7690-6

Li M, Gu J-D (2016b) Molecular evidence of the existence of anaerobic ammonia oxidation bacteria in the gut of polychaete (*Neanthes glandicincta*). Appl Environ Biotechnol 1(1):19-29. https://doi.org/10.18063/AEB.2016.01.011

Li M, Hong Y-G, Cao H-L, Gu J-D (2011c) Mangrove trees affect the community structure and distribution of anammox bacteria at an anthropogenic-polluted mangrove in the Pearl River Delta reflected by 16S rRNA and hydrazine oxidoreductase (HZO) encoding gene analyses. Ecotoxicology 20(8):1780–1790

Li M, Hong Y, Klotz MG, Gu J-D (2010b) A comparison of primer sets for detecting 16S rRNA and hydrazine oxidoreductase genes of anaerobic ammonium-oxidizing bacteria in marine sediments. Appl Microbiol Biotechnol 86(2):781–790

Ludwig W, Kirchhof G, Klugbauer N, Weizenegger M, Betzl D, Ehrmann M, Hertel C, Jilg S, Tatzel R, Zitzelsberger H (1992) Complete 23S ribosomal RNA sequences of Gram-positive bacteria with a low DNA G+ C content. Syst Appl Microbiol 15(4):487–501. https://doi.org/10.1016/S0723-2020(11)80107-4

Meng H, Yang Y-C, Lin J-G, Denecke M, Gu J-D (2017) Occurrence of anammox bacteria in a traditional full-scale wastewater treatment plant and successful inoculation for new establishment. Int Biodeterior Biodegrad 120:224–231

Mohamed NM, Saito K, Tal Y, Hill RT (2010) Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. ISME J 4(1):38–48

Moore TA, Xing Y, Lazenby B, Lynch MDJ, Schiff S, Robertson WD, Timlin R, Lanza S, Ryan MC, Aravena R, Fortin D, Clark ID, Neufeld JD (2011) Prevalence of anaerobic ammonium-oxidizing bacteria in contaminated groundwater. Environ Sci Technol 45(17):7217–7225. https://doi.org/10.1021/es201243t

Mulder A, Graaf A, Robertson L, Kuenen J (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol 16(3):177–184

Narita Y, Zhang L, Kimura Z-i, Ali M, Fujii T, Okabe S (2017) Enrichment and physiological characterization of an anaerobic ammonium-oxidizing bacterium '*Candidatus* Brocadia sapporoensis'. Syst Appl Microbiol 40(7):448–457. https://doi.org/10.1016/j.syapm.2017.07.004

Neef A, Amann R, Schlesner H, Schleifer K-H (1998) Monitoring a widespread bacterial group: in situ detection of planctomycetes with 16S rRNA-targeted probes. Microbiology 144(12):3257–3266. https://doi.org/10.1099/00221287-144-12-3257

Ni B-J, Hu B-L, Fang F, Xie W-M, Kartal B, Liu X-W, Sheng G-P, Jetten M, Zheng P, Yu H-Q (2010) Microbial and physicochemical characteristics of compact anaerobic ammonium-oxidizing granules in an upflow anaerobic sludge blanket reactor. Appl Environ Microbiol 76(8):2652–2656. https://doi.org/10.1128/AEM.02271-09

Nikolaev YA, Kozlov M, Kevbrina M, Dorofeev A, Pimenov N, Kallistova AY, Grachev V, Kazakova E, Zharkov A, Kuznetsov B (2015) *Candidatus* "Jettenia moscovienalis" sp. nov., a new species of bacteria carrying out anaerobic ammonium oxidation. Microbiology 84(2):256–262. https://doi.org/10.1134/S0026261715020101

Oshiki M, Satoh H, Okabe S (2016) Ecology and physiology of anaerobic ammonium oxidizing bacteria. Environ Microbiol 18:2784–2796

Oshiki M, Shimokawa M, Fujii N, Satoh H, Okabe S (2011) Physiological characteristics of the anaerobic ammonium-oxidizing bacterium '*Candidatus* Brocadia sinica. Microbiology 157(6):1706–1713. https://doi.org/10.1099/mic.0.048595-0

Park H, Rosenthal A, Jezek R, Ramalingam K, Fillos J, Chandran K (2010) Impact of inocula and growth mode on the molecular microbial ecology of anaerobic ammonia oxidation (anammox) bioreactor communities. Water Res 44(17):5005–5013

Penton CR, Devol AH, Tiedje JM (2006) Molecular evidence for the broad distribution of anaerobic ammonium-oxidizing bacteria in freshwater and marine sediments. Appl Environ Microbiol 72(10): 6829–6832

Pitcher A, Villanueva L, Hopmans EC, Schouten S, Reichart G-J, Damsté JSS (2011) Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone. ISME J 5(12):1896–1904. https://doi.org/10.1038/ismej.2011.60

Quan ZX, Rhee SK, Zuo JE, Yang Y, Bae JW, Park JR, Lee ST, Park YH (2008) Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. Environ Microbiol 10(11):3130–3139

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Gloeckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(D1):D590–D596. https://doi.org/10.1093/nar/gks1219

Risgaard-Petersen N, Nielsen LP, Rysgaard S, Dalsgaard T, Meyer RL (2003) Application of the isotope pairing technique in sediments where anammox and denitrification coexist. Limnol Oceanogr 1(1):63–73

Rothrock Jr MJ, Vanotti MB, Szögi AA, Gonzalez MCG, Fujii T (2011) Long-term preservation of anammox bacteria. Appl Microbiol Biotechnol 92(1):147–157

Russ L, Kartal B, Op Den Camp HJM, Sollai M, Le Bruchec J, Caprais J-C, Godfroy A, Sinninghe Damsté JS, Jetten MSM (2013) Presence and diversity of anammox bacteria in cold hydrocarbon-rich seeps and hydrothermal vent sediments of the Guaymas Basin. Front Microbiol 4. https://doi.org/10.3389/fmicb.2013.00219

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23):7537–7541. https://doi.org/10.1128/AEM.01541-09

Schmid M, Twachtmann U, Klein M, Strous M, Juretschko S, Jetten M, Metzger JW, Schleifer K-H, Wagner M (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. Syst Appl Microbiol 23(1):93–106

Schmid M, Walsh K, Webb R, Rijpstra WI, van de Pas-Schoonen K, Verbruggen MJ, Hill T, Moffett B, Fuerst J, Schouten S (2003) *Candidatus* "Scalindua brodae", sp. nov., *Candidatus* "Scalindua wagneri", sp. nov., two new species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol 26(4):529–538

Schmid MC, Hooper AB, Klotz MG, Woebken D, Lam P, Kuypers MM, Pommerening-Roeser A, Op Den Camp HJ, Jetten MS (2008) Environmental detection of octahaem cytochrome *c* hydroxylamine/hydrazine oxidoreductase genes of aerobic and anaerobic ammonium-oxidizing bacteria. Environ Microbiol 10(11): 3140–3149

Schmid MC, Risgaard-Petersen N, Van De Vossenberg J, Kuypers MM, Lavik G, Petersen J, Hulth S, Thamdrup B, Canfield D, Dalsgaard T (2007) Anaerobic ammonium-oxidizing bacteria in marine environments: widespread occurrence but low diversity. Environ Microbiol 9(6):1476–1484

Schrader C, Schielke A, Ellerbroek L, Johne R (2012) PCR inhibitors—occurrence, properties and removal. J Appl Microbiol 113(5):1014–1026

Schubert CJ, Durisch-Kaiser E, Wehrli B, Thamdrup B, Lam P, Kuypers MM (2006) Anaerobic ammonium oxidation in a tropical freshwater system (Lake Tanganyika). Environ Microbiol 8(10):1857–1863

Sinninghe Damsté JS, Rijpstra WIC, Geenevasen JA, Strous M, Jetten MS (2005) Structural identification of ladderane and other membrane lipids of planctomycetes capable of anaerobic ammonium oxidation (anammox). FEBS J 272(16):4270–4283

Sonthiphand P, Cejudo E, Schiff SL, Neufeld JD (2013) Wastewater effluent impacts ammonia-oxidizing prokaryotes of the Grand River, Canada. Appl Environ Microbiol 79(23):7454–7465. https://doi.org/10.1128/aem.02202-13

Sonthiphand P, Hall MW, Neufeld JD (2014) Biogeography of anaerobic ammonia-oxidizing (anammox) bacteria. Front Microbiol 5. https://doi.org/10.3389/fmicb.2014.00399

Speth DR, Lagkouvardos I, Wang Y, Qian P-Y, Dutilh BE, Jetten MS (2017) Draft genome of *Scalindua rubra*, obtained from the interface above the discovery deep brine in the Red Sea, sheds light on potential salt adaptation strategies in anammox bacteria. Microb Ecol 74(1):1–5. https://doi.org/10.1007/s00248-017-0929-7

Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS (1999) Missing lithotroph identified as new planctomycete. Nature 400(6743): 446–449

Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, Taylor MW, Horn M, Daims H, Bartol-Mavel D, Wincker P (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440(7085):790–794

Thamdrup B, Dalsgaard T, Jensen MM, Ulloa O, Farías L, Escribano R (2006) Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile. Limnol Oceanogr 51(5):2145–2156

Tsushima I, Kindaichi T, Okabe S (2007) Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR. Water Res 41(4):785–794. https://doi.org/10.1016/j.watres.2006.11.024

van de Vossenberg J, Rattray JE, Geerts W, Kartal B, van Niftrik L, van Donselaar EG, Damste JSS, Strous M, Jetten MSM (2008) Enrichment and characterization of marine anammox bacteria associated with global nitrogen gas production. Environ Microbiol 10(11):3120–3129. https://doi.org/10.1111/j.1462-2920.2008.01643.x

Wang J, Gu J-D (2013) Dominance of Candidatus Scalindua species in anammox community revealed in soils with different duration of rice paddy cultivation in Northeast China. Appl Microbiol Biotechnol 97(4):1785–1798. https://doi.org/10.1007/s00253-012-4036-x

Wang S, Zhu G, Peng Y, Jetten MS, Yin C (2012a) Anammox bacterial abundance, activity, and contribution in riparian sediments of the Pearl River estuary. Environ Sci Technol 46(16):8834–8842. https://doi.org/10.1021/es3017446

Wang Y, Sheng H-F, He Y, Wu J-Y, Jiang Y-X, Tam NF-Y, Zhou H-W (2012b) Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags. Appl Environ Microbiol 78(23):8264–8271. https://doi.org/10.1128/aem.01821-12

Wang Y, Zhu G, Harhangi HR, Zhu B, Jetten MS, Yin C, Op den Camp HJ (2012c) Co-occurrence and distribution of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in a paddy soil. FEMS Microbiol Lett 336(2):79–88

Ward B, Devol A, Rich J, Chang B, Bulow S, Naik H, Pratihary A, Jayakumar A (2009) Denitrification as the dominant nitrogen loss process in the Arabian Sea. Nature 461(7260):78–81

Woebken D, Fuchs BM, Kuypers MM, Amann R (2007) Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. Appl Environ Microbiol 73(14):4648–4657

Woebken D, Lam P, Kuypers MM, Naqvi SW, Kartal B, Strous M, Jetten MS, Fuchs BM, Amann R (2008) A microdiversity study of anammox bacteria reveals a novel *Candidatus* Scalindua phylotype in marine oxygen minimum zones. Environ Microbiol 10(11):3106–3119. https://doi.org/10.1111/j.1462-2920.2008.01640.x

Yoshinaga I, Amano T, Yamagishi T, Okada K, Ueda S, Sako Y, Suwa Y (2011) Distribution and diversity of anaerobic ammonium oxidation (anammox) bacteria in the sediment of a eutrophic freshwater lake, Lake Kitaura, Japan. Microbes Environ 26(3):189–197. https://doi.org/10.1264/jsme2.ME10184

Zhou Z, Chen J, Meng H, Dvornyk V, Gu J-D (2017) New PCR primers targeting hydrazine synthase and cytochrome *c* biogenesis proteins in anammox bacteria. Appl Microbiol Biotechnol 101(3):1267–1287. https://doi.org/10.1007/s00253-016-8013-7

Zhu G, Wang S, Wang W, Wang Y, Zhou L, Jiang B, den Camp HJO, Risgaard-Petersen N, Schwark L, Peng Y (2013) Hotspots of anaerobic ammonium oxidation at land-freshwater interfaces. Nat Geosci 6(2):103–107

Zhu G, Wang S, Wang Y, Wang C, Risgaard-Petersen N, Jetten MS, Yin C (2011) Anaerobic ammonia oxidation in a fertilized paddy soil. ISME J 5(12):1905–1912