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Review 综 述

Environmental adaptation basis and ecological function of deep-sea ammonia-oxidation archaea

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Abstract: Ammonia-oxidizing archaea is one of the most abundant microbial groups driving the nitrogen cycling on Earth. Especially, in the deep sea, its relative abundance can reach 20%–40% of the total prokaryotes. However, the lack of deep-sea ammonia-oxidizing archaea isolates hindered our comprehensive understanding of their physiology and ecological contribution. In this paper, we analyzed the relationship between characteristics of deep-sea environments and the adaptability of microorganisms, focusing on the potential survival strategies and metabolic preferences of deep-sea ammonia-oxidizing archaea. This knowledge will assist us to design suitable cultivation techniques on them. Moreover, the ammonia-oxidizing archaea habituated in the deep sea apparently distanced from those in soil or surface ocean, in terms of phylogeny as well as physiology. Therefore, we are about to reconsider the global oceanic nitrogen budget estimation.

Keywords: ammonia-oxidizing archaea, deep-sea, environment adaptation, geochemical cycle

The discovery of ammonia-oxidizing archaea (AOA) is an important milestone[1] that changed the understanding of archaea and the nitrogen cycle[2–4]. AOA uses energy produced by aerobic ammonia oxidation to synthesize organic matter, and its total stoichiometric amount of ammonia oxidation is indistinguishable from ammonia-oxidizing bacteria (AOB)[5]. Based on phylogenetics analysis, it is likely that AOA originated during the Great Oxygenation Event around 2.3 billion years ago. They evolved from anaerobic terrestrial non-AOA Thaumarchaeota, and their habitats have steadily expanded from land to the shallow and deep sea[6]. Lateral gene transfer (LGT) from bacteria to

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archaea has occurred continuously throughout the evolutionary history of AOA from land to deep sea[6], which may be one reason for their high diversity. The latest edition of the *Bergey's Manual of Systematics of Archaea and Bacteria* classifies AOA into four orders and nine genera[7]. AOA assigned to 18 branches of the genus level in addition to four main branches of the order level [NP (*Nitrosopumilales*), NT (*Ca.* Nitrosotaleales), NS (*Nitrososphaerales*), and NC (*Ca. Nitrosocaldales*)][8], based on the amino monooxygenase subunit A (*amoA*) gene sequences of archaea. At present, AOA have been isolated or enriched from natural and artificial ecosystems such as shallow seawater[9–11], shallow marine sediments[9,12], terrestrial hot springs[13–16], frozen soil[17], garden soil[18], agricultural soil[19–24], aquariums[1,25], and sewage treatment plants[26–28]. However, the cultured AOA mostly belong to a few evolutionary branches at the genus level, such as *Nitrososphaera* (NS-α), *Ca*. Nitrosocosmicus (NS-ζ), *Ca*. Nitrosocaldus (NC-α), *Ca.* Nitrosoarchaeum (NP-γ), *Nitrosopumilus* (NP-γ), *Ca*. Nitrosotenuis (NP-η), *Ca*. Nitrosopelagicus (NP-ε) and *Ca*. Nitrosotalea (NT-α) and these cultured groups are not the most abundant in the environment[8]. Many uncultured groups have high abundance in the environment, such as NS-γ, NS-δ, NP-α[8]. The ecological functions of these uncultured AOA in the environment are worth to be explored.

AOA have achieved great ecological success. Apart from ubiquitous on Earth, AOA also occupy a high number of cells in various environments. AOA account for 1%–5% of all prokaryotes in terrestrial ecosystems[29–30]. Their abundance is much higher than AOB in acidic or alkaline soils[31–32]. About 1×10^{28} AOA cells in the ocean account for 20%–40% of all prokaryotes in the seawater[33–34], as a dominant microbial taxon in deep seawater. The distribution of AOA in the ocean has a spatial distribution specificity. For example, In Challenger Deep of the Mariana Trench, the abundance of AOA is extremely low in seawater at depths less than 100 m. However, they are the most abundant microorganisms in the mesopelagic (200–1000 m), bathyal (1000–4000 m), and abyssal (4000–6000 m) zone, accounting for up to 45%–80% of the total prokaryotes (inferring to 16S rRNA gene abundances, similarly hereinafter). Even in the hadal zone (6000–11000 m), the relative abundance of AOA in prokaryotes can still reach 10%–30%[34]. At depths deeper than 200 m, AOA nearly occupy all archaeal biomass, accounting for as much as 96.0%–99.6% of the relative abundance of archaeal 16S rRNA gene abundance[34]. The cell number of prokaryotes in seawater decreases with increasing depth, but the cell number of AOA shows a trend of rising then falling, with the highest one in the 100–1000 m depth range[33–34]. In surface sediments, AOA accounts for up to 30% of the prokaryotes, but its abundance decreases from the seafloor down[35].

Marine AOA belong to the order *Nitrosopumilales* (Group I.1a, MG-I, NP). Based on the *amoA* gene sequence, *Nitrosopumilales* is divided into eight branches as NP-α, β, γ, δ, ε, ζ, η, θ at the genus level[8], and their distribution is dependent on the depth of the seawater (Figure 1). For instance, NP-α (dominant in the mesopelagic, bathyal, abyssal and hadal zone), NP-γ (lower abundance in all depths and relatively higher abundance in the hadal zone), NP-δ (present in surface waters, low abundance), and NP-ε (dominant in surface water)[8,36]. At present, most of the cultured marine AOA belong to NP-γ, although they inhabit seawater at depths of 50–10000 m, their relative abundance is very low (Figure 1). There is a lack of cultures that are dominant in the deep sea (Figure 1, Table 1), and their metabolic characteristics at the cellular level and the physiological basis for adaptation to the extreme environment of the deep sea have not been confirmed under laboratory conditions.

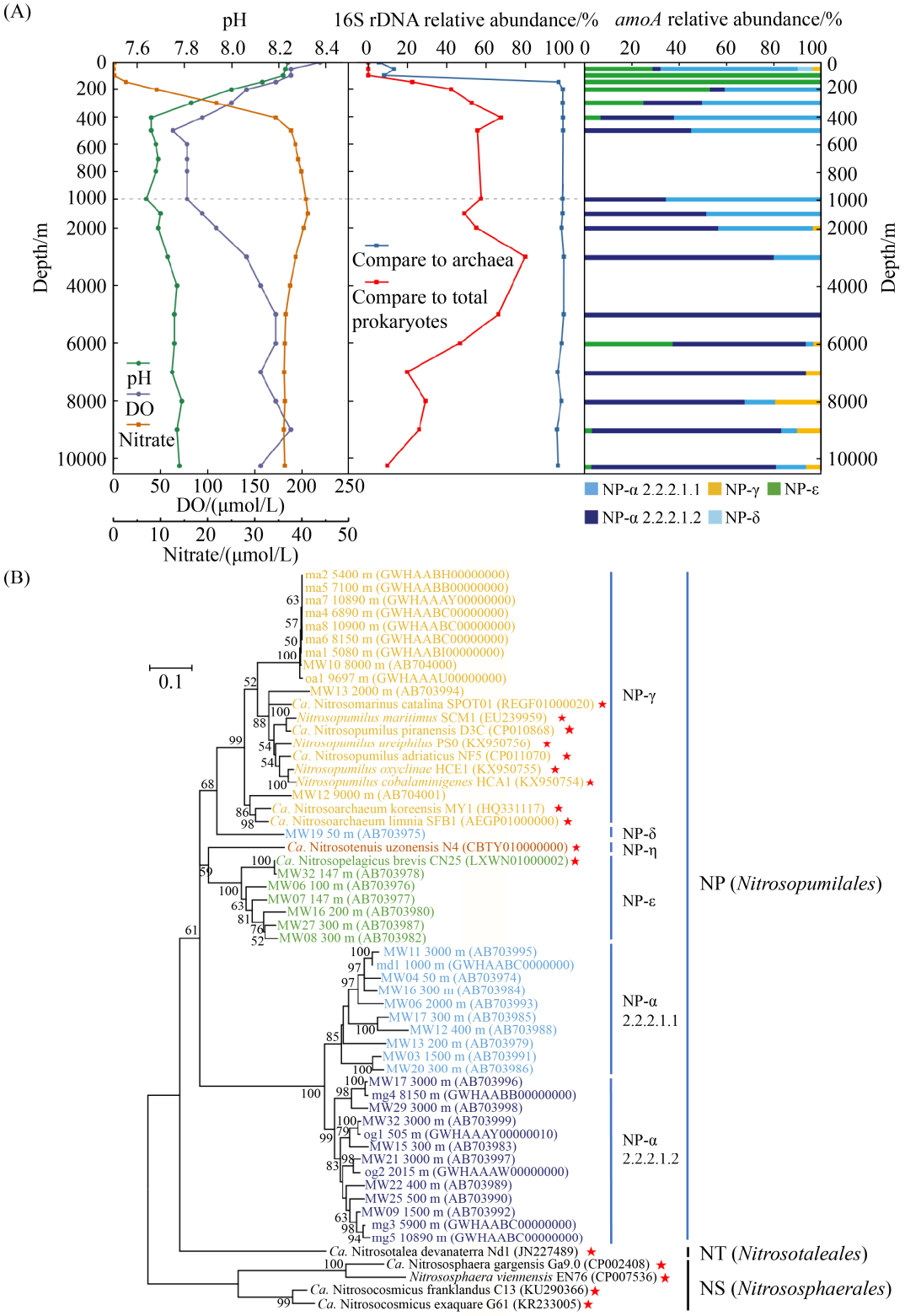

Figure 1. Variation of pH, oxygen, nitrate, AOA relative abundance with depth in Mariana Trench (A) and phylogeny of *amoA* genes in deep-sea environmental physicochemical indicators (B). AOA abundance data refer to Nunoura *et al*[34].

Table 1. Information of AOA isolates and enrichments

AOA strains	Lineage [a]	Year	Source (depth/m)	Relative abundance/%	Enrichment duration
Nitrosopumilus maritimus SCM1	NP-γ	2005	Marine aquarium	100	N/A
Ca. Nitrosopumilus koreensis AR1	NP-γ	2010	Marine sediment (78)	>80	2 years
Nitrosopumilus ureiphilus PS0	NP-γ	2014	Marine sediment (50)	100	N/A
Nitrosopumilus cobalaminigenes HCA1	NP-γ	2014	Seawater (50)	100	N/A
Ca. Nitrosopelagicus brevis CN25	NP-ε	2015	Seawater (25)	90–95	N/A
Nitrosopumilus maritimus NAO2	NP-γ	2015	Seawater (5)	100	N/A
Nitrosopumilus maritimus NAO6	NP-γ	2015	Seawater (5)	100	N/A
Ca. Nitrosopumilus piranensis D3C	NP-γ	2016	Seawater (0.5)	>99	2 years
Ca. Nitrosopumilus adriaticus NF5	NP-γ	2016	Seawater (0.5)	>99	2 years
Nitrosopumilus sp. DDS1	NP-γ	2016	Seawater (200)	100	2 years
Nitrosopumilus oxyclinae HCE1	NP-γ	2017	Seawater (17)	100	N/A
Ca. Nitrosopumilus sp. NM25	NP-γ	2011	Coastal sand	89	2 years
Ca. Nitrososphaera gargensis Ga9.2	NS-α	2008	Hot spring	50	6 years
Ca. Nitrosocaldus yellowstonii HL72	NC-α	2008	Hot spring	>90	2 years
Ca. Nitrosotenuis uzonensis N4	NP-η	2013	Hot spring	50	7 years
Ca. Nitrosocaldus islandicus 3F	NC-α	2018	Hot spring biofilm	85	N/A
Ca. Nitrosocaldus cavascurensis SCU2	NC-α	2018	Hot spring mud	92	4 years
Ca. Nitrosotenuis cloacae SAT1	NP-η	2016	Wastewater treatment plant	91	1 year
Ca. Nitrosocosmicus exaquare G61	NS-ζ	2017	Wastewater treatment plant	99	3 years
Ca. Nitrososphaera sp. OTU8	NS-α	2017	Wastewater treatment plant	91	N/A
Ca. Nitrosotenuis aquarius AQ6F	NP-η	2018	Freshwater aquarium biofilter	97–99	N/A
Ca. Nitrosoarchaeum limnia SFB1	NP-γ	2011	Estuarine sediment	84	N/A
Ca. Nitrosocosmicus oleophilus MY3	NS-ζ	2016	Coal tar-contaminated sediment	>99	N/A
Ca. Nitrosocosmicus arcticus Kfb	NS-ζ	2019	Frozen soil	72–93	5 years
Nitrososphaera viennensis EN76	NS-α	2011	Garden soil	100	2 years
Ca. Nitrosotalea devanaterra Nd1	NT-α	2011	Acid soil	90	N/A
Ca. Nitrosotalea sp. Nd2	NT-α	2014	Acid agricultural soil	100	3 years
Ca. Nitrosoarchaeum koreensis MY1	NP-γ	2011	Agricultural soil	90	2 years
Ca. Nitrososphaera sp. JG1	NS-α	2012	Agricultural soil	89	1 year
Ca. Nitrososphaera evergladensis SR1	NS-α	2014	Agricultural soil	50	1 year
Ca. Nitrosotenuis chungbukensis MY2	NP-η	2014	Agricultural soil	91	3 years
Ca. Nitrosocosmicus franklandus C13	NS-ζ	2016	Agricultural soil	100	N/A
Ca. Nitrosocosmicus agrestis SS	NS-ζ	2019	Agricultural soil	>97	150 days

Lineage [a]: compatible with taxonomy based on archaeal *amoA*[8].

1 Habitat of deep-sea ammonia-oxidizing archaea

1.1 Space

The ocean covers 71% of the Earth's surface area (3.62×10^8 km^2) and contains 97% of the total water volume (1.33×10^9 km^3), with an average depth of 3800 m and a depth approaching 11000 m in the Mariana Trench. The sea at a depth of 1000 m or more is called the deep sea, with an area of 88% (3.18×10^8 km^2) and a volume of 75% (1.143×10^9 km^3) of the total ocean[37], occupying the most extensive area of the biosphere. Despite the small area depth of the hadal zone at depths greater than 6000 m, which is equivalent to China's land area, it covers 45% of the ocean depth.

1.2 Hydrostatic pressure

Hydrostatic pressure is one of the distinguishing features of the ocean, and its magnitude is linked to the depth of seawater, and the hydrostatic pressure in the deep sea is up to 1.15×10^8 Pa. Pressure induces a phase change from liquid to solid. It affects Gibbs free energy, conducive to the chemical reaction accompanying the decrease in volume (Le Chatelier's principle). However, the pressure-induced volume change is generally small in non-gaseous reactions[38]. Most covalent bonds involved in the primary structure of proteins are pressure stable at least at $(1.0–1.5)\times10^8$ Pa[38]. Therefore, the pressure mainly affects the intermolecular forces of proteins, such as stabilizing hydrogen bonds, reducing electrostatic interactions, and breaking down hydrophobic interactions, thereby affecting protein hydration, folding, unfolding and aggregation of proteins, and even causing denaturation[39]. Under high hydrostatic pressure (HHP), the volume of liquid decreases, and the volume change is much greater for hydrocarbons relative to water[40]. Under the effects of HHP, the phospholipid bilayer is compressed, and the acyl chains are tightly packed, resulting in a phase change to gelatinous liquid crystals[41], leading to a decrease in fluidity. Increasing the pressure at different temperatures can denature or renature DNA[42], but under environmental parameters of deep sea, HHP acts to stabilize the double helix structure of DNA.

1.3 Low temperatures

Except for the space around hydrothermal vents, the deep-sea temperature is only 2–3 °C. In addition to pressure, kinetics of biochemical enzymatic reaction and phase transitions of biomacromolecules are closely related to temperature. Low temperature leads to a significant decrease in the rate of enzymatic reactions and membrane fluidity[43]. Like high pressure, low temperatures also affect the hydrophobic interactions and hydrogen bonds between protein tertiary structures, enhancing hydration and even leading to cold denaturation. Besides, increasing the solubility of oxygen at low temperatures may be traced to an increase in reactive oxygen species (ROS)[44].

1.4 Biogeochemistry

Marine photosynthesis occurs at a water depth of 0–200 m. As the depth increases, the sunlight gradually decreases until it completely disappears. There is a faint blue light in the range of 200–1000 m, while the space deeper than 1000 m has no sunlight at all. Usually, the deep sea is an oligotrophic environment, where the available organic matter is mainly derived from the ocean surface[45–46]. Nevertheless, only a small fraction of this generally settle to the deep sea. A study in Iquique, northern Chile, found that 82% of the proteins produced by photosynthesis were degraded at 0–30 m depth, another 15% degraded at 30–300 m, and only about 1% reached the surface sediments at 1200 m depth[47]. Many studies have shown that large AOA produce additional organic matter to the deep-sea environment by fixing inorganic carbon, about 400 million tons of carbon annually[48–49]. The

equilibrium of CO_2, HCO_3^- and CO_3^{2-} is pH-dependent, and inorganic carbon is predominantly in the form of HCO_3^- in seawater. In the deep sea, ammonia is a major electron donor. In most oceans, the primary source of ammonia is the euphotic layer or land[50]. Inorganic or organic nitrogen input from terrestrial runoff and ammonia synthesized by nitrogen-fixing microorganisms is utilized by plankton to synthesize biomass. These nitrogen-containing biomasses are decomposed after the death of the organisms, with some of them settling in the deep sea in the form of particulate organic matter (POM)[51–53]. The POMs rely on the high temperature of hydrothermal fluids or the mineralization by bacteria with extracellular enzymatic activity to release ammonia[54]. Recent studies have demonstrated that nitrogen fixation by methane anaerobic oxidation-sulfate reducing microorganisms may also be an essential source of ammonia in the deep sea[55]. In contrast to ammonia limitation, nitrate concentration in the deep sea is significantly higher under nitrification[56]. Influenced by low temperatures and high pressure, the oxygen concentration in deep seawater is lower than that of the surface layer of the ocean, but higher than the oxygen minimum zone (OMZ)[57] (Figure 1).

2 Deep sea is a shelter for AOA

The low cell number and extremely low relative abundance in the surface indicate that AOA does not possess competing advantage in the surface seawater. From the perspective of environmental factors, surface seawater temperature, pH, dissolved oxygen concentration, and light intensity are significantly higher than those in deep-sea seawater. AOA adapt to a wide temperature range, and the optimal growth temperature is generally greater than 20 °C. Therefore, the temperature of surface seawater does not limit the inhabitant of AOA.

The pH of seawater is alkaline, generally between 7.5 and 8.4. Surface seawater has the highest pH due to the influence of phytoplankton photosynthesis; as the depth increases, the seawater pH gradually decreases. The alkali tolerance of AOA is relatively low compared to AOB. It has been shown that the viable pH for marine AOA growth is in the range of 5.9–8.1, and the optimal pH is generally between 6.8–7.3[58]. The physiological characteristics of the isolated strains indicate that the high pH of shallow seawater likely inhibits AOA growth. In contrast, the deep sea provides a relatively low pH environment conducive to AOA growth.

Although AOA are aerobic microorganisms, currently isolated shallow-sea AOA are sensitive to ROS[59]. Because they do not have a complete antioxidant system (encoding superoxide dismutase, but lack of catalase, peroxidase). Ammonia oxidation of marine AOA are inhibited by 10 nmol/L H_2O_2[60], which may be related to the origin of AOA from anaerobic microorganisms[6]. The dissolved oxygen on the sea surface is close to saturation. Although dissolved oxygen concentration decreases rapidly with the increasing depth, it is still much higher than the deep sea in the range of 0–100 m deep, resulting in ROS stress on shallow-sea AOA

Illumination is one of the most different environmental factors between the deep and shallow oceans. Only about 1% of light energy can pass through 100 m of seawater. High light intensity markedly inhibits the activity of ammonia-oxidizing microorganisms, regardless of AOA and AOB. The mechanism of photoinhibition of ammonia-oxidizing microorganisms is that photooxidation destroys ammonia monooxygenase (AMO). Light induces a reaction between oxygen and organic matter[61], increasing the ROS concentration of light environment by 1–2 orders of magnitude. The reaction between oxygen and organic matter is photocatalyzed, increasing ROS concentration in the light environment by 1–2 orders of magnitude[60]. AOA may be more sensitive to light

owing to insufficient antioxidant capacity. Thus AOB occupied almost all of the ammonia oxidizers in seawater from 0–100 m depth[34].

In summary, AOA have difficulty surviving in eutrophic surface seawater, but the dark, deep sea serves as a vast living space for AOA and protects them from the inhibition of high pH, high dissolved oxygen, and high ROS.

3 The basis for deep-sea adaptation of AOA

Although AOA in the deep sea is protected from adverse conditions such as high pH and photooxidation, the deep sea, in general, is a harsh environment characterized with high pressure, low temperature, low nutrient availability for life. Therefore, it is likely that the AOA have specific basic characteristics, enabling them to adapt to environmental changes in expanding from land to the deep sea (Figure 2). Besides, evolution and LGT also increase their adaptation.

3.1 Sufficient membrane fluidity

Lipids are susceptible to stress. On average, they represent an order of magnitude more compressible than proteins. Many deep-sea microorganisms respond to HHP by regulating the composition of membranes. Such as producing higher content of monounsaturated or polyunsaturated fatty acid (M/PUFA), terminal branched fatty acids (TBFA), and fatty acids of short acyl chain length[62–63]. These changes increase membrane fluidity and avoid curing under high pressure and low-temperature conditions.

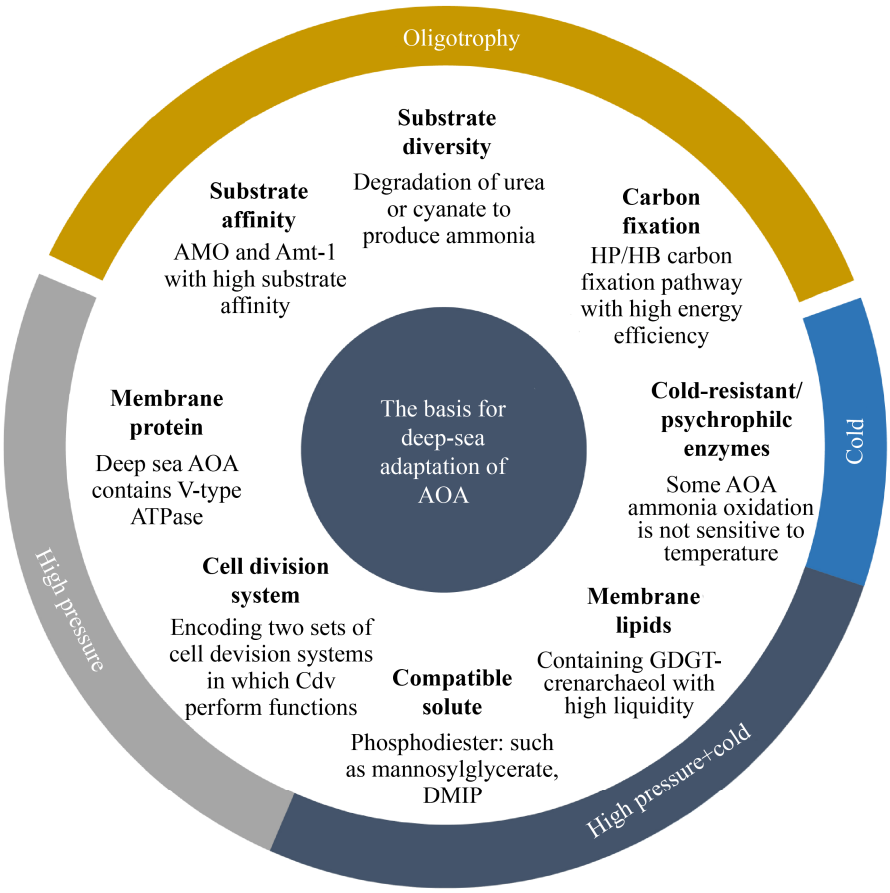

Figure 2. Potential adaptation of AOA to the deep-sea environment.

The membrane lipids of archaea differ from the bilayer membrane of bacteria, but rather glycerol dibiphytanyl glycerol tetraether membrane lipids (GDGTs) containing different core groups. They are monolayer phospholipids, consisting of a core membrane lipid and a polar head group. AOA all contain a high abundance of Thaumarchaeota-specific crenarchaeol core GDGTs. These membrane lipids are ubiquitous in the ocean[64]. Its molecular structure contains one cyclohexane and four cyclopentane rings, similar to the GDGT-4 synthesized by (super) thermophilic Crenarchaeota, but its internal cyclization double phytoalkane hydrocarbon chain forms a cyclohexane. Thus, the membrane fluidity is improved, being beneficial for AOA to adapt to the extreme environment in the deep sea[65]. The composition of GDGTs of different taxa of AOA and analogous AOA in altered metabolic states is highly variable[64], suggesting that the GDGTs have an essential role in the physiological function of AOA. For example, *Ca.* Nitrosotalea devanaterra has a high abundance of GDGT-4[66], resulting in their lower membrane permeability, giving them the ability to grow in low pH environments. GDGT-X is currently found only in *Ca.* Nitrososphaera gargensis Ga9.2, which is abundant in membranes, may be related to the thermophilic nature of *Nitrososphaerales* (Group I.1b)[67]. However, there is a lack of researches on GDGTs of AOA from the deep sea, the relationship among GDGTs composition, function, and deep-sea environment, and whether there is an undiscovered type of GDGTs still need further research and discussion.

3.2 Ability to maintain enzyme activity

Temperature is the main factor in determining the chemical reaction rate. The psychrophilic enzyme with high specific activity is often an order of magnitude higher in microorganisms living in low temperatures than mesophilic microorganisms[43]. The generally accepted hypothesis is that psychrophilic enzymes increase the flexibility of their structures to compensate for the "freezing effect" in cold habitats[68]. Although the structure of psychrophilic proteins is generally similar to their mesophilic or thermophilic homologous proteins, the active center of psychrophilic proteins is more accessible. Specifically: reducing the number of electrostatic interactions, hydrogen bonds, and hydrophobic interactions, decreasing subunit interactions, increasing interactions with solvents, reducing non-polar structures in the core, increasing the contact between non-polar residues and solvents, lessening the combination of cofactors, aggregating glycine residues and reducing proline and arginine contents[69]. It shows that the high catalytic constant (k_{cat}) of psychrophilic enzymes at low temperature is due to the decrease of activation enthalpy and activation entropy, which causes the enzyme-substrate complexes to display a broader conformation[70]. Therefore, enhancing the flexibility of psychrophilic enzymes also reduces the affinity between enzymes and substrates[71]. High pressures and low temperatures increase protein hydration, thus increasing the osmotic pressure inhibits protein expansion and exposing more hydrated surface area, which helps maintain a more compact structure and enzyme activity[72].

Although the optimum temperature for ammonia oxidation of AOA is generally greater than 20 °C, some studies based on *in situ* measurements have shown that temperature has no significant effect on the AOA ammonia oxidation activity in the range of 8–20 °C[73]. This indicates that there may be a particular type of AOA whose AMO can withstand low temperatures. Enhancing osmotic pressure reduces hydration and helps maintain protein activity under high pressure and low temperature. The presence of putative mannosy-l-3-phosphoglycerate synthase in the genome and proteome of terrestrial-derived

Nitrososphaerales-AOA indicates that this type of AOA can synthesize compatible solute mannosylglycerate from mannose-6-phosphate[74]. Based on genomic analysis, almost all deep-sea AOA have the potential to produce di-myo-inositol-phosphate (DMIP), which can act as an osmotic pressure regulator to deal with HHP and low temperature in the deep sea[75].

3.3 Stability of DNA structure and function

High pressure and low temperature make DNA melting difficult, affecting DNA replication and transcription. Genetic studies have shown that the DNA recombination repair system also plays a part in HHP adaptation. Many non-piezophilic microorganisms become filamentous at high pressures that allow cell growth, probably because cell division is more sensitive to stress than cell growth[76]. Similarly, piezophilic bacteria are filamentous when grown at pressures below or above their optimal pressure[76]. Proteins involved in cell division may be susceptible to changes in pressure. FtsZ is a kind of tubulin-like GTP hydrolyzed protein that is extensively present in prokaryotes. They control the division process of prokaryotic cells. In the early stage of the separation, FtsZ aggregates to form a ring in the center of the cell. In the cell of *Escherichia coli* incubated under high pressure, the FtsZ ring was almost absent, but it formed quickly after decompression[77].

AOA encode two replication systems, FtsZ and Cdv, but only the Cdv system controls the splitting process[78]. Members of Euryarchaeota contain FtsZ protein, while *Crenarchaeota* lacks FtsZ but contains Cdv protein. The sequenced AOA were all found to encode FtsZ and Cdv, indicating that AOA have evolved/obtained these two systems very early. Heterologous expression studies have shown that the Cdv protein of *Nitrosopumilus maritimus* SCM1 forms a stable complex in yeast compared to the rapid turnover of the FtsZ protein in *E. coli*. This may be related to the slow division cycle or oligotrophic living environments[79]. However, the characteristics of the Cdv of deep or shallow sea AOA under high pressure are still unclear.

3.4 Maintaining the function of membrane proteins

The pressure of 1×10^8 Pa causes a decrease in lipid fluidity and reversible conformational changes in transmembrane proteins resulting in disturbance of Na^+/K^+-ATPase function[80]. In comparison, the genomes of hadal AOA have two sets of ATPase (A-type and V-type). The V-type ATPase is discovered in all deep-sea AOA genomes. Due to the ability to pump out protons, V-type ATPase is also an acidophilic basis of *Ca.* Nitrosotalea. Hydrostatic pressure up to 2×10^7 Pa has completely inhibited the growth of shallow-sea AOA *Nitrosopumilus maritimus* SCM1 (without V-type ATPase gene)[75]. It shows that pumping out protons by V-type ATPase when energy is sufficient is one possible mechanism for high-pressure adaptability of deep-sea AOA. Still, more AOA strain need to be isolated and cultured from the deep sea to conclusively confirm the role of V-type ATPase in adaptation to high pressure.

3.5 Adaptation to oligotrophic environment

Although the total stoichiometry of AOA ammonia oxidation is equal to AOB, AOA have a very high affinity for ammonia. For instance, *N. maritimus* SCM1 oxidized unionized ammonia (NH_3) with the half-saturation constant (km) of about 3 nmol/L[81] (calculated by Emerson et al.'s formulas[82]). In addition to being oxidized on the outer side of the cytomembrane as an energy source, ammonia also serves as a substrate for synthesizing nitrogen-containing compounds, which require extracellular ammonium to be transported into cells through ammonium transporters (Amt). Amt transporters encoded in AOA genomes define two separate lineages, Amt-1 and Amt-2, while marine AOA have Amt-1 with high substrate affinity[83].

The superior affinity of AMO and Amt transporter indicates that AOA are advantages in the competition for ammonia nitrogen in oligotrophic environments.

AOA perform carbon fixation through a modified hydroxypropionate/hydroxybutyrate (HP/HB) cycle with the highest energy efficiency among aerobic autotrophic pathways[84], making it possible for them to multiply in low energy conditions.

Soluble organic nitrogen, such as urea and cyanate, is prevalent in the oceans, with urea concentrations roughly similar to ammonia and cyanate about an order of magnitude lower. AOA can degrade urea and cyanate, thus supplementing nitrogen and energy requirements under nitrogen-limited conditions[85]. The urease is often encoding in the marine AOA genomes[9,11,36]. However, the gene of cyanase is only found in *Nitrososphaera gargensis* Ga9.2 derived from terrestrial hot springs[86], even though the pure culture of *Nitrosopumilus maritimus* SCM1 without cyanase still utilizes cyanate as a substrate for ammonia oxidation[85].

Since ammonia oxidation in the deep sea is limited by cold, oligotrophic, and other extreme conditions. The *gtsABC* gene with glucose uptake function has been identified in the marine δ group (based on 16S rRNA gene classification)[36]. Moreover, the AOA genome encodes various transporters, indicating that multiple organic matters may be utilized as precursors[83]. Some strains of *Nitrosopumilus* stimulated by α-keto acid, showing potential mixotrophy[9]. However, the promotion of marine AOA by α-keto acids finally shown to act as a ROS scavenger. Through the ^{13}C isotope substrate labeling experiments, *Nitrosopumilus* sp. DDS1 and *Nitrososphaera viennensis* EN76 stimulated to grow by the organic matter are strictly autotrophic microorganisms[59]. The terrestrial AOA, *Ca.* Nirosocosmicus exaquare G61 has a robust antioxidant system and various organic substances stimulating its growth in enrichment, even if some of them do not eliminate ROS[28]. *Ca.* Nitrosocosmicus arcticus Kfb, originating from Arctic soil, grew faster at low temperatures (4–8 °C) than at moderate temperatures (20–28 °C); Unexpectedly, nitrite production was not detected unless increasing incubation temperature[17]. This evidence suggests that *Ca.* Nitrosocosmicus might have diverse metabolic capabilities. However, there is still a lack of culture-based experiments to study the trophic type of deep-sea AOA.

4 The role of AOA in deep-sea environment

The carbon cycle in the ocean plays a fundamental role in the habitability of the planet. Organic and inorganic carbon is transformed into each other regulating the atmospheric CO_2 concentration. The surface phytoplankton collect atmospheric CO_2 through photosynthesis. Then, they export particulate organic carbon (POC) or dissolved organic carbon (DOC) to the deep sea[87], that is the biological pumps.

However, organic carbon reaching the seafloor represents only about 0.3% of the primary productivity of the ocean[88]. Heterotrophic microorganisms consume the vast majority of them to produce CO_2 during the sedimentation process. Thus, organic carbon exists in the oceans mainly as recalcitrant dissolved organic carbon (RDOC) that is difficult to degrade[89].

RDOC as colossal carbon sinks is mainly generated by microbial degradation, synthesis, secretion. These processes release CO_2 and inorganic nitrogen and phosphorus[90], that is, the role of microbial carbon pump (MCP). AOA and other chemoautotrophic microorganisms use inorganic carbon and nutrient salts to synthesize

organic matter in the deep sea, recover CO_2 produced by the MCP and provide new organic carbon for the MCP, enhancing the effect of carbon sequestration.

Primary producers use light or chemical energy to convert CO_2 to organic metabolites while changing the stoichiometric composition of the cell to affect other element cycles, such as the nitrogen cycle[53]. Nitrification is an essential segment of the global nitrogen cycle. For a long time, researchers consider AOB is the only ammonia oxidizer group, but the discovery of AOA has led to a reassessment of the ammonia oxidation process. As a central process in the nitrogen cycle, nitrification linking the biological processes of nitrogen fixation and nitrogen loss (denitrification and anaerobic ammonia oxidation)[91]. It consists of two steps: ammonia oxidation and nitrite oxidation. Nitrification energizes two chemoautotrophs (ammonia oxidizer and nitrite oxidizer), driving the coupling between reduced inorganic nitrogen and newly generated organic carbon.

AOA is much more abundant in the deep sea than AOB[34,92]. *In situ* studies have shown that AOA are active in the marine environment[93–96]. Despite the ammonia oxidation rate of AOA is very low[5], the enormous biomass means that the contribution of marine AOA to the carbon and nitrogen cycle of the Earth is considerable. According to estimates, deep-sea nitrifying microorganisms can fix 1×10^{13}–2×10^{13} moles of carbon and oxidize 1×10^{14}–2×10^{14} moles of ammonia per year in the deep sea[97], which has a profound impact on climate change. Figure 3 macroscopically illustrates the involvement of AOA in the marine geochemical cycle. Settled nitrogenous organic matters are decomposed and mineralized by heterotrophic microorganisms, thereby releasing ammonia, urea, cyanate, which serve as energy sources and biosynthetic substrates for AOA. Nitrite-oxidizing bacteria (NOB) use nitrite produced by AOA as an oxidation substrate, and these two groups of nitrifier provide oxidized nitrogen for denitrification. AOA synthesize polysaccharides, lipids, nitrogenous organics, and other metabolites from inorganic carbon using the energy generated by ammonia oxidation (Figure 4). Heterotrophic organisms can use these primary metabolites as important energy support for deep-sea ecosystems. The refractory components dissolve in seawater or settle to the seafloor and no longer enter the atmosphere. Thus, AOA may play a key role in the process of carbon fixation and sequestration in the ocean as an essential complement to MCP.

AOA are also adaptable to toxic deep-sea environments, such as surviving in hydrothermal vents[98] and cold spring sediments[99] containing reduced sulfur, which is different from AOB sensitive to H_2S[100]. Also, co-culture with sulfur-oxidizing bacteria (SOB) in thiosulfate-containing media can promote the growth of AOA (probably SOB consumes the extra oxygen)[12]. It indicated that AOA is responsible for primary production based on ammonia oxidation and may significantly influence other primary production processes such as sulfur oxidation.

A great deal of research has been conducted in revealing the role of AOA in the marine geochemical cycle. However, whether AOA consume organic matter as energy sources, in addition to autotrophic ammonia oxidation, remains inconclusive. In addition to death or lysis by phages, AOA secrete DOC, which can be utilized by heterotrophic microorganisms, including amino acids, thymine, B vitamins[101]. The secreted DOC by AOA account for only 0.08%–1.05% of carbon requirement of heterotrophic prokaryotes[102]. They are crucial to some AOA-related auxotrophic microorganisms, suggesting that there may be close cooperation between AOA and some heterotrophic microorganisms.

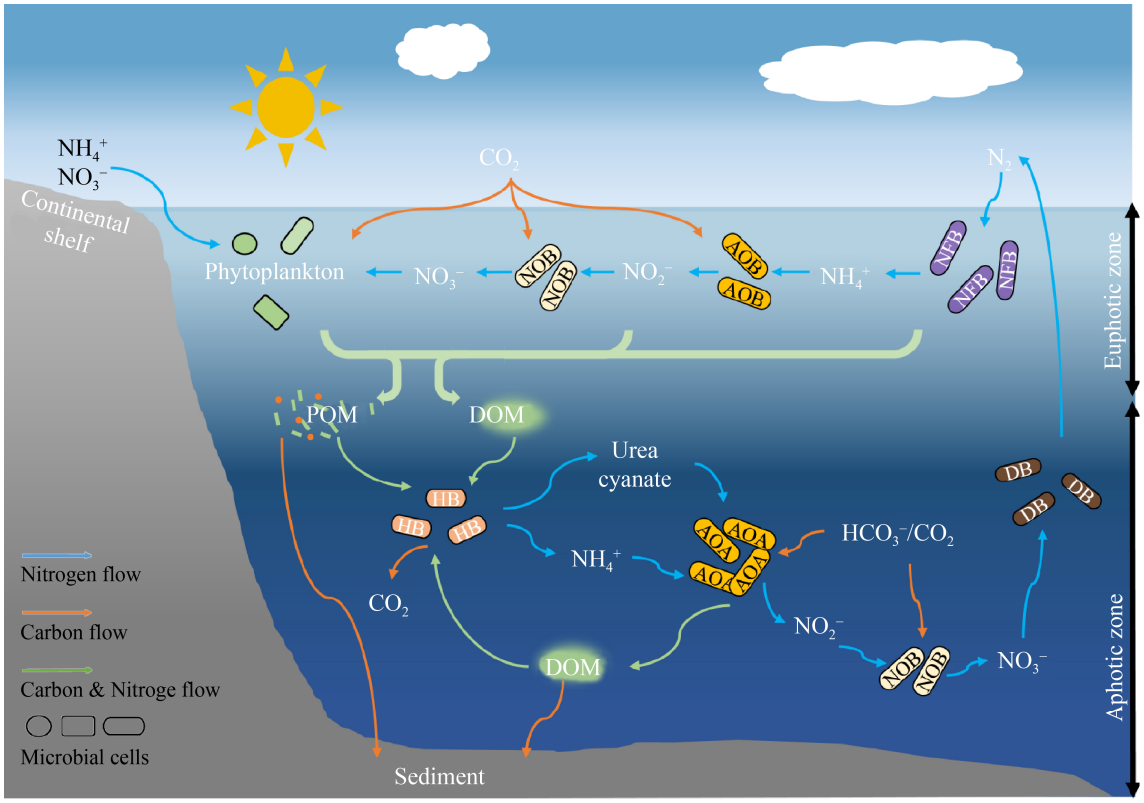

Figure 3. Ocean carbon cycle and the nitrogen cycle in which AOA is primarily involved. POM: particulate organic matter; DOM: dissolved organic matter; NFB: nitrogen-fixing bacteria; AOB: ammonia-oxidizing bacteria; NOB: nitrite-oxidizing bacteria; AOA: ammonia-oxidizing archaea); DB: denitrifying bacteria; HB: heterotrophic bacteria.

5 Perspectives

Given the irreplaceable role of marine AOA in the carbon and nitrogen cycles, the study of the metabolic properties of AOA also helps to improve our understanding on the geochemical cycles in which AOA participates. Unfortunately, at present, there is still limited knowledge on the metabolism of AOA, such as the processes of energy sources other than ammonia (e.g., urea and cyanate), energy metabolism (e.g., hydroxylamine oxidation), the composition of synthesized primary products, and the regulatory mechanism of carbon and nitrogen metabolism process (Figure 4).

As AOA expands its habitats into the deep sea, high species diversity has developed along with evolution and LGT along with environmental pressure. Culture-based research of AOA forms the core of our knowledge on this type of microorganisms and promotes understanding of the biochemical cycle in which they participate. AOA strains from different sources show significant differences in genome and metabolic functions. However, the cultured AOA only belong to a few branches of global AOA evolution trees (Figure 1), and a large number of uncultured AOAs from different environments are waiting for in-depth study.

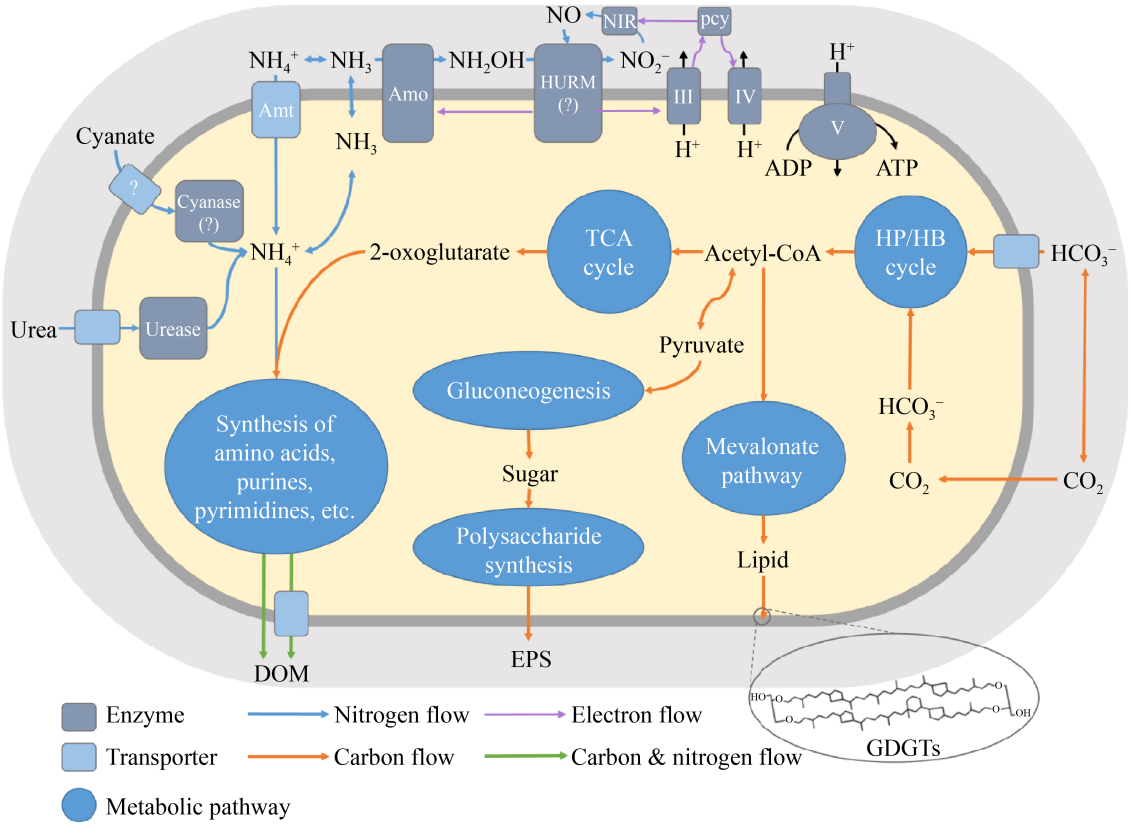

Figure 4. Carbon and nitrogen metabolism pathway related to the geochemical cycle of marine AOA. Amt: ammonium transporter; Amo: ammonia monooxygenase; HURM: hydroxylamine ubiquinone redox module; NIR: nitrite reductase; pcy: plastocyanin; III: complex III; IV: complex IV; V: ATPase; GTGDs: glycerol dibiphytanyl glycerol tetraether lipids.

Isolated AOA strains are crucial to confirming the physiological characteristics of AOA from different water depths or different groups. However, AOA are difficult-to-culture microorganisms and often require long-term enrichment to obtain cultures[103]. Many important findings are based on culture, such as the determination of the function[1], kinetics of AOA ammonia oxidation[5], a putative autotrophic ammonia oxidation model[104], α-keto acid facilitates antioxidation of AOA[59], and the identification of carbon fixation cycle[84]. Metagenomic techniques provide direct access to the genomes of microorganisms in the environment to predict their metabolic properties and have broad applications in mining the genetic resources of uncultured microorganisms. Auxotype determines how AOA participates in the carbon and nitrogen cycle, but *in situ* surveys and genomic-based analyses do not provide conclusive evidence for the auxotype of deep-sea AOA. Ammonia oxidation kinetics are also crucial to re-estimating global oceanic nitrogen fluxes. Current studies are mainly based on a few shallow-sea AOA strains. The differences in physiological characteristics between shallow and deep-sea AOA may dramatically impact the calculation of oceanic nitrogen fluxes. Culture-based methods in combine with *in situ* geochemical analysis will provide more reliable data to approaching a precise evaluation on the ecological function of these microorganisms in

terms of driving the nitrogen cycling in deep sea.

Apparently, AOA has long generation time, potential piezophilic properties, and interactions with other microorganisms; its growth is inhibited by ammonia[24] and ROS[59]. All these features lead us to develop novel approaches for deep-sea AOA cultivation. For example, we can use deep-sea simulation technology to achieve cultivation conditions that are similar to their natural habitats. Besides, a modified medium matching their metabolic potential based on the genetic analysis is also recommended. For example, a continuous supplementation with very low concentrations of ammonia nitrogen and ROS scavengers in a facility that can perform under high pressure and low temperatures. Studying physiological properties and metabolic processes of deep-sea AOA on this basis will play a major part in illuminating their contribution to the geochemical cycle, adaptation, and biological evolution of deep-sea life. The isolation of deep-sea AOA will also be beneficial to the study of the relationship between oligotrophy and high-pressure adaptation, which is helpful for us to understand the living strategy in deep biosphere.

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深海氨氧化古菌的环境适应基础及生态功能

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摘要：氨氧化古菌是地球上丰度最高的微生物类群之一，驱动氮循环。尤其在深海，其相对丰度可达原核生物的 20%–40%。然而，纯培养的缺乏严重阻碍了我们全面认知深海氨氧化古菌的生理特性和生态贡献。本文系统性地分析了深海环境特征与微生物适应性之间的关系，聚焦深海氨氧化古菌的潜在生存策略和代谢偏好。这些信息将有助于我们设计适用于深海氨氧化古菌的培养技术。此外，从系统发育和生理特性来看，深海氨氧化古菌与土壤或表层海洋来源的氨氧化古菌有显著区别，提示我们需要根据其特性重新估算全球海洋氮通量。

关键词：氨氧化古菌，深海，环境适应，地球化学循环

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