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Methanoperedenaceae archaea: a 20-year research journey

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Twenty years ago, an archaeal lineage capable of catalyzing nitrate-dependent anaerobic methane oxidation (n-damo) was identified. This archaeal lineage was originally grouped within the ANME-2d cluster, and later renamed as the *Methanoperedenaceae* family. Since then, researchers worldwide have advanced our understanding of the biology of this archaeal group by describing its phylogenetic diversity, versatile metabolic functions, unique kinetic and physiological properties, special growing patterns, extracellular electron transfer capability, and extrachromosomal genetic elements. In addition, the potential engineering and industrial applications of *Methanoperedenaceae* have been explored broadly, employing granular and biofilm platforms for wastewater treatment and other purposes. In this Review, we reflect on the history of *Methanoperedenaceae* research, covering both the biology and the potential applications, summarize main achievements and challenges, and propose avenues for future research and applications.

Recapping the history of *Methanoperedenaceae* research

Methane, the simplest hydrocarbon, has been present in Earth's atmosphere since its earliest days[1]. Today, a considerable portion of biogenic methane is produced in oxygen-depleted environments by methanogenic archaea, the majority of which is consumed by methanotrophic bacteria and archaea before escaping into the atmosphere (Box 1). Notably, all currently identified anaerobic methanotrophic archaea (ANME) belong to the *Halobacteriota* phylum, one of five divisions of the previously known *Euryarchaeota*[2], including lineages *Methanophagales* (formerly ANME-1), *Methanocomedenaceae* (ANME-2a/b), *Methanogasteraceae* (ANME-2c), *Methanoperedenaceae* (ANME-2d), and *Methanosarcinales* (ANME-3). While most ANME lineages originate in marine environments and rely on symbiotic bacterial partners, *Methanoperedenaceae* stand out as freshwater dwellers capable of conducting anaerobic oxidation of methane (AOM) independently.

Despite earlier hypotheses, the existence of ANME was first confirmed in marine environments at the very beginning of the twenty-first century[3–5]. These initial ANME species couple AOM with the reduction of sulfate, an important electron acceptor in the ocean. A turning point came in 2006 when Raghoebarsing et al. provided evidence of AOM coupled to nitrate reduction in freshwater sediments[6] (Fig. 1). The archaeon enriched by that study was initially grouped within the ANME-2d cluster and was later classified within the family *Methanoperedenaceae*. The discovery sparked an intense effort to enrich and characterize this new archaeal family, fueled by the rapid development of advanced molecular techniques. A landmark study by Haroon et al. in 2013 described a *Methanoperedenaceae*-dominated enrichment culture and inferred metabolic pathways of *Methanoperedenaceae* from meta-omic and single-cell sequencing data[7]. Over the subsequent decade, researchers have made remarkable strides in exploring the phylogenetic and functional diversity of *Methanoperedenaceae*. For instance, Ettwig

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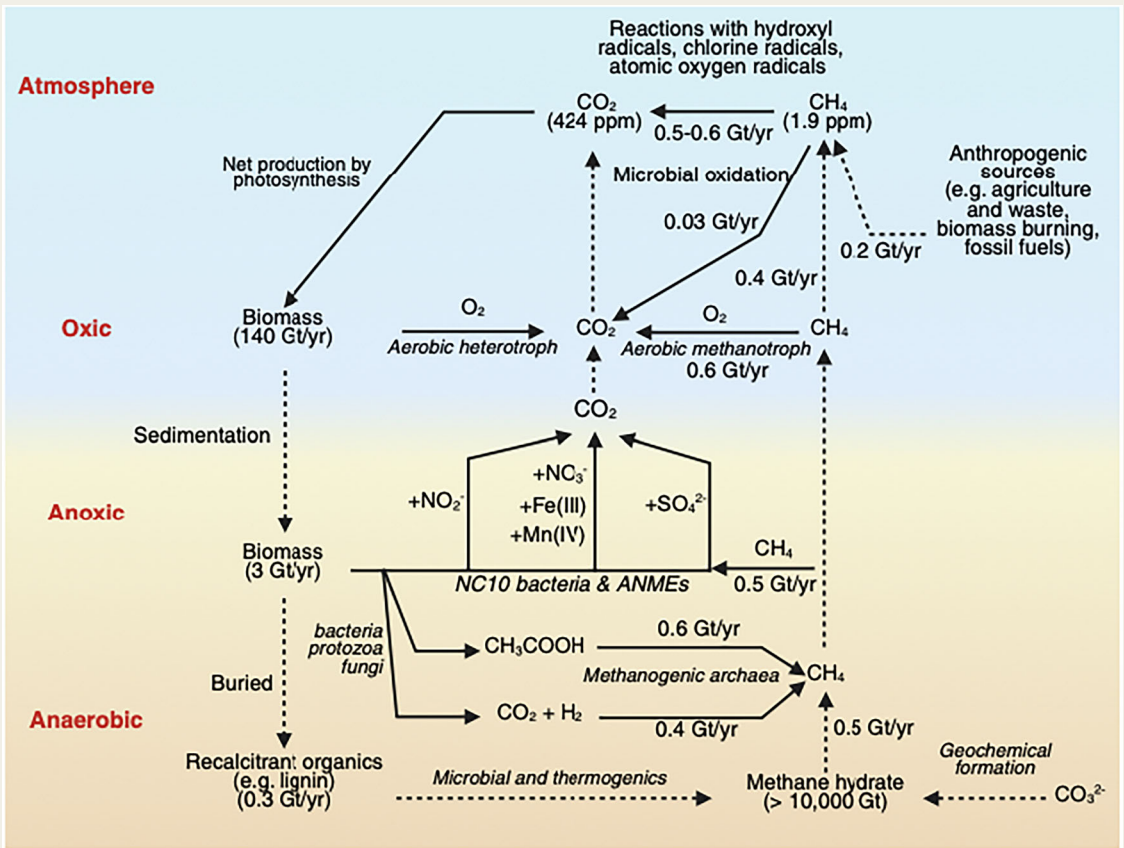
BOX 1

Methane cycle

In methanogenic anaerobic environments, approximately 1 Gt of methane is produced annually by methanogenic archaea. Together with an estimated 0.5 Gt of methane released each year from methane hydrates, the total global methane production is about 1.5 Gt year^{-1}. Before reaching the atmosphere, methane typically undergoes sequential oxidation in anoxic and oxic environments. In anoxic environments, anaerobic oxidation of methane (AOM) consumes about 0.5 Gt year^{-1}, converting methane into CO_2 through the use of various electron acceptors, available in different environmental niches. Two major groups of microorganisms are responsible: (1) NC10 bacteria, which couple AOM with nitrite and nitrate reduction; and (2) ANMEs, which perform AOM with sulfate, nitrate, Fe(III), and Mn(IV), either independently or in syntrophy with bacteria. Among ANMEs, the *Methanoperedenaceae* have been confirmed to carry out AOM with nitrate, Fe(III), and Mn(IV), and are also proposed to form partnerships with sulfate-reducing bacteria for sulfate-dependent AOM, highlighting their remarkable metabolic versatility. Many recent studies have confirmed the existence and examined the AOM rates associated with *Methanoperedenaceae* across freshwater systems[42,147–149], paddy ecosystems[150,151], and coastal aquatic systems[152,153]. In oxic environments, approximately 0.6 Gt of methane is oxidized annually into CO_2 by aerobic methanotrophs. Collectively, this results in a net annual release of about 0.4 Gt of methane from natural sources. Once in the atmosphere, ~90% of methane is oxidized to CO_2 by hydroxyl and other radicals, while a smaller fraction is consumed microbially in aerated surface soils. Data were collected from refs. 154–158, which were cross-checked with the report of the Global Methane Budget[159]. Figure of Box 1 was created in BioRender. Liu, T. (2026) https://BioRender.com/p4uh04i.



et al. reported the capability of *Methanoperedenaceae* to perform dissimilatory nitrate reduction to ammonia (DNRA) and iron-dependent AOM[8], while Cai et al.[9] and Leu et al.[10] enriched, over several years, additional *Methanoperedenaceae* species from freshwater sediments capable of iron/manganese-dependent AOM. In addition to phylogenetic diversity and functional versatility, the focus of fundamental research on *Methanoperedenaceae* included also extracellular electron transfer (EET) mechanisms[11,12], extrachromosomal genetic elements[13–15], and adaptation strategies against harsh environmental conditions[16,17]. Such versatility and adaptability may play roles in the geochemical cycling of various elements, far more than originally anticipated.

In parallel with fundamental exploration, researchers also explored the application potential of enriched *Methanoperedenaceae* cultures (Fig. 1). With their unique ability to remove nitrate and methane, *Methanoperedenaceae* have been proposed as a promising candidate for water remediation and greenhouse gas mitigation[18]. Over the past 10 years, this potential has driven efforts in innovative process design and its modeling and demonstration. In particular, efforts have been made to enhance the activity of

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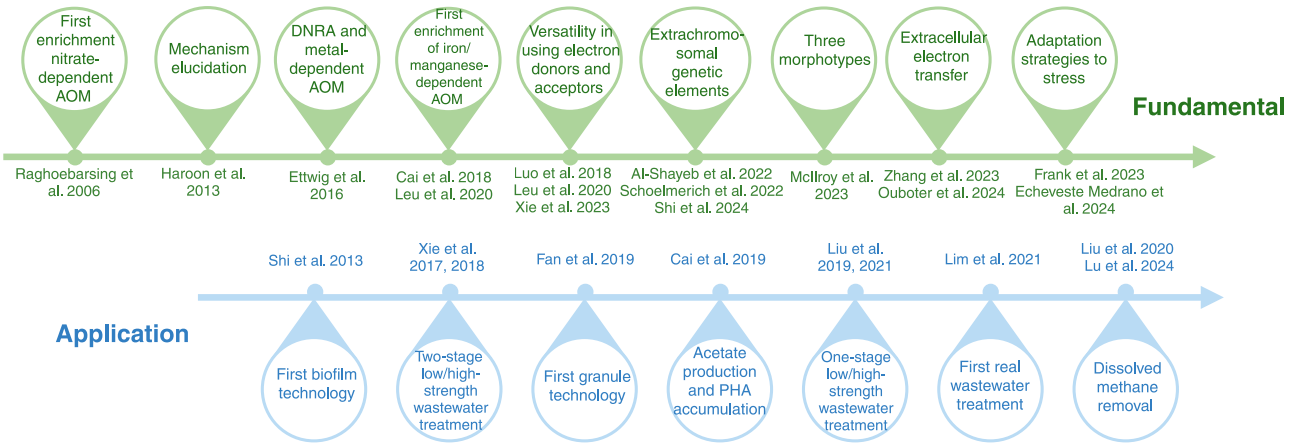

Fig. 1 | Milestones in research of *Methanoperedenaceae* archaea. 'Fundamental'[6–17,37,38,46,58] and 'Application'[26,73,76–78,82,89,90,98,146] may not comprehensively capture the scope of every study, as some of them delve into both aspects. The timeline and the arrangement of items are indicative. The authors acknowledge the collective contributions of all those involved in the 20-year research journey of *Methanoperedenaceae* archaea. Created in BioRender. Liu, T. (2026) https://BioRender.com/p4uh04i.

Methanoperedenaceae, which are inherently slow-growing and metabolically restrained. With process optimization, the processes leveraging enrichment cultures of *Methanoperedenaceae* have achieved volumetric rates two orders of magnitude higher than those of the initial enrichments (Table 1), meeting the required level for practical applications. In many applications, *Methanoperedenaceae* are frequently paired with anammox bacteria[19–23], another microbial group with unique metabolic abilities[24]. From an engineering perspective, this partnership may be useful for a widely applied nitrogen removal process, namely 'partial denitrification and anammox' (PdNA)[25]. Additionally, biotechnological applications aimed at converting methane into value-added products, such as biopolymers and fatty acids, have been proposed[26,27], although current production rates are far from meeting practical application requirements. Particularly, the production of biopolymers associated with the growth of *Methanoperedenaceae* is considered unrealistic. These conversions are achievable through direct biological conversions, where methane is built into the products, or indirect bioelectrochemical conversions, where electrons are harvested from methane at the anode and transferred to the cathode for the reduction of substrates to valuable compounds.

As research advances, *Methanoperedenaceae* are increasingly recognized as important players in the biogeochemical cycling of multiple elements, offering promising solutions to environmental challenges associated with greenhouse gas emissions, water remediation, and resource recovery. The 20-year research journey of *Methanoperedenaceae* archaea also highlights the vital role of interdisciplinary research. This review aims to synthesize two decades of *Methanoperedenaceae* research, highlighting key discoveries in both fundamental and applied contexts, and to envision future directions for investigating this unique archaeal group.

Progress in fundamental understanding in the past 20 years

Metabolic versatility

An increasing number of *Methanoperedenaceae* species have been enriched in laboratory bioreactors. The first characterized species, "*Candidatus* Methanoperedens nitroreducens", which couples AOM with nitrate reduction to nitrite (Fig. 2a), was reported by Haroon et al.[7]. A few years later, two distant species coupling AOM with dissimilatory Fe(III) reduction, namely "*Ca*. M. ferrireducens" and "*Ca*. M. psychrophilus", were enriched at room temperature (22 ± 2 °C) from freshwater sediments[9] and at 18 °C from the cold Zoige wetland[28], respectively. Further, "*Ca*. M. nitroreducens Vercelli" was enriched from paddy fields[29], and two new species, "*Ca*. M. manganicus" and "*Ca*. M. manganireducens", were recovered in a bioreactor performing Mn(IV)-dependent AOM[10]. While these different *Methanoperedenaceae* species were enriched with different electron acceptors, each species appears to be versatile in utilizing various electron acceptors. For instance, two phylogenetically distant nitrate-reducing species, "*Ca*. M. nitroreducens BLZ1" and "*Ca*. M. nitroreducens", are also capable of using Fe(III) as an alternative electron acceptor[8,30]. This metabolic flexibility is deemed to be related to their EET capability (Fig. 2b), which may enable them to reduce other extracellular electron acceptors, including Mn(IV)[8], humic substances[31], and artificial electrodes[11,32–34]. Genomic evidence also suggests the potential of *Methanoperedenaceae* to reduce toxic metals (e.g., chromate[35,36]) and metalloids (selenate[37]).

In addition to EET, genes encoding obligate reductases (Fig. 2a), which are hypothesized to be acquired through horizontal gene transfer (HGT), have been described in these organisms[38]. For example, some *Methanoperedenaceae* species appear to have acquired nitrate reductase genes (*nar*GHI) from bacteria via HGT[7,39]. Given the broad substrate range of nitrate reductases, it is possible that *Methanoperedenaceae* may use this enzyme to reduce perchlorate, bromate and vanadate[40], although physiological validation is lacking. Nitrate-reducing *Methanoperedenaceae* also harbor a putative nitrite reductase gene (*nrf*A), and they have been physiologically demonstrated to be able to perform DNRA[8,41]. Another obligate reductase encoded in *Methanoperedenaceae* genomes is a putative arsenate reductase (Arr) of the dimethyl sulfoxide (DMSO) reductase family, which might enable arsenic reduction[38,42]. As the Arr protein complex has been proven to provide prokaryotes a biochemical foundation for dissimilatory antimony reduction[43,44], it is legitimate to speculate that Arr-harboring *Methanoperedenaceae* may also be capable of antimony reduction. Additionally, genomic analyses have identified a gene encoding a putative arsenite oxidase (Arx)[38], previously implicated in reversible arsenite oxidation and arsenate reduction in vitro by the bacterial isolate *Alkalilimnicola ehrlichii*[45]. Future physiological assays are therefore warranted to validate the biochemical role of Arr and Arx-like proteins in *Methanoperedenaceae*.

Apart from their flexibility in metabolizing various electron acceptors, members of the *Methanoperedenaceae* carry genes encoding putative formate dehydrogenase and hydrogenase complexes (Fig. 2c), which suggests their potential to oxidize various electron donors, such as formate and hydrogen. "*Ca*. M. nitroreducens" has been recently shown to utilize formate as an alternative electron

Table 1 | Comparison of studies reporting *Methanoperedenaceae*-driven nitrogen removal process

Biomass type	Reactor type	Functional populations	Temperature °C	Nitrogen composition and concentration mg N L^{-1}	Nitrate reduction rate mg N L^{-1} d^{-1}	Methane source	Reference
Suspended biomass	SBR	Anammox bacteria + *Methanoperedenaceae* archaea	22	Ammonium: 70–140 Nitrate: 70–140	15–20	Gaseous methane	7
	SBR	NC10 bacteria + *Methanoperedenaceae* archaea	27	Nitrate: 17.5–70	4	Gaseous methane	29
	MBR	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	35	Ammonium: 2300 Nitrite: 2700	250–300	Gaseous methane	87
Biofilm	MBfR	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	22	Ammonium: 300 Nitrate: 600	190	Gaseous methane	73
	MBfR	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	22	Ammonium: 25 Nitrite: 25	10	Gaseous methane	89
	MBfR	AOB+Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	30	Ammonium: 1000	170	Gaseous methane	76
	Biotrickling filter	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	35	Ammonium: 500 Nitrite: 500	400–500	Gaseous methane	74
	MBfR	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	22	Ammonium: 10-20 Nitrite: 40–50 Nitrate:40–50	80	Dissolved methane	78
	MBfR	AOB+Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	22	Ammonium: 50	30	Dissolved methane	98
Granule	UASB	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	30	Ammonium: 500 Nitrite: 500	100	Gaseous methane	83
	UASB	Anammox bacteria + NC10 bacteria + *Methanoperedenaceae* archaea	35	Ammonium: 500 Nitrite: 500	1900	Gaseous methane	82

References were selected based on different biomass types, reactor configurations, operational temperatures, feed composition and concentrations, methane sources, functional populations, and nitrate reduction rates. The table shows that the volumetric nitrate reduction rates increased by approximately two orders of magnitude relative to the initial enrichment.
SBR sequential batch reactor, *MBR* membrane bioreactor, *MBfR* membrane biofilm reactor, *UASB* upflow anaerobic sludge blanket.

donor, with a putative formate dehydrogenase gene (*fdh*AB) complex highly expressed[46]. Unlike marine ANME clades, which generally lack hydrogenase genes, a member of *Methanoperedenaceae* sampled from deep continental aquifers was found to harbor a group 1 membrane-bound [NiFe] hydrogenase gene[47], suggesting a capability to utilize molecular hydrogen as an alternative electron donor for the respiratory chain and energy conservation. Several *Methanoperedenaceae* genomes also encode group 3 cytoplasmic [NiFe] hydrogenases, which are typically involved in bidirectional hydrogen metabolism and might function in redox balancing through NAD(P)H coupling[38]. Despite these genomic features, direct physiological evidence for hydrogen metabolism in *Methanoperedenaceae* and the specific roles of these hydrogenase types are lacking. Furthermore, the storage of polyhydroxyalkanoate (PHA) via PHA synthase (*pha*CE) was recently confirmed in nitrate-reducing *Methanoperedenaceae* members[32,33], providing an auxiliary electron sink for AOM. Identifying the factor to manage the electron flow towards PHA will be key to understanding the metabolic flexibility and environmental resilience of this archaeal lineage.

Extracellular electron transfer pathways

One of the most important traits identified for *Methanoperedenaceae* in the last decade is their EET capability (Fig. 2b), though the mechanisms remain incompletely understood. A critical question is how to distinguish a non-syntrophic direct EET pathway from a diffusion-dependent indirect EET pathway. The presence of genes encoding multi-heme c-type cytochromes (MHCs), ubiquitously conserved in *Methanoperedenaceae* species, suggests EET mechanisms resembling those of well-known electroactive microorganisms such as *Geobacter* and *Shewanella*. Field observations further reinforce this hypothesis: the absence of traditional metal-reducing organisms in *Methanoperedenaceae*-dominated Fe(III)-dependent AOM consortia indicates that *Methanoperedenaceae* can independently mediate respiratory Fe(III) reduction[48,49]. Further, laboratory enrichment of multiple *Methanoperedenaceae* species has provided additional support, demonstrating their transcriptomic dominance when reducing insoluble Fe(III) and Mn(IV) oxides[9,10]. Recently, Zhang et al. used fluorescence and single-cell electron microscopy and in vivo electrochemical characterization to provide physiological evidence at the single-cell level supporting that MHC-based systems enable direct EET in *Methanoperedenaceae*[12].

However, a full picture of such direct EET systems remains unclear. It has been proposed that both archaea and bacteria can form extracellular cytochrome nanowires by optimized heme packing arrangement[50–55]. As *Methanoperedenaceae* possess homologs of extracellular cytochrome nanowires found in a bacterium (*Geobacter sulfurreducens*)[51] and in an archaeon (*Archaeoglobus veneficus*)[50], it is plausible that *Methanoperedenaceae* may be able to assemble MHCs into cytochrome-based conductive filaments for long-range direct EET, an intriguing aspect to be investigated. By contrast, a prior hypothesis posits that *Methanoperedenaceae* may use conductive archaeal flagella (i.e., archaella) for long-range EET[10,56], similar to their close relatives, the methanogens[57]. This idea is supported by the finding that planktonic *Methanoperedenaceae* cells upregulate

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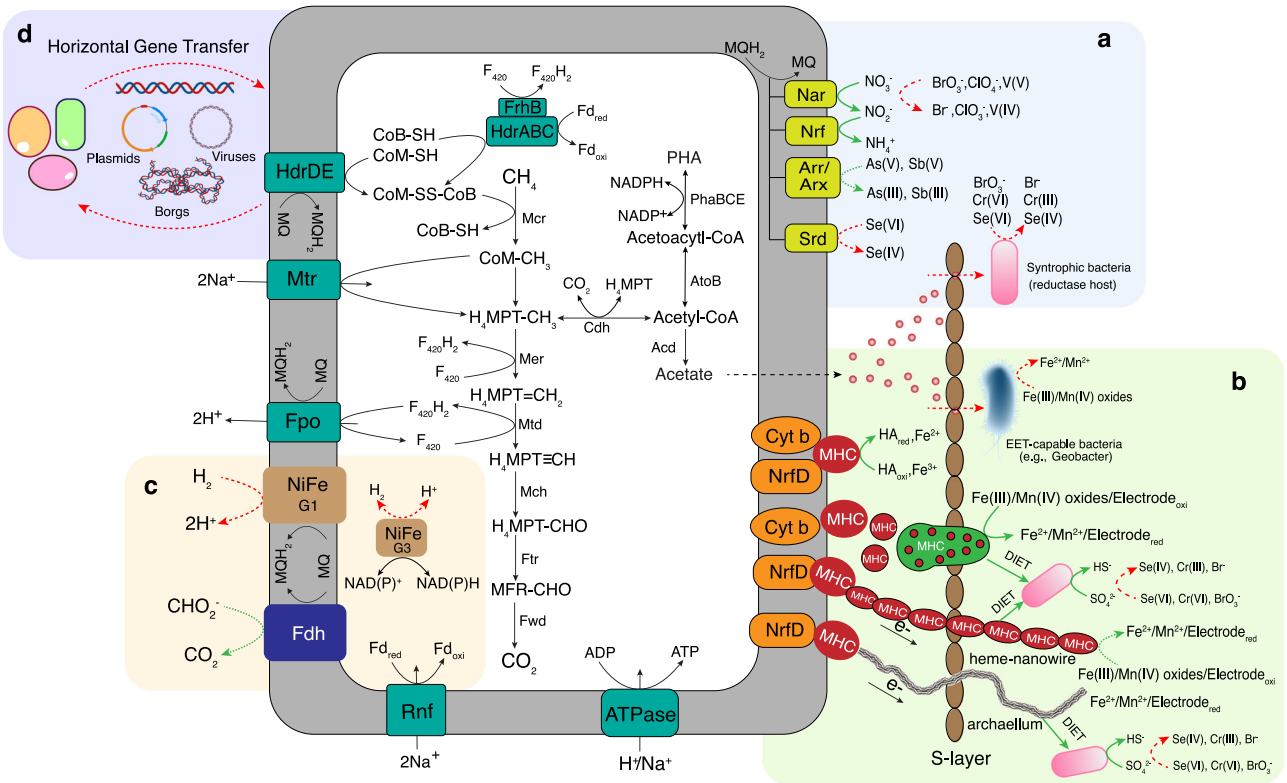

Fig. 2 | Fundamental breakthroughs in the understanding of *Methanoperedenaceae* archaea. Fundamental discoveries include, but are not limited to, the identification of diverse electron acceptors (**a**), extracellular electron transfer mechanisms (**b**), diverse electron donors (**c**), and extrachromosomal genetic elements (**d**). The level of evidence for the depicted findings and pathways is indicated as follows: red dashed arrows indicate putative pathways based on genomic prediction; green dotted arrows indicate pathways supported by microbial activity assays, genomic, and transcriptomic data; green solid arrows indicate well-defined pathways with robust physiological and biochemical evidence. Notably, not all of the depicted metabolic functions are conserved across different *Methanoperedenaceae* species. For instance, the nitrate reductases (*nar*GH) are present in "*Ca*. M. nitroreducens" but absent in "*Ca*. M. ferrireducens"[9]. Likewise, the formate and hydrogen-related genes were only detected in several species, such as "*Ca*. M. MGW-1"[47]. Instead, the EET ability enabled by MHCs appears to be universal across all *Methanoperedenaceae* archaea.

archaellum-related genes, possibly for motility purposes[58], which might promote chemotaxis towards extracellular insoluble electron acceptors[59]. Interestingly, Ouboter and colleagues recently found that genes encoding flagellin (*fla*B) were upregulated when *Methanoperedenaceae* utilized a poised electrode in place of nitrate as the terminal electron acceptor[11]. As the poised electrode can provide a constant electron acceptor and also a platform for the static biofilm attachment rather than planktonic movement, their results are consistent with a conductivity function for the archaellum in long-range direct EET in *Methanoperedenaceae*. In comparison to filaments observed for other ANME clades[60], the filamentous structure has yet to be visualized in *Methanoperedenaceae*. Future studies are required to distinguish the potential conductive models (cytochrome nanowire vs. conductive archaellum) for long-range EET in *Methanoperedenaceae*.

Genomic analysis suggests that *Methanoperedenaceae* possess a pathway for acetate production[61], leading to the hypothesis that they may produce and secrete acetate as a diffusive intermediate and electron carrier, which can be fed to a syntrophic partner for a diffusion-dependent indirect EET pathway (Fig. 2b). This pathway was previously observed for methanogen-bacteria syntrophy, in which acetate was proposed to be produced from AOM by engineered methanogens[62,63] and was utilized by *Geobacter* for electrode reduction. A similar acetate-linked interaction has been proposed between *Methanoperedenaceae* and *Geobacter*[33,64]. However, isotopic tracer experiments revealed that acetate was not directly derived from methane but from the fermentative degradation of intracellular or extracellular polymeric substances of *Methanoperedenaceae* biomass[33]. These findings suggest that a diffusion-dependent syntrophic pathway may not be obligate for EET in *Methanoperedenaceae*. A likely explanation is the comparatively high abundance of EET-relevant genes (MHCs) in *Methanoperedenaceae* relative to methanogens to favor a direct EET mode[65,66]. This evolutionary direct EET pathway could enable more favorable energy conservation of *Methanoperedenaceae*, as energy loss caused by the diffusive flux of intermediates can be avoided. Thus, direct EET may be advantageous when *Methanoperedenaceae* directly interact with insoluble, spatially structured electron acceptors (e.g., Fe(III)/Mn(IV) oxides and electrodes). However, the acetate-dependent indirect EET pathway could not be fully ruled out, which could be advantageous in specific niches or under certain conditions by overcoming long-range spatial electron transfer. For instance, when electron acceptors are dispersed, poorly accessible, or present in forms that constrain direct physical contact, producing a diffusible intermediate may enable *Methanoperedenaceae* to offload reducing equivalents to proximal syntrophic partners, thereby bypassing the geometric constraints of long-range electron conduction.

Environmental adaptation

The widespread occurrence of *Methanoperedenaceae* across diverse natural and engineered ecosystems reflects their remarkable physiological adaptability to complex environmental conditions, including variations in salinity, oxygen, temperature, and pH. So far, most *Methanoperedenaceae* have been identified in freshwater environments or enriched in laboratory bioreactors with freshwater inoculum sources. Thus, salinity has long been thought to be a differentiating factor for *Methanoperedenaceae* from other ANME clades that are

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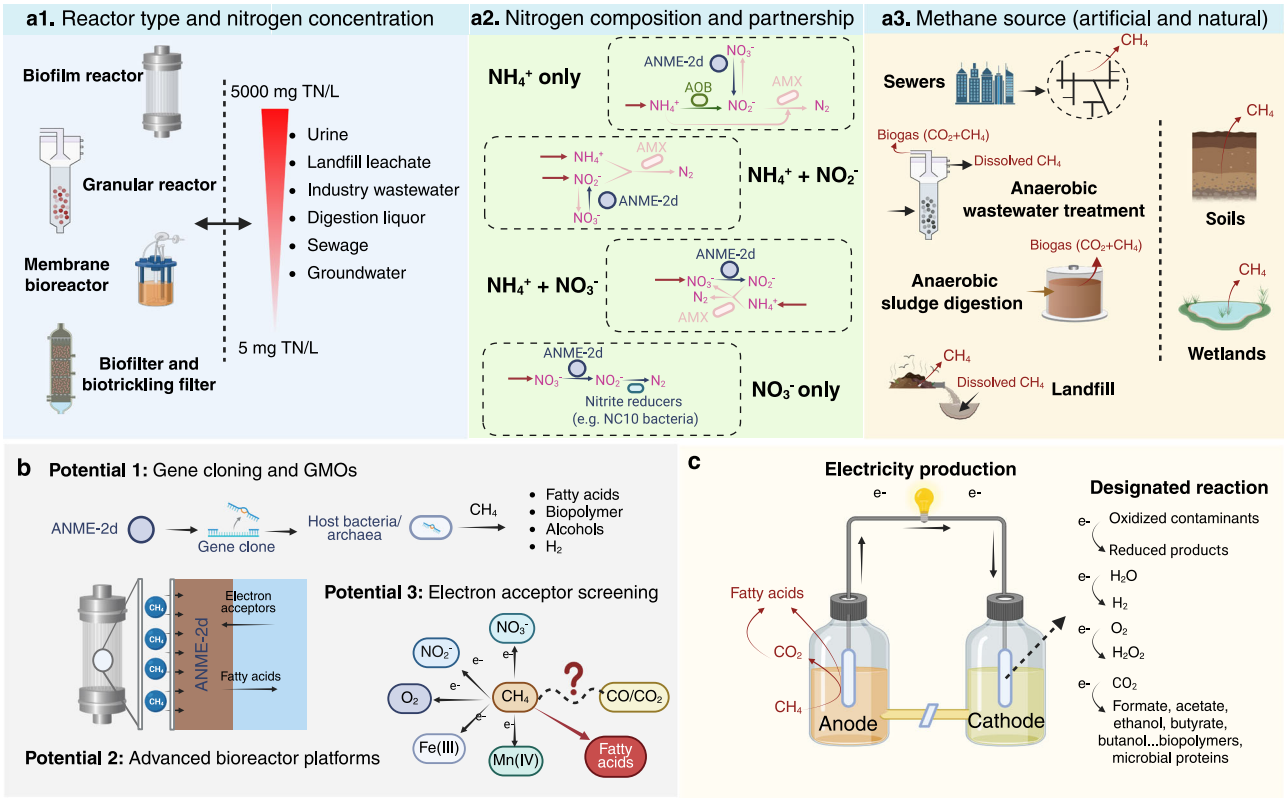


Fig. 3 | Engineering progresses of *Methanoperedenaceae* archaea. *Methanoperedenaceae* archaea have been explored for a range of environmental and biotechnological applications, including water and wastewater treatment, greenhouse gas mitigation, and the production of value-added chemicals. In the context of water and wastewater remediation (**a**), *Methanoperedenaceae* have been employed in various reactor configurations to facilitate nitrogen removal across a wide spectrum of compositions and concentrations. Both gaseous and dissolved methane serve as viable substrates to support their metabolic activity. For methane mitigation, *Methanoperedenaceae* offer flexible solutions targeting both point-source and non-point-source emissions. For the production of value-added products (**b**), advanced bioreactor design, optimal electron acceptor selection, and gene cloning and genetically modified organisms (GMOs) serve as potential strategies to enhance the production rate. Bioelectrochemical technologies provide a platform that can address these targets and offer distinct advantages by enabling independent regulation of oxidation and reduction processes (**c**). Created in BioRender. Liu, T. (2026) https://BioRender.com/p4uh04i.

usually derived from marine environments. However, recent field studies have identified *Methanoperedenaceae* in marine sediments[67–69], and several *Methanoperedenaceae* species that were originally enriched at low-salinity conditions were later shown to have tolerance to high-salinity conditions[16,17,70]. Physiological and genetic evidence have demonstrated that *Methanoperedenaceae* employ the osmolyte Nε-acetyl-β-L-lysine as a salinity adaptation mechanism, with genes encoding this osmoregulatory pathway widely distributed across the lineage[16]. Beyond salinity, *Methanoperedenaceae* also show resilience to oxygen exposure (5% v/v in the headspace), with elevated expression of reducing enzymes likely contributing to cellular repair mechanisms under oxidative stress[71]. Furthermore, recent findings indicate that freshwater *Methanoperedenaceae* can adapt to long-term sulfide exposure, potentially through sulfide detoxification mediated by Group III sulfite reductases (Dsr-LP)[72]. These findings collectively highlight the ecological plasticity and robustness of *Methanoperedenaceae*, enabling their occurrence across a wide range of habitats.

Emerging genetic and physiological traits

Recently, additional genetic and physiological traits have been reported. *Methanoperedenaceae* broadly host diverse extrachromosomal genetic elements (Fig. 2d), including Borgs[14], viruses[58], and plasmids[15]. These extrachromosomal elements carry genes with distinct evolutionary origins, and have been speculated to contribute to gene acquisition of *Methanoperedenaceae* via HGT, thus augmenting or extending their energy metabolism and the conditions under which they can function[13]. Another new finding is that *Methanoperedenaceae* show a pleomorphic life cycle with three distinct morphotypes, including archetypal coccobacilli microcolonies, planktonic rods, and coccobacilli cells with PHA stored[58]. This morphotypic variation may aid their survival and dispersal in the face of sub-optimal environmental conditions.

Progress in engineering applications in the past 20 years

Water and wastewater remediation

Nitrogen is a common contaminant in a wide range of water and wastewater streams, and the use of *Methanoperedenaceae* is being explored for nitrogen remediation using methane. Since the first process demonstration in 2013[73], *Methanoperedenaceae*-driven processes have been extensively demonstrated in different scenarios. A critical engineering challenge across these processes is optimizing biomass retention within bioreactors, given the inherently slow growth rate of *Methanoperedenaceae*. Engineering solutions include specialized reactor designs (Fig. 3a), such as biofilm reactors[20,21,74–80], granular reactors[22,23,81–83], membrane bioreactors (MBR)[84–86], and biofilters/biotrickling filters[74]. Such approaches have demonstrated encouraging volumetric reaction rates, reaching levels suitable for application, such as 1.2 kg NO_3^--N m^{-3} d^{-1} achieved in a granular system[82]. Based on these reactor configurations, numerous processes have been developed for the treatment of water and wastewater with different nitrogen compositions (Fig. 3a), e.g., ammonium only[76,77], ammonium and

nitrite[20,82,87–89], ammonium and nitrate[73,79,90], and nitrate only[91]. Different nitrogen compositions facilitate different microbial collaborations. For instance, in ammonium-only wastewater (e.g., sewage and sludge digestion liquor), ammonia-oxidizing bacteria (AOB) first oxidize approximately 50% ammonium to nitrite, which are then removed by anammox bacteria with nitrate as a byproduct. *Methanoperedenaceae* subsequently reduce the nitrate back to nitrite, which is reintroduced into the anammox process, thereby achieving nearly complete nitrogen removal.

These processes have exhibited flexibility in removing nitrogen from high-strength streams containing up to 5000 mg N L^{-1}[87] to low-strength streams with concentrations as low as 5 mg N L^{-1}[91], showing their versatile applicability in practice. Two primary methane sources are identified for these processes: gaseous methane in biogas and dissolved methane present in some liquid streams (Fig. 3a). While biogas offers a more concentrated supply of methane, it needs to be first dissolved in liquid and its low solubility poses a technical challenge. Efficient gas-to-liquid transfer often necessitates specialized designs, such as using hollow fiber membrane modules to enhance the methane transfer efficiency[92]. A previous plant-scale mass balance analysis indicates that approximately 5% of the biogas generated from sludge digestion in a typical domestic wastewater treatment plant would be sufficient to support both mainstream and sidestream nitrogen removal through the coupled activity of anammox and n-DAMO microorganisms[18]. Alternatively, wastewaters that undergo anaerobic treatment or originate from anaerobic environments often contain saturated or even supersaturated levels of dissolved methane[93,94]. These include effluents from anaerobic wastewater treatment (e.g., anaerobic lagoons, upflow anaerobic sludge blanket reactors, and anaerobic membrane bioreactors), wastewater in sewers, anaerobic sludge digestion liquor, and landfill leachate. The up-scaling of *Methanoperedenaceae*-driven nitrogen removal processes is highly expected in the near future.

Methanoperedenaceae have also been employed to remove other contaminants, including perchlorate, bromate, selenate, arsenate, and chromate, with methane as the electron donor[35,37,91,95,96]. While it was proven feasible, contaminant removal rates were relatively low in most cases, e.g., <8 mg Se L^{-1} d^{-1} for selenate removal. Furthermore, long-term operations revealed considerable shifts in microbial community, with *Methanoperedenaceae* decreasing to low abundance or even disappearing in several instances[91]. This raises critical questions about the actual role of *Methanoperedenaceae* in the removal of these oxyanions. While *Methanoperedenaceae* may couple AOM with the direct reduction of oxyanions via obligate reductases and direct EET, they may also interact with other bacterial species through the diffusion-dependent indirect EET and cross-feeding organic intermediates. Indeed, fatty acids have been detected in some of these processes, potentially as intermediate electron donors in these cross-feeding interactions.

Greenhouse gas mitigation

As a potent atmospheric greenhouse gas, methane has a global warming potential 28–36 times that of carbon dioxide over a 100-year horizon[97]. *Methanoperedenaceae* represent a potential agent to help mitigate methane emissions across various environmental and engineering settings. Within wastewater treatment plants, anaerobic processing of wastewater and sludge supports bioenergy recovery. However, 20–50% of the methane produced remains dissolved in effluent (10–30 mg CH_4/L) (Fig. 3a) and will be stripped into the atmosphere easily, contributing to greenhouse gas emissions. Two primary strategies leveraging *Methanoperedenaceae* archaea have been developed for dissolved methane mitigation in anaerobically treated wastewater. In the two-stage anoxic/oxic process, nitrate recirculated from the oxic stage drives dissolved methane removal by *Methanoperedenaceae* in the anoxic stage, as demonstrated in biofilms[78], granules[93], and MBR[84]. Alternatively, a one-stage process, which integrates AOB, anammox bacteria, and *Methanoperedenaceae* within a single reactor, can also remove dissolved methane from wastewater efficiently (>99%)[98]. Recently, research has explored strategies of dosing nitrate and nitrite to reduce methane emissions from sewer networks[94,99], another substantial source of methane emissions in urban areas. Notably, the volumetric methane oxidation rate up to 1 kg CH_4 m^{-3} d^{-1} was achieved in a *Methanoperedenaceae*-dominated granular system, which is sufficient to mitigate methane emissions in various anaerobic wastewater[82].

Diffuse or non-point methane emissions from landfills, soils, and wetlands may also be addressed by stimulating AOM in situ (Fig. 3a)[100–102]. For example, the application of biochar, a widely used soil amendment, has been shown to enhance AOM activity by *Methanoperedenaceae*, providing a potential strategy for methane mitigation in terrestrial systems[100,103]. For point sources where gaseous methane is too diluted for economic recovery or reuse, such as sewer off-gases and emissions from anaerobic lagoons, biofilters and biotrickling filters offer promising mitigation processes. These systems, widely used to control volatile sulfur compounds, are well-suited to incorporate *Methanoperedenaceae* for microbial methane abatement in the future.

Methane to value-added chemicals

Advances in drilling and extraction technologies have considerably expanded natural gas reserves, while the increasing anaerobic digestion of various organic feedstocks also leads to a growing availability of biogas. Gas-to-liquids (GTL) is a desirable process to enhance the value of methane. However, many methane sources are small and distributed, making centralized processing economically unfeasible. In this context, biological conversions, which operate under mild conditions, present a compelling alternative for transforming methane into biofuels and value-added bioproducts[104,105]. Aerobic methane oxidation, though extensively studied, is characterized by low carbon and energy efficiencies, as a large portion of carbon is converted to CO_2, and substantial energy is expended for biomass growth[106]. AOM, however, has shown promise for improved efficiencies. Genetic data suggest that *Methanoperedenaceae* may be able to produce acetate via the reductive acetyl-CoA pathway and produce formate by the reversible formate dehydrogenases (FdhAB) (Fig. 2). Further experimental studies with *Methanoperedenaceae*-dominated cultures have confirmed not only acetate production but also the accumulation of PHA, a polymer with potential applications in biodegradable plastic synthesis[26]. Nonetheless, for PHA production, the reaction rates reported so far are several orders of magnitude lower than those needed for industrial-scale application (at least 1 g L^{-1} d^{-1})[107,108], thus being considered unrealistic for these slow-growing anaerobic methanotrophs. Regarding GTL, the initially observed acetate productivity of *Methanoperedenaceae*-dominated cultures was only 1–2 mg L^{-1} d^{-1}[26], likely constrained by the methane mass-transfer efficiency, slow metabolism of *Methanoperedenaceae* and microbial community shifts.

However, opportunities exist to overcome these limitations (Fig. 3b):

- Advanced bioreactor platforms, such as membrane biofilm reactors (MBfRs), have demonstrated their capacity to enhance methane conversion rates. For instance, an MBfR seeded with an enriched *Methanoperedenaceae* culture achieved a production rate of 9.6 g L^{-1} d^{-1} for short-chain fatty acids from methane[109], nearly two orders of magnitude higher than previously reported rates and approaching the threshold required for industrial applications (12–120 g L^{-1} d^{-1})[110]. This substantial improvement was primarily attributed to the use of hollow fiber membranes, which enabled high methane flux and effectively formed densified biofilms. Reactor optimization (e.g., membrane material, surface area-to-volume ratio, and hydrodynamic conditions) is expected to further improve the production rate, while operational

optimization (e.g., pH and oxygen concentration) is anticipated to improve product selectivity.

- Enhancing methane bioconversion can be achieved by optimizing electron acceptors. In a study using enriched *Methanoperedenaceae* cultures as inoculum, researchers showed that the addition of trace oxygen as an additional electron acceptor triggered the methane conversion to fatty acids[111]. However, the electron balance indicated a notable deficiency in electron acceptors, which led to the hypothesis that the previously neglected CO_2 may serve as additional electron acceptors ($CH_4 + CO_2 \rightarrow CH_3COOH$; $\Delta G_0' = 36\ kJ\ mol^{-1}$ of CH_4). Additional ATP is required to trigger this reaction, which might be derived from methane oxidation with nitrate. This CO_2 utilization has important implications for methane-supported biotechnology, as it enhances the carbon efficiency by incorporating both CH_4 and CO_2 into products.
- Genetic and metabolic engineering of ANMEs is currently infeasible due to the absence of isolated ANME strains and challenging growing conditions. However, ANME genes can be used to generate potentially useful genetically modified organisms. For example, introduction of ANME-1 methyl-coenzyme M reductase (Mcr) genes into the methanogen host *Methanosarcina acetivorans*[112] allowed this engineered organism to perform reverse methanogenesis, converting methane to acetate at a rate of 2.0 mmol L^{-1} d^{-1}. Further metabolic engineering enabled the modified *M. acetivorans* to produce lactate with a yield of 0.1 mol lactate/mol methane[113], representing a tenfold increase compared to aerobic lactate synthesis from methane[114].

In summary, recent studies have proven the GTL concept using *Methanoperedenaceae*. Interestingly, while these studies employed enriched *Methanoperedenaceae* cultures as inocula and showed the necessity of such cultures for initiating methane conversion to fatty acids, the relative abundance of *Methanoperedenaceae* decreased considerably in most experiments after extended operation[109,111]. To advance our understanding of anaerobic methane conversion to fatty acids, comprehensive molecular analyses are essential to uncover the biochemical pathways involved in methane transformation. These analyses should focus on stoichiometric pathways, particularly the roles of CO_2 and thermodynamically favorable electron acceptors (e.g., nitrate, Fe(III), and Mn(IV)) that may favor fatty acid synthesis by *Methanoperedenaceae*. Additionally, the microbial shift, and particularly the roles of the non-methanotrophic microorganisms enriched during fatty acid production, remain to be investigated. To mitigate undesirable community shifts, potential strategies include implementing more precise process control (e.g., carefully regulating trace oxygen, which can stimulate fatty acid production but destabilize *Methanoperedenaceae* if oversupplied), optimizing product separation to prevent inhibition and suppress the growth of heterotrophs fueled by accumulated fatty acids, and integrating *Methanoperedenaceae* into bioelectrochemical systems (BESs) that spatially separate redox reactions (see more details in the next section).

Bioelectrochemical technology

Due to their EET capabilities, *Methanoperedenaceae* have emerged as promising biocatalysts for CH_4-based bioelectrochemical applications. Indeed, the flexible configuration of BESs enables the tailored integration of *Methanoperedenaceae* for diverse applications (Fig. 3c), particularly for methane-to-electricity conversion, production of value-added chemicals, and advanced environmental bioremediation. For example, *Methanoperedenaceae* have been demonstrated as anodic biocatalysts in microbial fuel cells (MFCs) for bioelectricity generation[11,32,34,115]. In contrast to conventional systems relying on finite chemical electron acceptors for AOM, the use of electrodes in BESs offers an inexhaustible electron sink, as demonstrated in electro-fermentation systems for the sustainable production of formate and acetate[33,116,117]. Another key advantage of BESs lies in their compartmentalized design, which allows for the spatial separation of anodic AOM catalyzed by *Methanoperedenaceae* from cathodic reactions. This separation not only enhances product selectivity and diversification, leveraging well-characterized electrocatalysts at the cathode, but also enables operational control. In bioremediation contexts, such separation protects *Methanoperedenaceae* from direct exposure to toxic pollutants, which are instead targeted at the cathode by more resilient contaminant-reducing microbes. Nevertheless, despite the exciting prospects of employing *Methanoperedenaceae* in BESs, kinetic constraints remain. The inherently slow enzymatic activity associated with methane oxidation and electron transfer poses a critical bottleneck, highlighting the need for future research to focus on enhancing the catalytic efficiency of *Methanoperedenaceae* pathways in the anode to improve overall system efficiency.

Challenges and perspectives

Fundamental questions to be answered

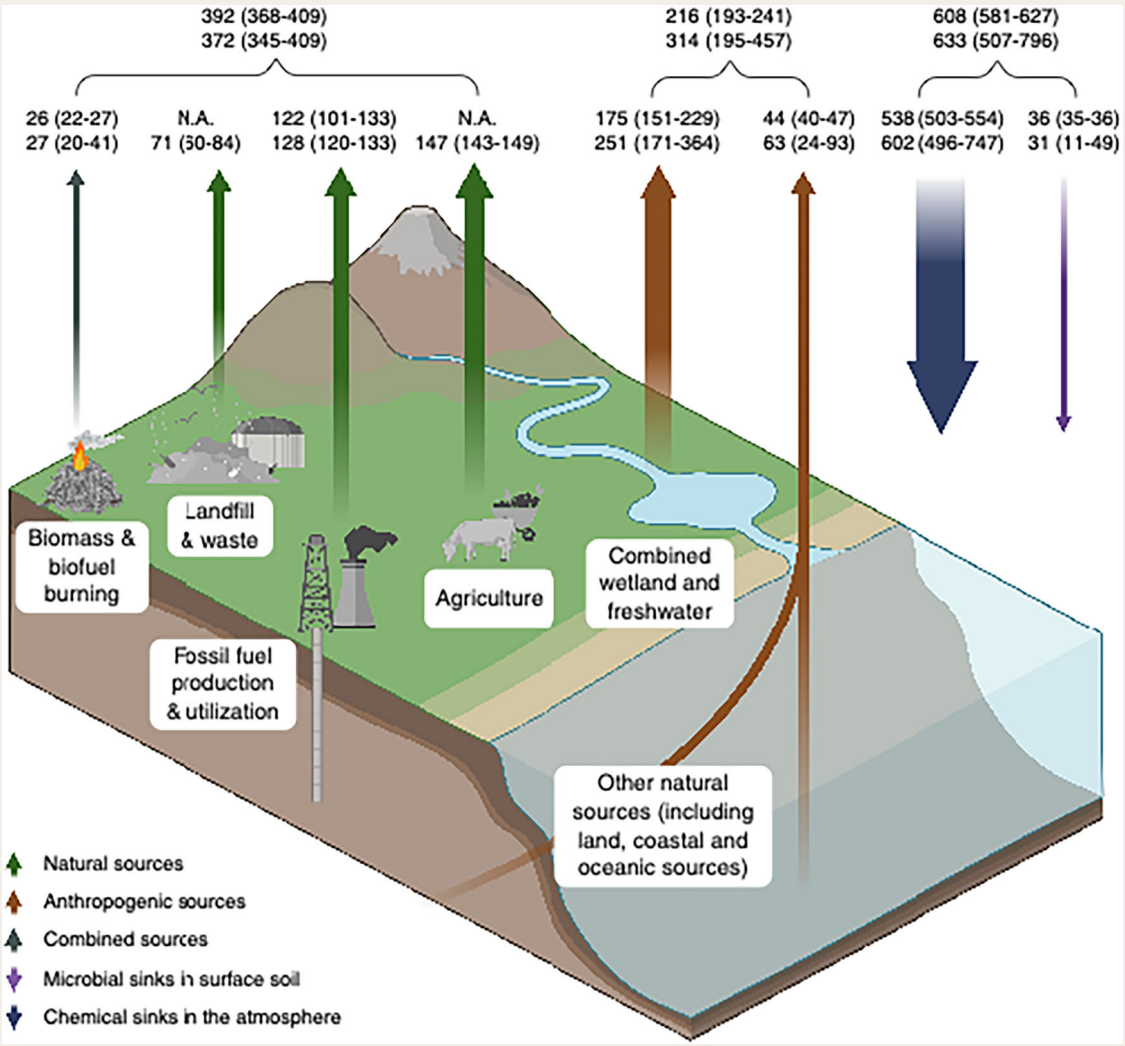
The absence of pure cultures remains one of the most critical obstacles to direct physiological and biochemical characterization. Although emerging techniques such as flow cytometry and fluorescence-activated cell sorting[118], as well as recent findings (e.g., antimicrobial intervention)[119], may provide opportunities for isolating strains, most fundamental advances are still expected to be achieved based on enriched mixed cultures. Critical knowledge gaps remain, presenting valuable opportunities for future investigation and exploration:

- Freshwater prevalence and beyond: as one of the most important greenhouse gases, methane is dynamically produced and consumed (approximately two-thirds by AOM) across a wide range of natural and human-influenced environments (Box 2). As the known phylogenetic and functional diversity of *Methanoperedenaceae* continues to expand, the ecological distribution and, more importantly, their contribution to the global methane budget should be revisited. Originally identified as nitrate-dependent anaerobic methanotrophs, members of this archaeal lineage are now recognized for their potential capacity to use a broad spectrum of electron acceptors and donors (some to be confirmed experimentally as shown in Fig. 2), suggesting that their environmental roles are likely being underestimated. Despite a few exceptions, most observations and nearly all enrichment of *Methanoperedenaceae* to date are derived from freshwater systems. The mechanisms driving this freshwater prevalence remain unresolved but may include physiological limitations in salinity tolerance, competitive exclusion in saline environments by other anaerobic methanotrophs, or variations in substrate availability across habitats. However, the recently reported adaptation strategies of *Methanoperedenaceae* against salinity and their co-occurrence with sulfate-reducing bacteria (SRB) encourage the investigation of *Methanoperedenaceae* in ecosystems other than freshwater[47,69,120,121].
- Confirming the versatility in electron donor and acceptor utilization: overall, the metabolic versatility and flexibility of *Methanoperedenaceae* represent an opportunity, providing both a biological advantage and an engineering leverage in natural and engineered systems where the availability of electron acceptors and donors fluctuates spatially and temporally. From an evolutionary perspective, this versatility may result from environmental selection pressures, enabling *Methanoperedenaceae* to thrive under fluctuating redox conditions. However, direct physiological evidence supporting these pathways remains limited and underlying biochemical mechanisms remain largely speculative. Importantly, the presence of relevant genes, or even transcriptional activity, does not equate to bona fide functional capability. Thus, providing rigorous physiological evidence instead of merely

BOX 2

Global sources and sinks of methane

The estimated methane fluxes (Tg CH_4 $year^{-1}$) for each methane source and sink category are based on the 2020 data reported by Saunois et al.[159]. Values are presented as the average with the associated range (maximum-minimum). Values derived from top-down approaches based on atmospheric observations and inverse modeling are presented at the top, and those derived from bottom-up approaches based on inventories, process-based models, and field measurements are presented at the bottom. It is important to note that the microbial sink in these estimates refers exclusively to the sink of atmospheric methane: ~90% by the reaction with hydroxyl radicals, with the rest by microbial oxidation in surface soils, reactions with chlorine radicals and atomic oxygen radicals in the stratosphere, and reactions with chlorine radicals from sea salt in the marine boundary layer[158]. The microbial consumption occurring at the source, as depicted in Box 1, is not accounted for. For example, in freshwater wetlands, AOM is estimated to consume approximately 200 Tg CH_4 $year^{-1}$, a value nearly equal to the annual methane emissions from wetlands themselves[160] but not presented in the figure. Likewise, in ocean settings, one of the reasons for the low methane emissions is that AOM consumes more than 90% methane produced from the seafloor[161–163], making the ocean being only a minor methane source (< 2% of the global flux). Figure of Box 2 was created in BioRender. Liu, T. (2026) https://BioRender.com/p4uh04i.



relying on genomic analysis would be critical to verify these putative pathways. Such evidence would clarify the environmental triggers and regulatory mechanisms governing their activation, determine their contribution to energy conservation and growth, and evaluate whether they can sustain long-term growth or lead to shifts in microbial community structure over time.

- A better understanding of EET mechanisms: while recent studies have confirmed the role of MHCs in mediating EET in *Methanoperedenaceae*, the full picture of EET remains incompletely resolved. Critical gaps persist in understanding the spatial organization of MHCs, particularly for those enabling long-range EET via putative conductive cellular appendages such as archaella or

nanowires. High-resolution imaging techniques, such as cryo-electron microscopy and atomic force microscopy, can be leveraged to determine whether these conductive appendages exist and serve as physical conduits for long-range electron transport. Second, differentiating between the non-syntrophic direct EET pathway and the diffusion-dependent indirect EET pathway requires more physiological studies. To differentiate these two pathways, multi-omics should be combined with bioelectrochemical monitoring to capture the dynamic regulation of EET pathways in real-time. Stable-isotope tracing can clarify whether acetate originates from methane-derived carbon or from biomass turnover and whether it serves as an active electron-transfer intermediate. Beyond individual physiology, the ecological implications of EET merit attention, as electron flow through *Methanoperedenaceae* may influence the assembly, structure, and function of surrounding microbial communities. Field studies, microcosm experiments, and community-scale modeling efforts may help assess the broader biogeochemical roles and environmental relevance of EET.

- Adaptation strategies: *Methanoperedenaceae* inhabit a range of dynamic and often harsh anoxic environments, requiring effective adaptation strategies to cope with variable redox conditions, nutrient availability, and environmental stressors. Previous studies suggest that these archaea may employ diverse mechanisms[16,17,58,71,72], such as storage compound production and consumption, osmoprotectant production, morphological and phenotypic variation, and metabolic pathway rewiring, to survive in fluctuating environments. Notably, many of these strategies appear to be linked with each other. For example, against high salinity or sulfide exposure, *Methanoperedenaceae* consume intracellular PHA and lean towards shifting from PHA-abundant granular shape to planktonic free-living rod-shaped morphotypes. Further explanation for these observations is expected to better understand the adaptation strategies. Moreover, the ability to form biofilms and to potentially associate with syntrophic partners may confer more stability and resilience in complex microbial communities.
- Extrachromosomal genetic elements and their functional roles: recent genomic analyses suggest that extrachromosomal genetic elements may harbor key genes involved in EET, nitrogen and methane metabolism, and stress response, potentially conferring selective advantages to their hosts. However, the specific gene content of *Methanoperedenaceae*-associated extrachromosomal genetic elements, their mechanisms of replication and transmission, and their roles in facilitating HGT remain unclear. Of particular interest is how extrachromosomal genetic elements mediate the acquisition and expression of auxiliary metabolic genes, thereby enhancing the versatility of their host's metabolic repertoire. Despite these intriguing genomic insights, the actual physiological roles of extrachromosomal genetic elements require experimental validation through targeted studies, such as phenotypic assays and in vitro tests. Moreover, the application of advanced imaging techniques could provide visual confirmation of the presence, localization, and potential dynamics of extrachromosomal genetic elements.

Engineering challenges to be addressed

Engineering challenges remain significant for the application of *Methanoperedenaceae*. One primary constraint is their inherently slow growth rate, with an estimated doubling time (t_d) of weeks[6,29], indicating the need for an extended solid retention time (SRT) to ensure their retention and functionality within bioreactors, as elaborated above and delineated in Fig. 3a. In addition to the approaches that have been tested, cell immobilization techniques, i.e., gel entrapment and encapsulation, could be alternatives, as previously applied for other slow-growing archaea, such as ammonia-oxidizing archaea[122] and methanogenic archaea[123]. Overall, these engineering efforts have advanced the application of the slow-growing *Methanoperedenaceae*, transforming what was once deemed "impossible" into "possible". Further exploration, optimization and scaling-up of these approaches represent important future research directions for *Methanoperedenaceae*-based biotechnologies.

Nonetheless, although these "top-down" strategies have achieved high volumetric rates by accumulating greater densities of *Methanoperedenaceae*, they do not directly address their underlying slow growth rate. Consequently, the start-up phase required for achieving such high volumetric rates is often as long as months or even years[19,82], especially when the initial *Methanoperedenaceae* cultures are limited or unavailable. This triggers an intriguing question: can we enhance the inherent growth rate and shorten the doubling time of *Methanoperedenaceae*? Several examples suggest that the answer may be affirmative. Anammox bacteria, initially thought to have doubling times exceeding 10 days, have recently been shown to double every 2.1 days[124,125]; and bacterial anaerobic methanotrophs ("*Ca*. Methylomirabilis lanthanidiphila"), originally estimated to double every 2–8 weeks, have seen this reduced to just 5 days in a recent study[126]. Understanding the reasons behind the faster growth rates requires a "bottom-up" analysis of the microbial-specific growth rate (μ):

$$\mu = q_{\max} \times Y \times S/(S + K_S) - k_d$$

where $q_{\max}$ is the maximum specific substrate uptake rate (g-NO_3^--N g-VSS^{-1}d^{-1}), Y is the biomass growth yield (g-VSS g-NO_3^--N^{-1}), S is the substrate concentration (e.g., nitrate concentration, mg N L^{-1}), Ks is the substrate affinity (e.g., nitrate affinity constant, mg N L^{-1}), and k_d is the decay rate (d^{-1}). The doubling time (t_d) serves as a measurable indicator of microbial growth performance and is directly linked to μ ($t_d = \ln 2/\mu$). Thus, to shorten t_d and to increase μ, a change in one or more terms must occur. For example, when $q_{\max}$ or Y is doubled, the resulting increase in μ leads to a halving of the doubling time. For the substrate term (S), due to the inherently low methane affinity of these microorganisms, the Monod expression $S/(S + K_S)$ approaches a first-order form ($k \times S$) when methane concentrations are below 100 mg CH_4 L^{-1}[127]. Under this condition, doubling S effectively doubles μ, likewise resulting in a halved doubling time.

In this context, we propose several potential strategies for future investigation (Fig. 4):

- Increasing Y: the microbial yield reflects the stoichiometric link between anabolism and catabolism, governed by the proportion of energy available for biosynthesis after subtracting maintenance and dissipation losses. Given that the catabolic energy yield of *Methanoperedenaceae* is relatively fixed under defined environmental conditions, increasing the microbial yield can be achieved either by minimizing energy demands for cellular maintenance or by improving the energy transfer efficiency. The maintenance energy demand, for cell functions such as motility, osmotic regulation and heat loss, is typically accounted for in the endogenous decay coefficient k_d[128], and can be minimized by avoiding inhibitory factors, for example, oxygen[71], salinity[16,17], and extreme pH[129]. The electron transfer efficiency is assumed to be relatively low in *Methanoperedenaceae*, due to the spatial separation between the cytoplasmic electron generation (via reverse methanogenesis) and pseudoperiplasmic electron acceptance (e.g., nitrate reductase). This long electron transport chain involves a multi-compartmental series of redox carriers, including cofactor F_{420} in the cytoplasm, quinones in the cytoplasmic membrane and cytochrome c in the pseudoperiplasm, while the membrane-bound electron transport chain includes $F_{420}H_2$ dehydrogenase and an atypical Rieske/cytochrome b complex[39]. Targeted genetic or metabolic engineering to streamline electron flow could

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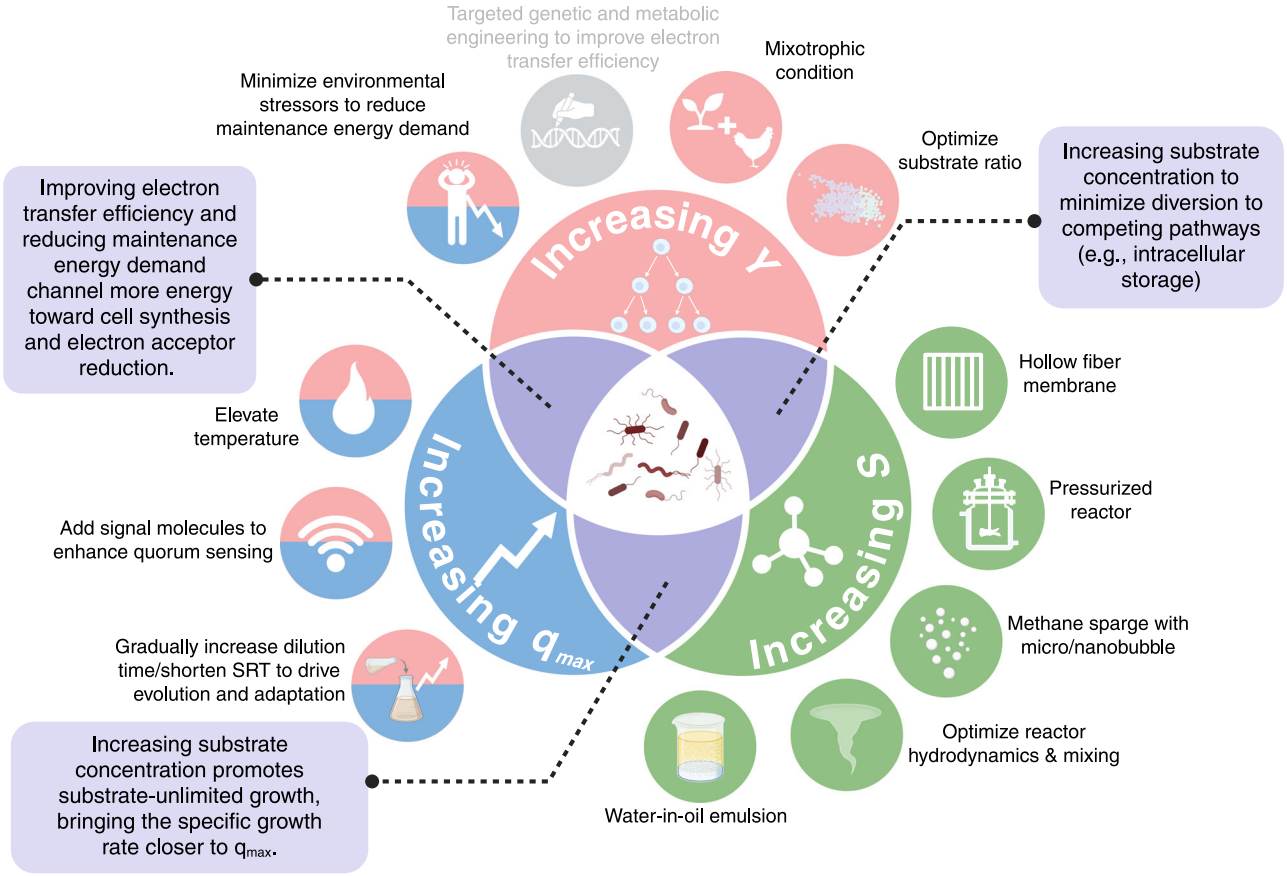

Fig. 4 | Potential strategies to enhance the growth rate of *Methanoperedenaceae*. Please note that these strategies are proposed based on theoretical analysis, and many have not yet been experimentally validated for *Methanoperedenaceae*. The authors remain cautious about the feasibility of certain approaches, such as genetic and metabolic engineering. Created in BioRender. Liu, T. (2026) https://BioRender.com/p4uh04i.

theoretically improve electron transfer efficiency and thus the biomass yield[130,131]. However, implementing such interventions in mixed cultures, particularly targeting the archaeal cells, remains unrealistic to achieve. Regarding *Methanoperedenaceae* living on EET (e.g., metal-dependent *Methanoperedenaceae*), improving the EET process by adding electron shuttles (e.g., humic acids and biochars) could be a potential solution to improve the energy transfer efficiency and thus the yield.

- Increasing q_{max}: the maximum specific substrate uptake rate (q_{max}) is also related to energy/electrons produced and the proportion directed to cell synthesis[128]. Thus, improving the electron transfer efficiency mentioned above should also contribute to the improvement of q_{max}. Additionally, temperature plays a significant role in regulating q_{max}. Empirically, a 10 °C increase in temperature can approximately double q_{max} ($q_T = q_{20} \times 1.07^{(T-20)}$). As shown in short-term tests, the nitrate reduction rate of an enriched *Methanoperedenaceae* culture increased by ~3 times from 27 °C to 35 °C[129]. Hence, elevating the operational temperature, within limits that avoid thermal inhibition, can serve as an effective strategy to enhance q_{max} of *Methanoperedenaceae*. Moreover, metabolic adaptation or directed evolution through long-term reactor operation may also contribute to an increased q_{max}. One potential method is the gradual reduction of SRT or the gradual increase of dilution time in chemostat systems. Shortening SRT has been shown to impose selective pressure that upregulates key functional genes involved in metabolism and electron transfer, which has been used to enrich fast-growing aerobic heterotrophic bacteria[132], anammox bacteria[124], methanogenic archaea[133], and many others. Another potential strategy is to add signaling molecules, which regulate the expression of related genes. Very recent results showed that adding C6-HSL (0.2 μM), a key acyl-homoserine lactone signaling molecule, immediately enhanced *Methanoperedenaceae* activity by over 65%[134].
- Increasing *S*: to ensure that the net growth rate approaches the maximum value, the substrate availability is essential. Particularly, *Methanoperedenaceae* exhibit an extremely low affinity for methane, with first-order kinetics observed at methane concentrations below 100 mg CH_4 L^{-1}[127]. This poor affinity indicates that methane, the key electron donor driving catabolic energy production, is often a limiting factor under ambient conditions. Indeed, the low methane affinity appears to be a common physiological feature of ANMEs, which all use the reverse methanogenesis pathway to activate methane[135], and increasing methane pressure has been proven effective in increasing the activities of ANMEs almost linearly[136,137]. This might explain the favorable use of pressurized hollow fiber membranes to enrich *Methanoperedenaceae*, as the pressurized methane inside fibers (1.1–2.3 atm) leads to high methane concentrations close to the membrane surface. Moreover, custom-designed high-pressure bioreactors, allowing safe supply of methane at pressures exceeding 200 atm[136,137], could serve as a promising microbial factory for accelerating the growth of *Methanoperedenaceae*. Nonetheless, at such high hydrostatic pressure, whether the archaeal cells will undergo deformation and whether the metabolic pathway will vary (e.g., towards intracellular compound storage) require further investigation. Additionally, to increase mass transfer rates, vigorous mixing or agitation is typically used to create small gas bubbles and good hydraulic conditions that

provide increased gas–liquid interfacial area. Some studies also proposed using water-in-oil emulsion to increase the methane solubility[138].

Another engineering interest is the development of effective preservation and reactivation strategies for *Methanoperedenaceae* biomass. Preservation is critical for maintaining biomass viability during periods of operational downtime, minimizing reactivation lag, and ensuring rapid system recovery. General preservation methods include refrigeration, freezing, lyophilization, immobilization, or any combination of these. Investigating the preservation approach will drive research into understanding the decay mechanisms of *Methanoperedenaceae* under various environmental and operational conditions. The decay of *Methanoperedenaceae* is hypothesized to involve two distinct processes: (1) activity decay, where the metabolic functions of the archaea decline without immediate cell death, potentially reversible under favorable conditions, and (2) archaeal death, where cells undergo irreversible degradation and loss of viability. Neither of these decay processes nor the conditions influencing them has been investigated.

The story of *Methanoperedenaceae* inspires more studies into the underexplored archaeal domain

The archaeal contribution to global biogeochemical cycles, as well as their phylogenetic, functional, and physiological diversity, is substantially underestimated compared to their bacterial counterparts. This is largely due to the limited availability of tools to study these low-abundance and hard-to-cultivate microorganisms. While culture-independent techniques leveraging advanced sequencing methods can shed light on some potential, culture-based methodologies still remain of interest and indeed indispensable. The study of *Methanoperedenaceae* offers a compelling lens through which to explore the broader, often enigmatic, archaeal domain, emphasizing the need to:

- Explore archaeal diversity in moderate environments: early archaea research was predominantly associated with extreme environments, such as hypersaline lakes[139], high-temperature terrestrial springs[140], and hydrothermal vents in the deep sea[141], that were thought to be inhospitable to most forms of life[142]. This perception limited their perceived importance in "conventional" ecosystems and hindered a comprehensive exploration of their ecological contributions. Indeed, ANMEs were also initially discovered in marine ecosystems with high salinity, high osmotic pressure, likely extreme temperatures and oligotrophic conditions. The subsequent discovery of *Methanoperedenaceae* from freshwater sediments with moderate conditions challenges this view, exemplifying how archaea, once overlooked, can play important roles in such settings. Notably, accumulating evidence now highlights that diverse archaeal groups are widespread in non-extreme environments, including conventional terrestrial soils, freshwater ecosystems, and anthropogenically influenced environments, where they play key roles in carbon, nitrogen, and metal cycling. Continued exploration across these moderate environments is likely to reveal additional archaeal lineages and provide a more comprehensive understanding of their contributions to key biogeochemical processes.
- Explore archaeal functional diversity and evolution: many biochemical reactions now attributed to archaea, such as nitrate reduction by *Methanoperedenaceae*, would not have been possible in the early Earth's environment due to the absence of such substrates. This indicates that many archaea have acquired their metabolic repertoire over billions of years of evolution, often through mechanisms such as HGT and retained via vertical gene transfer[7,38]. While the processes that led to this remarkable functional diversification occurred over geological timescales and cannot be mimicked or repeated in laboratory settings, understanding the evolutionary pathways and adaptive strategies of archaea remains profoundly important[143]. While advanced molecular techniques and new sequencing approaches in conjunction with powerful bioinformatics are capable of shedding light on this evolutionary trajectory, emerging machine learning techniques, already applied to predicting protein structure and direct protein engineering[144,145], hold potential for elucidating long-term evolutionary trends.
- Integrate interdisciplinary approaches: no isolates of ANMEs are currently available despite tremendous efforts; thus, most knowledge of this archaeal group comes from field measurements, enrichment cultures, and genomic analyses. This suggests that to fully understand and utilize archaea, it is essential to integrate perspectives from ecology, biochemistry, microbiology, and engineering. Ecological studies conducted on-site can elucidate the natural distribution and potential contributions of archaea to the studied site. Microbiological and genomic analyses could shed light on the enzymatic pathways and regulatory mechanisms that underpin their metabolic versatility, which could be partially corroborated by biochemical research. Engineering disciplines can facilitate the design of reactors and optimize operational conditions for their enrichment and translate these fundamental findings into applications. Moreover, leveraging computational tools like machine learning and system biology can model archaeal interactions within complex microbial consortia, predicting their behavior under various environmental scenarios. The interdisciplinary approach is expected to unlock the full potential of archaea, bridging the gap between fundamental research and applications.

The lessons learned from *Methanoperedenaceae* in the past 20 years not only expand our understanding of a single archaeal family, but also lead to a shift in how we view and study the archaeal domain. By addressing the gaps in our knowledge, we can unlock the untapped potential of archaea, advancing our understanding of life's origins and functionality on Earth and solving pressing environmental challenges.

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Competing interests

The authors declare no competing interests.

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