Progress in understanding the methane sink and methanotrophs in karst caves

Sitong Gong [a,b], Weijun Luo[a,c,d], Likai Hao[a], and Shijie Wang[a,c]

[a]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550081, China; [b]University of Chinese Academy of Sciences, Beijing, 100049, China; [c]Puding Karst Ecosystem Research Station, Chinese Academy of Sciences, Puding, 562100, China; [d]College of Rural Revitalization Research Center of Guizhou, Anshun, 561000, China

Corresponding author: **Weijun Luo** (email: luoweijun@vip.gyig.ac.cn)

Abstract

Karst caves are considered sinks for atmospheric CH_4 due to their sub-atmospheric CH_4 concentrations. Isotopic and molecular analyses suggest that methanotrophic bacteria (*MOB*) play an important role in consumption of CH_4 in caves. Previous research has established that the biogeographic patterns of microorganisms exist widely and is created and maintained by different ecological processes. Considering the great potential of cave *MOB* in CH_4 oxidation, understanding the assembly and working mechanisms will have practical implications for understanding and utilizing cave ecological resources. In this review, we have summarized the information collected thus far on CH_4 sources and sinks in caves, *MOB* phylogeny, and *MOB* ecophysiology. We applied this knowledge to discuss the factors that may influence the composition and distribution of cave *MOB* communities in the context of karst geology. Finally, we review the relevant methods and theories and discuss the opportunities and challenges involved in the predictive description of microbial processes of cave CH_4 consumption.

Key words: karst, cave, methane (CH_4), methanotrophic bacteria, environmental drivers, biogeochemistry

1. Introduction

Methane (CH_4) is currently the second-most abundant greenhouse gas in the atmosphere and has a much higher warming potential than carbon dioxide (CO_2) (IPCC 2013; Tollefson 2022). Global warming may result in a 1.5 °C increase in global temperatures in the short term, which will inevitably lead to an increase in severe climate disasters and pose multiple risks to ecosystems. Limiting this warming to 1.5 °C is essential to future work on global climate change, and requires a reduction in greenhouse gas emissions (IPCC 2022). Recent research shows that the concentration of CH_4 in the atmosphere has reached 1900 ppb, which is nearly triple the pre-industrial levels, highlighting the importance of curbing CH_4 emissions (Tollefson 2022).

Caves associated with karst landscapes may be overlooked sinks for atmospheric CH_4. Karst rocks occupy approximately 20% of the Earth's ice-free landmass (Ford and Williams 2007, p. 5). Karst is used to describe a particular form of landscape, characterized by caves and extensive groundwater systems, particularly those developed from soluble rocks such as limestone, marble, and gypsum (Ford and Williams 2007, p. 1). Water–rock reactions occur in underground caves, fissures, and cracks connected to the surface, creating channels for the exchange of above-ground and sub-surface materials and air (Ford and Williams 2007). The amount of air exchange occurring in subsurface pores and crevices may be insignificant compared to that occurring in caverns. Therefore, sur-

face caverns may represent a widely distributed subsurface atmosphere (Fernandez-Cortes et al. 2015). In addition, caves serve as an ideal place for the study of subsurface atmosphere owing to their accessible entrances (Fernandez-Cortes et al. 2015).

Caves are considered an important sink for atmospheric CH_4 because of their sub-atmospheric CH_4 concentrations (Mattey et al. 2013; Fernandez-Cortes et al. 2015; Ojeda et al. 2019). Most studies support the involvement of methanotrophic bacteria (*MOB*) as the organisms primarily responsible for CH_4 oxidation in caves owing to the dominance of *MOB* communities in cave walls, sediments, and soils. Furthermore, the enrichment of $\delta^{13}C$-CH_4 in the cave atmosphere indicates the involvement of *MOB* (Mattey et al. 2013; Zhao et al. 2018; Ojeda et al. 2019; Cheng et al. 2021). *MOB* oxidize CH_4 to methanol using the methane monooxygenase (MMO), which is further oxidized to formaldehyde, formate, or CO_2 by appropriate dehydrogenases. This step is followed by carbon assimilation through ribulose monophosphate, serine, or Calvin–Benson–Bassham pathways (Semrau et al. 2010).

Previous studies have shown that four key ecological processes—selection, drift, diffusion, and diversification—play an important role in the assembly of microbial communities (Martiny et al. 2006; Hanson et al. 2012; Gao et al. 2020). Different ecological processes can interact to create and maintain microbial biogeographical patterns (Hanson et al. 2012). Multiple studies have shown that there is a

298

Environ. Rev. **31**: 298–309 (2023) | dx.doi.org/10.1139/er-2022-0034

strong exchange of material and energy (e.g., CH_4, Cu, N, C, and moisture content) in karst critical zones that results in the spatiotemporal heterogeneity of karst cave habitats (Hartland et al. 2012; Li et al. 2012; Ortiz et al. 2014; Webster et al. 2018). Environmental selection pressure can affect the balance between different ecological processes, which in turn affect the composition, distribution, and ecological functions of microbial communities (Ferrenberg et al. 2013; Gao et al. 2020).

Considering the great potential of cave *MOB* in CH_4 oxidation, understanding their biogeographical patterns and assembly mechanisms will have practical implications for explaining the spatial heterogeneity of cave CH_4 and utilizing cave ecological resources. However, the biogeographic pattern and stochasticity of cave *MOB* communities is rarely considered. In this review, we summarize the information collected to date on CH_4 fluxes in caves as well as the factors that may create and maintain *MOB* diversity in karst caves. In addition, we discuss the opportunities and challenges in studying the microbial processes of CH_4 oxidation in karst caves.

2. The cave sink for atmospheric methane

The area below the subsoil and above the water table is called the vadose zone. This zone is a domain for biogeochemical reactions, responsible for regulating gas exchange between the subsurface and atmosphere (Fernandez-Cortes et al. 2015). The subsurface layers in the vadose zone are characterized by a large amount of unconsolidated sediment, empty-water pores, caves, fissures, and cracks (Ford and Williams 2007; Ojeda et al. 2019). Significant gas exchange exists in caves compared to fissures and voids, which may represent a widely distributed subsurface atmosphere beneath terrestrial ecosystems (Fernandez-Cortes et al. 2015). Owing to extensive gas exchange and human-accessible portals, caves serve as ideal sites for in situ monitoring and mechanistic studies of cave CH_4 (Fernandez-Cortes et al. 2015).

Analysis of the air inside caves may reveal further details regarding the changes in cave CH_4 fluxes. For example, a previous study has observed CH_4 cycles to 1000 ppb in a few hours by tracing the aerodynamics of CH_4 in caves (Waring et al. 2009). Seasonal depletion of CH_4 has been observed in an Australia cave, from ambient ~1775 ppb to near zero during summer and to ~800 ppb in winter (Waring et al. 2017). High CH_4 consumption rates (from −0.1 to −0.4 ppm) for every 100 ppm increase in CO_2 levels has been observed in some caves in Spain (Fernandez-Cortes et al. 2015). A significant consumption of CH_4 has also been found in caves around the world, exceeding 2500 ppb in some of the US and Vietnam caves (Mattey et al. 2013; Nguyễn-Thuỳ et al. 2017; Webster et al. 2018; ojeda et al. 2019). Nguyễn-Thuỳ et al. (2017) estimated that 150 000 metric tons of atmospheric CH_4 are microbially oxidized annually in the ~29 000 km^2 of Vietnamese tropical karst.

Current studies suggest that CH_4 in caves has both atmospheric and microbial sources (Mattey et al. 2013; McDonough et al. 2016). Long-term tracking of the geochemical processes of CH_4 provides evidence that supports a microbial mechanism for CH_4 synthesis. The $^{12}C/^{13}C$ ratio of CH_4 is sensitive to changes in the production, oxidation, or transportation in the environment (Whiticar 1999). *MOB* preferentially consume $^{12}CH_4$, which results in ^{13}C enrichment of residual CH_4, while methanogenesis results in the enrichment of $^{12}CH_4$ (Whiticar 1999). According to a 4 year monitoring study, there are seasonal fluctuations in the abundance of $^{13}CH_4$ and the concentrations of CH_4 in caves. In addition, an analysis of the $^{13}CH_4$ depletion suggested that CH_4 from the microbial pathway frequently enters caves (Mattey et al. 2013). Similarly, the variation in the abundance of $\delta^{13}C$-CH_4 (−54‰ to −93‰) in boreholes in the vadose zone provides direct evidence of the contribution of microorganisms to CH_4 production (Ojeda et al. 2019). Based on carbon and hydrogen stable isotope evidence for CH_4, the exogenous CH_4 in the caves may be produced through acetic acid fermentation and CO_2 reduction (Whiticar 1999; Webster et al. 2016). Methanogens have a competitive advantage in anaerobic and humid environments such as soil voids and bedrock fissures (Angel et al. 2012; Mattey et al. 2013). Several researchers have reported that CH_4 in caves may partly stem from the surrounding bedrock cracks and soil pores (Mattey et al. 2013; Ojeda et al. 2019). Soil samples from the soil layer above caves have been reported to harbor methanogenic bacteria, and a comparatively high relative abundance (5.1%) of the obligate acetoclastic *Methanosaeta sp.* shows that the acetate pathway is significant in the subsoil (McDonough et al. 2016). In addition, the concentrations and stable isotope abundances of CO_2 in the cave reflect the residence time of air and the ventilation pattern of the cave; therefore, they are often employed to track the cave ventilation-mediated change in CH_4 concentrations (Fernandez-Cortes et al. 2015). Season and temperature play an important role in the ventilation of caves, when the cave temperature is higher than that of the outside atmosphere as a result of alternating seasons, the outside air is denser than the cave air, thus forcing the outside air into the cave (Fernandez-Cortes et al. 2015). It is not surprising that this process reverses as the seasons alternate. Furthermore, a negative correlation between CO_2 and CH_4 concentrations is a common phenomenon in a cave environment (Mattey et al. 2013; Webster et al. 2016; Webster et al. 2018; Ojeda et al. 2019). These findings imply that as seasons change, CH_4 from the outside environment enters via cave ventilation and is consumed in the caverns. This methane-depleted air is exchanged with the surrounding air.

A previous study suggested that the consumption of CH_4 in caves could be related to radiolysis, because researchers identified a significant correlation between CH_4 and ion concentration in cave air, but no obvious microbial intervention was identified (Fernandez-Cortes et al. 2015). However, a subsequent study has disproved this conclusion. Lennon et al. (2017) mixed powdered uranium (U) metal with CH_4 and discovered that even at radioactivity levels approximately 70 times those of Spanish cave air, CH_4 was oxidized at a rate of only 0.197 ng m^{-3} day^{-1}. In contrast, CH_4 may be oxidized at a rate of 1.3–2.7 mg m^2 day^{-1} by *MOB* colonizing cave rocks. Similarly, no CH_4 losses were observed at Rn concentrations 1000 times higher than those in the Spanish caves. However,

complete depletion of CH_4 levels was observed from the cave sediments after 20 days cultures. In contrast, there was no noticeable depletion of CH_4 in the sterilized samples (Waring et al. 2017).

Analysis of cave environments through the use of stable isotopes and thermodynamics revealed a negative association between cave CH_4 concentrations and ^{13}C, which further validates the involvement of microbial processes in cave CH_4 oxidation (Mattey et al. 2013). For isotopic exchange reactions in geochemistry, the equilibrium constant K is usually replaced by the fractionation factor (α), which can be used to reflect changes in the stable isotopic composition of CH_4 during its oxidation (Hoefs 2021, p. 7). Laboratory and field studies of *MOB* indicate that the value between 1.003 and 1.035 is the threshold value for the aerobic oxidation of CH_4 (Grant and Whiticar 2002). The α of CH_4 in different caves has been identified as 1.008–1.019 (Mattey et al. 2013; McDonough et al. 2016; Ojeda et al. 2019), which is lower than the reported values for wetlands forested and upland temperate forested but is within the range of that in subtropical wetlands (1.003–1.0132), pical-forested soils (1.012–1.023), and rice paddies (1.013–1.033) (Jeffrey et al. 2021). There is some spatial heterogeneity of CH_4 consumption in karst caves. As a previous study showed that even caves adjacent to each other may exhibit different CH_4 consumption (McDonough et al. 2016).

These previous studies have suggested the potential of caves as CH_4 sinks. However, extrapolating the potential of caves as CH_4 sinks at larger spatial scales remains largely uncertain, owing to the temporal and spatial variabilities in CH_4 dynamics exhibited across caves. Current estimates of CH_4 uptake by soils may include the subsurface atmosphere (Fernandez-Cortes et al. 2015). In fact, CH_4 may first be consumed by aerobic methanotrophs in the overlying karst soil of the cave and then completely consumed by the *MOB* on the cave surface (Waring et al. 2017). Eddy covariance would be able to provide an estimate of the flux of CH_4 across the landscape and would be able to account observe the total CH_4 flux in soils and caves (Janssens et al. 2001; Baldocchi 2003). However, the CH_4 flux of caves may be more important in karst areas than that of non-karst areas due to the development of caves. It is necessary to individually assess the capacity of karst caves as CH_4 sinks. As the understanding of the process of CH_4 oxidation in caves improved, the *MOB* community was identified as the primary contributor to CH_4 oxidation in caves. An extensive investigation of the microbial dynamics and environmental drivers of *MOB* may, therefore, provide a potential solution for the independent study of the potential of caves as CH_4 sinks.

3. Methanotrophs in karst caves

3.1. Phylogenetic classification of methanotrophic bacteria

Methanotrophs are a subset of methylotrophs known for their ability to utilize CH_4 as the source of carbon and energy (Hanson and Hanson 1996). In 1906, Söhngen was the first to identify the methanotrophic bacterium *Bacillus methanicus* (Söhngen 1906). However, it was not until 1970 that researchers were able to widely separate, enrich and cultivate *MOB* from the environment, allowing detailed phylogenetic and physiological analyses of these cells (Whittenbury et al. 1970).

Currently, almost all *MOB* identified in pure cultures belong to proteobacteria, which can be further classified into Gammaproteobacteria (type I *MOB*) and Alphaproteobacteria (type II *MOB*) (Dedysh and Knief 2018). The classification of type I and type II *MOB* was initially based on physiological, morphological, ultrastructural, and chemotaxonomic characteristics (Hanson and Hanson 1996; Semrau et al. 2010). However, as novel branches of *MOB* continue to be discovered, researchers have found that while carbon assimilation continues to be one of the most important features for distinguishing between type I and type II *MOB*, some features of the inner membrane system or phospholipid fatty acids are no longer unique to a certain class of *MOB* (Knief 2015). Therefore, the original concept of type I and type II *MOB* can no longer be used to classify all known aerobic methanotrophs and is recommended to be abandoned (Semrau et al. 2010). These terms can only be considered synonymous with the phylogenetic groups of methanotrophic Alphaproteobacteria and Gammaproteobacteria (Knief 2015).

Additionally, methanotrophs belonging to the *Verrucomicrobiota* phylum was identified in a geothermal environment and is known for its resistance to high temperatures and acids (Pol et al. 2007). The anaerobic oxidation of methane is primarily mediated by anaerobic methanotrophic archaea (ANME), which act through the reduction of nitrate and sulfate (Timmers et al. 2016). The ANME-2d archaea and NC10 phylum bacteria are responsible for denitrifying anaerobic methane oxidation (Padilla et al. 2016; Lomakina et al. 2020; Dang et al. 2021). By tagging the functional genes of *MOB*, several culture-independent experiments have identified uncultured and "high-affinity" *MOB* taxa known to possess the ability to oxidize CH_4 at atmospheric concentrations (Holmes et al. 1999; Knief 2015; Zhao et al. 2018).

3.2. Diversity and function of methanotrophs in caves

To understand the relationship between cave *MOB* communities and CH_4 cycling, we surveyed previous literature on cave *MOB*. Table 1 lists recent studies on cave *MOB* taxonomy, abundance, environmental drivers, and effects. However, these descriptions of the taxonomic composition of *MOB* are not entirely consistent. Several studies have demonstrated that the "high-affinity" USC-γ predominates within *MOB* taxa in caves (Zhao et al. 2018; Cheng et al. 2021; Webster et al. 2022). In contrast, one study has shown that the main *MOB* taxa in caves are γ- and α-proteobacteria (Waring et al. 2017). These *MOB* communities reportedly thrived in the cave's soil, sediment, rock wall, or biofilm, and the quantification of the *pmoA* gene revealed an *MOB* abundance of 10^4–10^9 copies g^{-1} samples, which were collected from different caves (Table 1). In karst caves in central and southern China, this value can reach up to 10^5–10^9 g^{-1} samples, which is higher than forest and grassland soils with 10^5–10^7 copies g^{-1} samples, and even on par with peat soils with an *MOB*

Table 1. Methanotrophs are widely distributed in cave environments and mediate methane oxidation.

Caves	Samples	Methods	*MOB* taxa	Species/Gene abundance	Impact of environmental factors	Relationship to cave CH$_4$	References
Heshang Cave	The surfaces of rocks; the sediments in stream	Quantitative PCR; amplicon sequencing; amplification primers: A189f/mb661r for *pmoA* gene.	USC-γ and USC-α	The *pmoA* gene ranged from 10^7–10^8 copies · g^{-1} sample; *MOB* accounted for 0.04–20.2% of the prokaryote communities.	None	None	Zhao et al. 2018
Chifley Caves	Soils and wall surfaces	Quantitative PCR; amplicon sequencing; amplification primers: A189f/mb661 for *pmoA* gene, A189/Gam634r for USC-γ, and A189/CL1603R for Cluster 1 clade.	α-Proteobacteria and γ-Proteobacteria	*MOB* accounted for 2–12% of total bacteria.	None	The rate of methane oxidation was 0.51 mg m^{-2} h^{-1}	Waring et al. 2017
Nerja cave	Water and sediments	Denaturing gradient gel electrophoresis; amplification primers: MB10Q/R1378r for type I *MOB*, and MB9K/R1378r for type II *MOB*.	γ-Proteobacteria and α-roteobacteria	None	None	None	Ojeda et al. 2019
Panlong Cave, Xincuntun Cave, and Luohandu Cave	Weathered crust and rocks	Quantitative PCR; amplicon sequencing; amplification primers: A189/Gam634r for USC-γ, A189/forest675r for USC-α, and A189f/A650r for atmospheric *MOB* (*atmMOB*).	USC-γ, USC-α, and Deep-sea 2 clades	USC ranged from 10^4–10^9 copies · g^{-1} dry weight and accounted for 5.72% to 20.27% of the *MOB* communities.	CH$_4$, CO$_2$, pH and Cl$^-$.	Correlation with the CH$_4$ concentration	Cheng et al. 2021
20 caves in North America	Soils	Amplicon sequencing; amplification primers: 515 F/806R for 16S rRNA.	USC-γ and USC-α	The maximum relative abundance of *MOB* was 6.25% and the median relative abundance was 0.88%.	Local scale mineralogy of soils.	Correlation with the CH$_4$ concentration	Webster et al. 2022
Hoa Cương cave and Minh Châu Cave	The rocks biofilm	Quantitative PCR; amplification primers: A189f/mb661r for *pmoA* gene.	None	The *pmoA* gene ranged from 1–1.5 $\times$ 10^4 copies · g^{-1} rock; *MOB* accounted for 0.16–1.48% of total bacterial.	None	The rates of CH$_4$ oxidation were 1.3–2.7 mg m^{-2} day^{-1}	Lennon et al. 2017

MOB, methanotrophic bacteria.

abundance of 10^7–10^8 copies g^{-1} sample (Zhao et al. 2018; Cheng et al. 2021). In these caves, *MOB* constitutes 2%–20% of the bacteria, which is comparable to the 2%–12% of bacteria in Australian caves (Waring et al. 2017; Zhao et al. 2018; Cheng et al. 2021). Abundant *MOB* contribute to the rapid depletion of CH$_4$ in the cave air. Several studies have shown a significant association between the concentration of CH$_4$ and cave *MOB* (Cheng et al. 2021; Webster et al. 2022). In the Chifley Cave of Australia, the *MOB*-mediated CH$_4$ oxidation rate of cave rocks can reach 0.51 mg m^{-2} h^{-1}, which is much higher than that of aerobic highland soil (0.1 mg m^{-2} h^{-1}) (Waring et al. 2017). In addition, an analysis of this oxidation rate in two Vietnamese caves reported values of 1.3 and 2.7 mg m^{-2} day^{-1}, which equaled or exceeded the reported rates in soils from agricultural systems, grasslands, mature forests, and arctic tundra (Lennon et al. 2017). While the *MOB* in the Vietnamese caves constituted only 0.16%–1.48% of the total bacteria, they accounted for 2%–12% of the bacteria in the Chifley Cave (Lennon et al. 2017; Waring et al. 2017). These findings partly reflect that *MOB* may occupy different ecological niches in karst caves and result in variations in CH$_4$ dynamics.

4. Biogeography and assembly of methanotrophs in caves

4.1. Assembly of microbial communities

Research in biogeography has a long history. Many ecologists have argued that microbes have broad biogeographical patterns across different geographic locations or/and habitat types (Martiny et al. 2006). This leads us to ask whether there is a biogeographic pattern in cave *MOB* communities, and what factors contribute to this? The "niche" theory holds that ecological communities are a limited combination of members, and interspecific biotic interactions and abiotic conditions are the main factors affecting community composition and diversity (Leibold 1995; Soberón 2007). In contrast, the "neutral" theory argues that the stochastic colonization, death, and extinction of species determine the diversity and composition of microbial communities (Hubbell 2011). These two theories describe two extreme cases where either fully deterministic niche-based processes or fully stochastic neutral processes determine the structure of microbial communities. However, the actual situation is more likely to be somewhere in between. There may be different ecological processes behind biogeography, which together determine the structure of microbial communities. Four key processes—selection, drift, dispersal, and diversification—are thought to play an important role in the assembly of microbial communities (Hanson et al. 2012). These processes interact to create and maintain microbial geographic patterns (Hanson et al. 2012). Among them, selection is considered deterministic, which means that environmental conditions will select taxes that are relatively more adapted to local conditions. Drift is considered completely stochastic and represents the stochastic birth, death, and colonization of microorganism. Dispersal and diversification are, respectively, the ability of a species to move to a certain location or direc-

tion and the local genetic differences of a species, which are both stochastic and deterministic (Hanson et al. 2012; Gao et al. 2020). Selection will no doubt result in variations in microbial community structure in a changing environment (Ferrenberg et al. 2013). Similarly, drift enhances this variation, but it often interacts with dispersal. Without dispersal limitations, microorganisms may exhibit a patchy distribution (Chase and Myers 2011; Hanson et al. 2012). The actual microbial community is co-regulated by niche assembly, dispersal limitation, and drift (Stegen et al. 2013). Therefore, an important question about cave *MOB* communities will be: how the different ecological processes interact relative to each other, and what factors influence the balance between them.

4.2. Environmental selection in methanotrophic bacteria communities in karst caves

In karst areas, water–rock reactions take shape in underground caves, fissures, and cracks connected to the surface, creating channels for the exchange of above-ground and subsurface materials (Ford and Williams 2007).

Previous studies have shown that the binding and transport of natural organic matter (NOM) to metals is widespread in karst cave drip water and that the competitive binding of heavy metals to NOM results in changes in the trace metals content in cave drip water, while Cu as a transition metal is regulated by this process (Hartland et al. 2011; Hartland et al. 2012). In addition, soils developed in karst areas are characterized by high background concentrations of heavy metals owing to the co-regulation of secondary enrichment and parent rock inheritance (Yang et al. 2021). During weathering, elements such as Cr, Mn, Ni, and Cu are regulated by the ratio of carbonate rock to clastic rock, which may be lost from the parent rock to the soil with an increase in the carbonate rock ratio (Yang et al. 2021). Hence, the composition of mineral elements in caves may vary both temporally and spatially. Among them, Cu may be the most important factor in controlling the activity of *MOB*. Previous research has established that the amount of Cu was critical in determining the oxidation of substrates by *MOB* that only expressed pMMO (Lontoh and Semrau 1998). In fact, Cu can bind to different subunits of pMMO, and the Cu sites bound to *pmoB* and *pmoC* are likely responsible for catalyzing the oxidation of CH$_4$ (Balasubramanian et al. 2010; Semrau et al. 2010). Until recently, a study confirmed that the Cu site bound to the *pmoC* subunit plays a key role in catalyzing the oxidation of CH$_4$ (Ross et al. 2019).

When considering ecosystem structure and function, another fundamental factor is the response of microbial communities to changes in nutrients. In karst areas, the dissolution rate of limestone is 1.5–2-folds of that of dolomite, and tensile fractures are usually formed along the long axis of limestone fragments (Wang et al. 2004). Limestone areas may have greater groundwater flow and nutrient loading (e.g., C, N, P); therefore, the nutrients in the topsoil are more likely to migrate downwards and accumulate (Li et al. 2017; Zhang et al. 2020; Bai et al. 2021). A previous study has shown that

N and organic C can enter the cave with dripping water and support the operation of the complex cave microbial system (Ortiz et al. 2014). The effects of N on the *MOB* community may either positively or negatively. A previous study showed that the addition of N fertilizers to paddy soils could suppress or stimulate CH_4 emissions from soils and are regulated by irrigation patterns and fertilizer type (Banger et al. 2012). Another study suggested that the addition of nitrate significantly stimulated the oxidation rate of CH_4 at low CH_4 concentrations but inhibited this oxidation process at high CH_4 concentrations (Jang et al. 2011). As MMO and ammonia monooxygenase are similar, *MOB* might utilize ammonium ions as a substrate and thus inhibit CH_4 oxidation (Dunfield and Knowles 1995). However, because *MOB* has a much lower affinity for ammonium than for CH_4, they only convert substrates from CH_4 to ammonia if the quantity of ammonia in the soil is much higher than that of CH_4 (Bédard and Knowles 1989; Banger et al. 2012). In summary, the effect of N may depend on the niche and fitness differences of *MOB*. Recently, attention has also been paid to the positive and negative effects of P on *MOB*. A systematic review indicated that adding P to rice fields, agricultural soils, landfills, peat bogs, permafrost, and forests showed differential results: by sometimes suppressing CH_4 oxidation and sometimes stimulating this process while at times having no effects on oxidation (Veraart et al. 2015). The presence/absence patterns of genes in different *MOB* types reflect their differences in the recognition and transport of P. However, the effect of P on *MOB* is not directly related to the phylogeny of *MOB*, and the environment may be the principal regulator (Veraart et al. 2015).

Another aspect of these researches was related to microorganisms. Morse et al. (2021) conducted a longitudinal analysis of cave aquatic planktonic bacteria and compared them with those of nearby surface waters. The results showed that the diversity of the microbial community had a burst in the cave during the rainy season, whereas the microbial community was more similar to the surface before the burst. Additionally, a previous study has shown that microbes are ubiquitous in the atmosphere and can be transported over intercontinental distances through dust (Barberán et al. 2015). Hence, bacteria may enter the cave from the surface or the interstitial zone through dripping water and airborne particles. Knowledge of the symbiotic relationship between *MOB* and heterotrophs is based on empirical studies that have investigated how methanotrophic communities respond to changes in the abundance of heterotrophs (Ho et al. 2014; Veraart et al. 2018). One of the studies indicated a significant increase in CH_4 oxidation with increasing heterotrophic abundance, suggesting that complex interactions in co-cultures lead to a stimulation of methanotrophic activity (Ho et al. 2014). A volatileomics study of the interaction of each methanotroph with specific heterotrophs revealed the presence of a complex mixture of volatiles, including dimethylsulfide, dimethyldisulfide, and bicyclic sesquiterpene, suggesting that volatile organic compounds originate from heterotroph-stimulated-CH_4 oxidation (Veraart et al. 2018). *MOB* construct a CH_4-driven food web to support the growth of alien bacteria, which provides nutrients that can be utilized by *MOB* (Iguchi et al. 2011; Stock et al. 2013).

The effect of pH on *MOB* has been demonstrated in different habitats. For instance, one study showed that pH could explain why type I and type II *MOB* in a hydrological continuum from soil to lake communities had distinct niches (Crevecoeur et al. 2019). Another study showed that soil pH is the principal regulator of the composition of the *MOB* community in mountain ecosystems (Li et al. 2021). In karst area, the effect of pH may be more important, as the dissolution of carbonate rocks can release cations (Ca^{2+} and Mg^{2+}) and neutralize the acidity (Ford and Williams 2007). Not only that, stronger N deposition may exist in the limestone area and affect the concentration of exchangeable cations (Cai et al. 2015). Hence, pH may be an important factor affecting the structure of cave *MOB* community. As a previous study showed that USC-α in caves shows affinity for neutral pH, while USC-γ shows tolerance to alkaline conditions (Cheng et al. 2021).

On the other hand, temperature was considered an important factor affecting the activity of *MOB* in previous studies. For example, Mohanty et al. (2007) showed that the maximum CH_4 consumption of *MOB* in forest soil occurs at 25–35 °C, but no activity was recorded at >40 °C. Knoblauch et al. (2008) showed that the maximum rates of CH_4 oxidation by *MOB* in permafrost-affected soils occurred at 22–28 °C, while the active community of soil samples changed significantly at 0–22 °C. It is often said that the cave temperature is constant. However, a previous study showed that cave temperature is not static and constant, and its change depends on the physical characteristics and structure of caves (Russell and Maclean 2008). Nevertheless, the temperature change in the cave is far lower than that in the forest soil, and thus that the ventilation caused by the temperature difference inside and outside the cave may be more important than the temperature itself (Russell and Maclean 2008).

Additionally, *MOB* are also able to modulate population dynamics to accommodate changes in water content. For example, a previous study showed that soil moisture can affect CH_4 uptake rates by modulating methanotrophic population dynamics (Shrestha et al. 2012). Hence, water is not only an important medium for material transportation from the surface to the cave, but also an environmental selection pressure.

Taken together, more complex ecological processes may be involved if the hydrological change and the ventilation of the cave on are comprehensively considered. Hence, we focus on the combined effects of these factors for the following section.

4.3. Stochasticity in cave methanotrophic bacteria communities

It is difficult to formulate hypotheses about the assembly of cave *MOB* communities in the absence of prior knowledge. Fortunately, new methodologies make it possible to assess and quantify different ecological processes (Chase et al. 2011; Stegen et al. 2013). Based on these methods previous studies have provided deeper insights into microbial community assembly, which guides the prediction of microbial community assembly in a natural context. For example,

Ferrenberg et al. (2013) used a null model to study the assembly of bacteria in soil under fire disturbance. The results showed that fire significantly altered the structure and diversity of bacterial communities and soil chemistry. Bacterial communities in the burned soil were more stochastic than unburned soils 4 weeks after the end of fire stress, but more deterministic after 16 weeks. This result is in line with previous studies that stochasticity dominates when environmental pressure is removed in a system subject to strong environmental selection (Gao et al. 2020). However, a recent study showed that drought stress did not cause an increase in stochasticity in the fungal community in the crop-soil system. Instead, plants and time impose a stronger selection on it (Gao et al. 2020). Although the stochasticity caused by environmental selection pressure has been confirmed, there is no-uniform conclusion.

In a natural setting, cave habitats are regulated by seasonal turnover. The seasons turnover leads to changes in climate and rainfall, which can create semi-arid cave and lead to the input of exogenous substances (e.g., Cu, Zn, N, and C) with dripping water and airborne particles (Hartland et al. 2011; Hartland et al. 2012; Ortiz et al. 2014). On the other hand, the temperature difference inside and outside the cave caused a reversal of the cave ventilation pattern, which in turn affected the CH_4 concentration in the cave air (Waring et al. 2017; Webster et al. 2018). Hence, the cave *MOB* community may simultaneously face drought stress, nutrient deficiency and interference from exogenous substances, which may increase the stochasticity in the cave *MOB* community. Stochastic components in microbial communities include drift, dispersal and diversification (Hanson et al. 2012; Gao et al. 2020). For microorganisms, dispersal means multiplying and exhibiting metabolic activity in a new location, while species exhibit dispersal limitation when establishment in a new location is hindered (Hanson et al. 2012). The interaction of dispersal and drift will reduce the impact of drift on microbial community structure. Therefore, drift may be more important in a dispersal-limited environment (Hanson et al. 2012). It is obvious that microorganisms in cave environments do not spread as far as continuous water and soils. However, a previous study showed that the high-affinity *MOB* in the caves has a strong dispersal ability (Webster et al. 2022). Different distance-decay patterns may be observed at different spatial scales (Hanson et al. 2012). Hence, the interplay between drift and dispersal in *MOB* communities remains an interesting topic at large spatial scales or in subterranean environments such as caves.

Like selection, drift distinguishes microbial composition on a spatial or/and temporal scale, but it is a completely stochastic process (Zhou and Ning 2017). Identifying and evaluating drift is generally difficult in a natural context, as this first needed to exclude the interference of selection, dispersal, and diversification (Gao et al. 2020). Nonetheless, the combination of selection, dispersal, and drift more closely reflects the true picture of microbial community assembly.

Taken together, we illustrate our hypotheses about the assembly of the *MOB* in karst cave through a schematic diagram (Fig. 1).

5. Scientific questions, technologies, and future directions

Considering the great potential of cave *MOB* in CH_4 oxidation, understanding the assembly and the biogeographic patterns will have practical implications for explaining the spatial heterogeneity of cave CH_4 and utilizing cave ecological resources. Previous research has shown that biogeographic patterns are widespread, reflecting how microbial communities create and maintain species diversity under the influence of historical and current environmental conditions (Martiny et al. 2006). In general, biogeographic patterns can be assessed by combining biotic-similarity matrix, environmental-similarity matrix, and geographic-distance matrix at different geographic locations and/or habitat types (Martiny et al. 2006). However, spatial distance is rarely used as an explanatory variable to study biogeographic patterns of cave *MOB*.

Behind biogeographic patterns different ecological processes (selection, drift, dispersal, and diversification) may drive changes in the structure of cave *MOB* communities (Hanson et al. 2012). Of these, only the selection is deterministic, while the drift, dispersal, and diversification all involve stochasticity (Zhou and Ning 2017). Although the impact of environmental selection on cave *MOB* communities has been confirmed in several studies, the current studies rarely consider the stochasticity in cave *MOB* communities at the spatio-temporal scale (Cheng et al. 2021; Cheng et al. 2022; Webster et al. 2022). Methods currently used to quantify and assess stochasticity in microbial communities, including the Raup-Crick Index, the beta Nearest Taxon Index, and null deviation analysis (Chase et al. 2011; Stegen et al. 2013). If assuming a given α-diversity and γ-diversity have a corresponding β-diversity, the turnover and stochasticity in a microbial community can be assessed by the null model (Chase et al. 2011; Stegen et al. 2013). As mentioned above, different ecological processes can interact and produce seemingly identical biogeographic patterns (Hanson et al. 2012). Hence, quantifying and evaluating the relative importance of different ecological processes will have practical implications for understanding the assembly mechanism of cave *MOB* communities.

Additionally, the application of multi-omics technology can provide new insights into the transcriptional and metabolic mechanisms of cave *MOB* (Bashiardes et al. 2016; Jameson et al. 2017; Knight et al. 2018). Understanding the composition of microbial communities is not an end point of the study. The integration of multi-omics data, including amplicon sequencing, genomes, transcriptomes, proteomes, metabolomes, and other technologies, is essential for a deeper understanding of the function and composition of microbial communities (Knight et al. 2018). On the one hand, multi-omics technologies can reveal the composition and metabolic pathway of microbial community without culture, which makes it possible to study the "high affinity" *MOB* in caves that is difficult to cultivate (Singleton et al. 2018). On the other hand, the low number of hits for methane cycling functional biomarkers highlights the importance of the application of multi-omics technology to prop-

Fig. 1. Illustration of our hypotheses. Mineral and metallic elements in the surface environment may be affected by weathering and parent rock composition. Differences in rock solubility may lead to differences in water-holding and fertilizer-retention capabilities. Differences in ionic composition may cause differences in pH. The changing hydrology in karst areas leads to the formation of the semi-arid caves. Nutrient elements, mineral elements, and microorganism may enter the cave along with dripping water and particles in the air. Changes in ventilation patterns can lead to differences in CH_4 concentrations in cave air. Nutrient deficiency, drought stress, and interference of exogenous substances may work together on the cave MOB community and determine its assembly as well as the biogeographic patterns. The structure of the karst critical zone refers to the previous study (Jiang et al. 2020). MOB, methanotrophic bacteria.
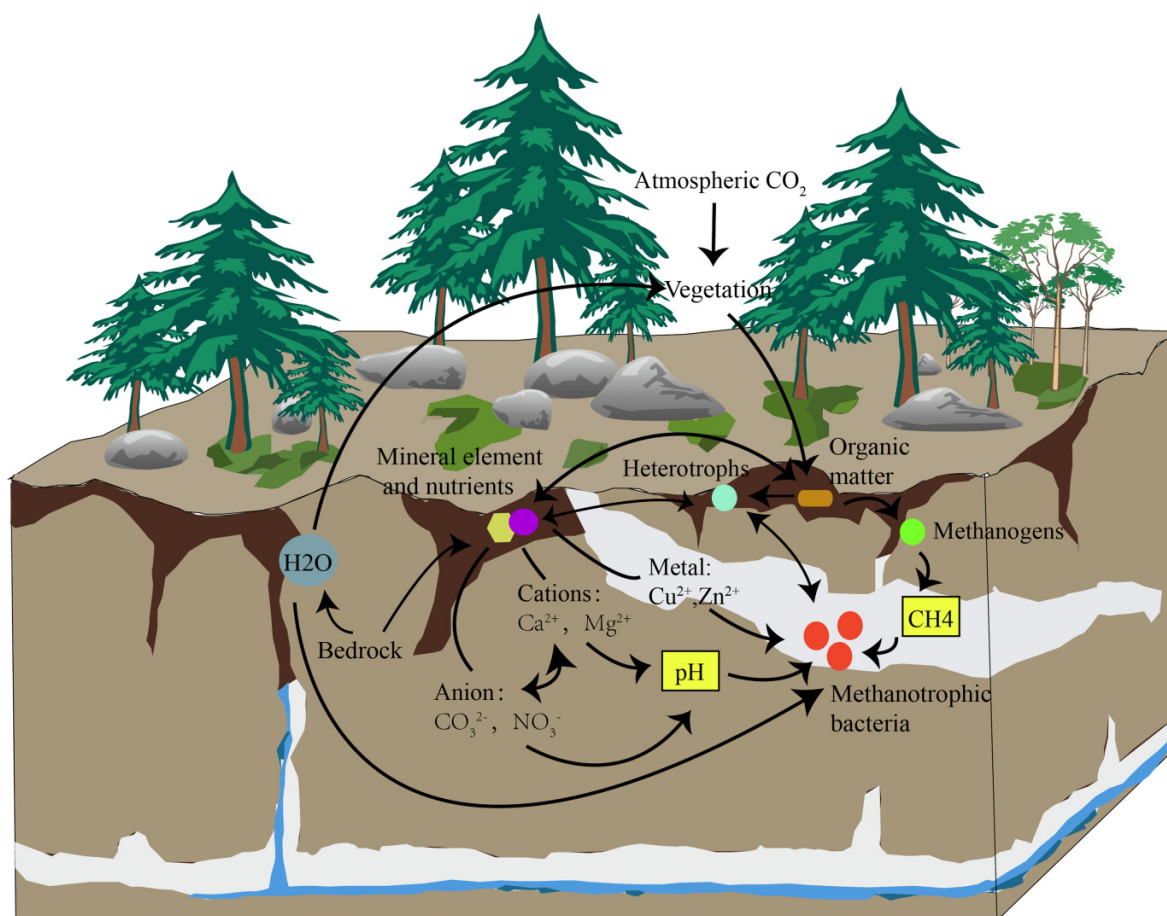

erly capture the diversity and relative abundance of methane cycling organisms (Allenby et al. 2022). The sampling of active species and high sequencing depth also provide technical support for the study of stochasticity and interspecies relationships of cave *MOB* communities (Hanson et al. 2012; Zengler and Zaramela 2018). In addition, genetic engineering advancements allow an association of specific microbial lineages to biogeochemical processes, isotopic dynamics, and/or microbial dynamics to acknowledge the changes in microbial diversities and functions at various temporal and spatial scales and predict the potential environmental consequences (McCalley et al. 2014; Singleton et al. 2018).

The development of genetic engineering and modeling techniques offers the possibility of speculating the underlying gene regulatory network-determined bacterial cell dynamics, as well as environmentally driven patterns of microbial geographic dispersion (Nazaries et al. 2018; Lopatkin and Collins 2020). Machine learning and artificial intelligence can effectively summarize and simplify models for enormous and complex ecological systems. These may reduce the number of parameters needed for modeling and/or the difficulty of obtaining reliable values, allowing an effective extraction of biologically relevant parameters (Larsen et al. 2012; Lopatkin and Collins 2020). These methods aid the prediction of unknown settings and provide solutions to issues that are challenging to address in conventional ecological models (Jørgensen and Bendoricchio 2001). However, machine learning and artificial intelligence still struggle with overfitting, bias, and lack of interpretability, highlighting the significance of the judicious application of these techniques (Lopatkin and Collins 2020). A more logical option would be to combine machine learning, artificial intelligence, microbial dynamics models, and simulation experiments (Lopatkin and Collins 2020). On the one hand, verifying the relationship between variables can make the model widely applicable and minimize its degree of uncertainty. Traditional kinetic models, on the other hand, improve the ability of the model to be

Fig. 2. Conceived experiments. Biotic-similarity matrix, environmental-similarity matrix, and geographic-distance matrix is used to assess the biogeographical patterns of cave MOB in different geographical locations. The RCI, the βNTI, and null deviation analysis is used to assess and quantify the biological processes behind the biogeography of cave MOB. The combination of microbial ecology, aerodynamics and stable isotope geochemistry of caves is used to understand the microbial process of CH_4 oxidation in caves. The multi-omics technologies, microcosmic experiment, microbial kinetics, and mathematical model are used to explore the assembly and working mechanism of cave MOB community. βNTI, beta Nearest Taxon Index; MOB, methanotrophic bacteria; RCI, Raup-Crick Index.
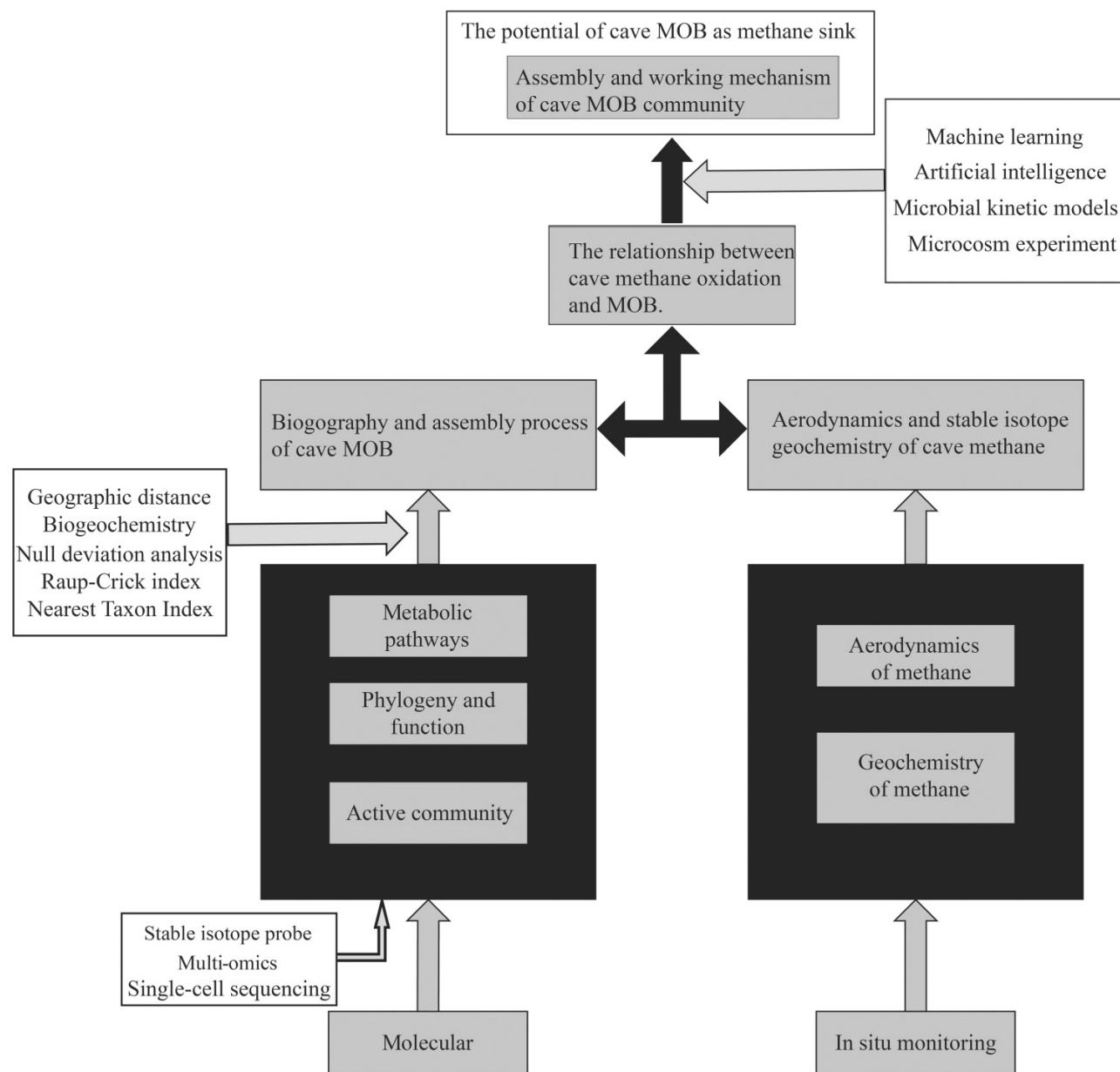
understood in terms of the kinetic characteristics of microorganisms.

An understanding of the assembly process and working mechanism of cave *MOB* serves as a foundation for the management and conservation of subterranean ecosystems. More significantly, cave *MOB* may be a significant CH_4 sink. Hence, knowledge and quantification of microbiological processes of CH_4 oxidation in caves will allow easier comprehension of the differences in CH_4 flux between karst and non-karst regions, and better coordinate the relationship be-

tween economic development and environmental conservation. Additionally, it has made it easier to develop novel methods of removing atmospheric CH_4 from subterranean environments to reduce CH_4 emissions while maintaining cost efficiency (Fernandez-Cortes et al. 2015). In summary, new techniques and approaches at the environmental, cellular, and mathematical levels will guide a mechanistic and predictive characterization of cave *MOB* communities and enhance the conservation and utilization of cave ecology (Fig. 2).

Acknowledgements

Article information

History dates

Copyright

Data availability statement

This manuscript does not report data.

Author information

Author ORCIDs

Sitong Gong https://orcid.org/0000-0002-1246-1336

Author contributions

Conceptualization: WL, SW
Funding acquisition: WL, SW
Writing – original draft: SG
Writing – review & editing: WL, LH

Competing interests

There is no conflict of interest.

References

Allenby, A., Cunningham, M.R., Hillebrand-Voiculescu, A., Comte, J-C., Doherty, R., and Kumaresan, D. 2022. Occurrence of methane-oxidizing bacteria and methanogenic archaea in earth's cave system—a metagenomic analysis. Front. Ecol. Evol. **10**: 909865. doi:10.3389/fevo.2022.909865.

Angel, R., Claus, P., and Conrad, R. 2012. Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J. **6**(4): 847–862. doi:10.1038/ismej.2011.141. PMID: 22071343.

Bai, Y., He, Q., Liu, Z., Wu, Z., and Xie, S. 2021. Soil nutrient variation impacted by ecological restoration in the different lithological karst area, Shibing, China. Glob. Ecol. Conserv. **25**: e01399. doi:10.1016/j.gecco.2020.e01399.

Balasubramanian, R., Smith, S.M., Rawat, S., Yatsunyk, L.A., Stemmler, T.L., and Rosenzweig, A.C. 2010. Oxidation of methane by a biological dicopper center. Nature **465**(7294): 115–119. doi:10.1038/nature08992. PMID: 20410881.

Baldocchi, D.D. 2003. Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. Glob. Change Biol. **9**(4): 479–492. doi:10.1046/j.1365-2486.2003.00629.x.

Banger, K., Tian, H., and Lu, C. 2012. Do nitrogen fertilizers stimulate or inhibit methane emissions from rice fields? Glob. Change Biol. **18**(10): 3259–3267. doi:10.1111/j.1365-2486.2012.02762.x.

Barberán, A., Ladau, J., Leff, J.W., Pollard, K.S., Menninger, H.L., Dunn, R.R., and Fierer, N. 2015. Continental-scale distributions of dust-associated bacteria and fungi. Proc. Natl. Acad. Sci. U.S.A. **112**(18): 5756–5761. doi:10.1073/pnas.1420815112.

Bashiardes, S., Zilberman-Schapira, G., and Elinav, E. 2016. Use of meta-transcriptomics in microbiome research. Bioinform. Biol. Insights **10**: BBI.S34610. doi:10.4137/BBI.S34610.

Bédard, C., and Knowles, R. 1989. Physiology, biochemistry, and specific inhibitors of CH4, NH4+, and CO oxidation by methanotrophs and nitrifiers. Microbiol. Rev. **53**(1): 68–84. doi:10.1128/mr.53.1.68-84.1989. PMID: 2496288.

Cai, Z., Wang, B., Xu, M., Zhang, H., He, X., Zhang, L., and Gao, S. 2015. Intensified soil acidification from chemical N fertilization and prevention by manure in an 18-year field experiment in the red soil of southern China. J. Soils Sediments **15**(2): 260–270. doi:10.1007/s11368-014-0989-y.

Chase, J.M., and Myers, J.A. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philos. Trans. R. Soc. B **366**(1576): 2351–2363. doi:10.1098/rstb.2011.0063.

Chase, J.M., Kraft, N.J.B., Smith, K.G., Vellend, M., and Inouye, B.D. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere **2**(2): art24. doi:10.1890/ES10-00117.1.

Cheng, X., Wang, H., Zeng, Z., Li, L., Zhao, R. Bodelier, P.L.E., et al. 2022. Niche differentiation of atmospheric methane-oxidizing bacteria and their community assembly in subsurface karst caves. Environ. Microbiol. Rep. **14**(6): 886–896. doi:10.1111/1758-2229.13112.

Cheng, X.Y., Liu, X.Y., Wang, H.M., Su, C.T., Zhao, R. Bodelier, P.L.E., et al. 2021. USC γ dominated community composition and cooccurrence network of methanotrophs and bacteria in subterranean karst caves. Microbiol. Spectr. **9**(1): e00820–e00821. doi:10.1128/Spectrum.00820-21. PMID: 34406837.

Crevecoeur, S., Ruiz-González, C., Prairie, Y.T., and Giorgio, P.A. 2019. Large-scale biogeography and environmental regulation of methanotrophic bacteria across boreal inland waters. Mol. Ecol. **28**(18): 4181–4196. doi:10.1111/mec.15223. PMID: 31479544.

Dang, C.C., Xie, G.J., Liu, B.F., Xing, D.F., Ding, J., and Ren, N.Q. 2021. Heavy metal reduction coupled to methane oxidation: mechanisms, recent advances and future perspectives. J. Hazard. Mater. **405**: 124076. doi:10.1016/j.jhazmat.2020.124076. PMID: 33268204.

Dedysh, S.N., and Knief, C. 2018. Diversity and phylogeny of described aerobic methanotrophs. In M.G. Kalyuzhnaya and X.H. Xing(Eds.). *Methane Biocatalysis: Paving the Way to Sustainability*. Springer, Switzerland. pp.17–42. doi:10.1007/978-3-319-74866-5_2.

Dunfield, P., and Knowles, R. 1995. Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a humisol. Appl. Environ. Microbiol. **61**(8): 3129–3135. doi:10.1128/aem.61.8.3129-3135.1995. PMID: 16535109.

Fernandez-Cortes, A., Cuezva, S., Alvarez-Gallego, M., Garcia-Anton, E., Pla, C. Benavente, D., et al. 2015. Subterranean atmospheres may act as daily methane sinks. Nat. Commun. **6**(1): 7003. doi:10.1038/ncomms8003. PMID: 25912519.

Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S. Bradley, D., et al. 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. ISME J. **7**(6): 1102–1111. doi:10.1038/ismej.2013.11. PMID: 23407312.

Ford, D., and Williams, P. 2007. *Karst Hydrogeology and Geomorphology*. John Wiley & Sons Ltd., West Sussex, England. doi:10.1002/9781118684986.

Gao, C., Montoya, L., Xu, L., Madera, M., Hollingsworth, J. Purdom, E., et al. 2020. Fungal community assembly in drought-stressed sorghum shows stochasticity, selection, and universal ecological dynamics. Nat. Commun. **11**(1): 34. doi:10.1038/s41467-019-13913-9. PMID: 31911594.

Grant, N.J., and Whiticar, M.J. 2002. Stable carbon isotopic evidence for methane oxidation in plumes above Hydrate Ridge, Cascadia Oregon Margin. Glob. Biogeochem. Cycles. **16**(4): 1124. doi:10.1029/2001GB001851.

Environ. Rev. **31**: 298–309 (2023) | dx.doi.org/10.1139/er-2022-0034

307

Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., and Martiny, J.B.H. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nat. Rev. Microbiol. **10**(7): 497–506. doi:10.1038/nrmicro2795. PMID: 22580365.

Hanson, R.S., and Hanson, T.E. 1996. Methanotrophic bacteria. Microbiol. Rev. **60**(2): 439–471. doi:10.1128/mr.60.2.439-471.1996. PMID: 8801441.

Hartland, A., Fairchild, I.J., Lead, J.R., Borsato, A., Baker, A., Frisia, S., and Baalousha, M. 2012. From soil to cave: transport of trace metals by natural organic matter in karst dripwaters. Chem. Geol. **304–305**: 68–82. doi:10.1016/j.chemgeo.2012.01.032.

Hartland, A., Fairchild, I.J., Lead, J.R., Zhang, H., and Baalousha, M. 2011. Size, speciation and lability of NOM–metal complexes in hyperalkaline cave dripwater. Geochim. Cosmochim. Acta **75**(23): 7533–7551. doi:10.1016/j.gca.2011.09.030.

Ho, A., de Roy, K., Thas, O., De Neve, J., Hoefman, S. Vandamme, P., et al. 2014. The more, the merrier: heterotroph richness stimulates methanotrophic activity. ISME J. **8**(9): 1945–1948. doi:10.1038/ismej.2014.74. PMID: 24785289.

Hoefs, J. 2021. *Stable Isotope Geochemistry*. Springer, Heidelberg. doi:10.1007/978-3-030-77692-3.

Holmes, A.J., Roslev, P., McDonald, I.R., Iversen, N., Henriksen, K., and Murrell, J.C. 1999. Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Appl. Environ. Microbiol. **65**(8): 3312–3318. doi:10.1128/AEM.65.8.3312-3318.1999. PMID: 10427012.

Hubbell, S.P. 2011. *The Unified Neutral Theory of Biodiversity and Biogeography (MPB-32)*. Princeton University Press, Princeton. doi:10.1515/9781400837526.

Iguchi, H., Yurimoto, H., and Sakai, Y. 2011. Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. Appl. Environ. Microbiol. **77**(34): 8509–8515. doi:10.1128/AEM.05834-11. PMID: 21984248.

IPCC. 2013. *Climate Change 2013: The Physical Science Basis*. United Nation, Geneva, Switzerland.

IPCC. 2022. *Climate Change 2022: Impacts, Adaptation and Vulnerability Summary for Policymaker*. United Nation, Geneva, Switzerland.

Jameson, E., Taubert, M., Coyotzi, S., Chen, Y., Eyice, Ö. Schäfer, H., et al. 2017. DNA-, RNA-, and protein-based stable-isotope probing for high-throughput biomarker analysis of active microorganisms. In W.R. Streit and R. Daniel (Eds.). Springer, New York. pp. 57–74. doi:10.1007/978-1-4939-6691-2_5.

Jang, I., Lee, S., Zoh, K.D., and Kang, H. 2011. Methane concentrations and methanotrophic community structure influence the response of soil methane oxidation to nitrogen content in a temperate forest. Soil Biol. Biochem. **43**(3): 620–627. doi:10.1016/j.soilbio.2010.11.032.

Janssens, I.A., Kowalski, A.S., and Ceulemans, R. 2001. Forest floor CO_2 fluxes estimated by eddy covariance and chamber-based model. Agric. For. Meteorol. **106**(1): 61–69. doi:10.1016/S0168-1923(00)00177-5.

Jeffrey, L.C., Maher, D.T., Chiri, E., Leung, P.M., Nauer, P.A. Arndt, S.K., et al. 2021. Bark-dwelling methanotrophic bacteria decrease methane emissions from trees. Nat. Commun. **12**(1): 2127. doi:10.1038/s41467-021-22333-7. PMID: 33837213.

Jiang, Z., Liu, H., Wang, H., Peng, J., Meersmans, J. Green, S.M., et al. 2020. Bedrock geochemistry influences vegetation growth by regulating the regolith water holding capacity. Nat. Commun. **11**(1): 2392. doi:10.1038/s41467-020-16156-1. PMID: 32404911.

Jørgensen, S.E., and Bendoricchio, G., 2001. Fundamentals of Ecological Modelling. Elsevier, Amsterdam.

Knief, C. 2015. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. Front. Microbiol. **6**. doi:10.3389/fmicb.2015.01346. PMID: 26696968.

Knight, R., Vrbanac, A., Taylor, B.C., Aksenov, A., Callewaert, C. Debelius, J., et al. 2018. Best practices for analysing microbiomes. Nat. Rev. Microbiol. **16**(7): 410–422. doi:10.1038/s41579-018-0029-9. PMID: 29795328.

Knoblauch, C., Zimmermann, U., Blumenberg, M., Michaelis, W., and Pfeiffer, E.M. 2008. Methane turnover and temperature response of methane-oxidizing bacteria in permafrost-affected soils of northeast Siberia. Soil Biol. Biochem. **40**(12): 3004–3013. doi:10.1016/j.soilbio.2008.08.020.

Larsen, P.E., Field, D., and Gilbert, J.A. 2012. Predicting bacterial community assemblages using an artificial neural network approach. Nat. Methods **9**(6): 621–625. doi:10.1038/nmeth.1975. PMID: 22504588.

Leibold, M.A. 1995. The niche concept revisited: mechanistic models and community context. Ecology **76**(5): 1371–1382. doi:10.2307/1938141.

Lennon, J.T., Nguyễn-Thùy, D., Phạm, T.M., Drobniak, A., Tạ, P.H. Phạm, N.Đ., et al. 2017. Microbial contributions to subterranean methane sinks. Geobiology **15**(2): 254–258. doi:10.1111/gbi.12214. PMID: 27671735.

Li, C., Tu, B., Kou, Y., Wang, Y., Li, X., Wang, J., and Li, J. 2021. The assembly of methanotrophic communities regulated by soil pH in a mountain ecosystem. Catena **196**: 104883. doi:10.1016/j.catena.2020.104883.

Li, D., Wen, L., Zhang, W., Yang, L., Xiao, K., Chen, H., and Wang, K. 2017. Afforestation effects on soil organic carbon and nitrogen pools modulated by lithology. For. Ecol. Manag. **400**: 85–92. doi:10.1016/j.foreco.2017.05.050.

Li, T., Li, H., Xiang, X., Kuo, T.-S., Li, J. Zhou, F., et al. 2012. Transportation characteristics of δ 13C in the plants-soil-bedrock-cave system in Chongqing karst area. Sci. China Earth Sci. **55**(4): 685–694. doi:10.1007/s11430-011-4294-y.

Lomakina, A., Pogodaeva, T., Kalmychkov, G., Chernitsyna, S., and Zemskaya, T. 2020. Diversity of NC10 bacteria and ANME-2d archaea in sediments of fault zones at Lake Baikal. Diversity **12**(1):. doi:10.3390/d12010010. PMID: 34168518.

Lontoh, S., and Semrau, J.D. 1998. Methane and trichloroethylene degradation by methylosinus trichosporium OB3b expressing particulate methane monooxygenase. Appl. Environ. Microbiol. **64**(3): 1106–1114. doi:10.1128/AEM.64.3.1106-1114.1998. PMID: 16349516.

Lopatkin, A.J., and Collins, J.J. 2020. Predictive biology: modelling, understanding and harnessing microbial complexity. Nat. Rev. Microbiol. **18**(9): 507–520. doi:10.1038/s41579-020-0372-5. PMID: 32472051.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A. Green, J.L., et al. 2006. Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol. **4**(2): 102–112. doi:10.1038/nrmicro1341. PMID: 16415926.

Mattey, D.P., Fisher, R., Atkinson, T.C., Latin, J.P., Durrell, R. Ainsworth, M., et al. 2013. Methane in underground air in Gibraltar karst. Earth Planet. Sci. Lett. **374**: 71–80. doi:10.1016/j.epsl.2013.05.011.

McCalley, C.K., Woodcroft, B.J., Hodgkins, S.B., Wehr, R.A., Kim, E.H. Mondav, R., et al. 2014. Methane dynamics regulated by microbial community response to permafrost thaw. Nature **514**(7523): 478–481. doi:10.1038/nature13798. PMID: 25341787.

McDonough, L.K., Iverach, C.P., Beckmann, S., Manefield, M., Rau, G.C., Baker, A., and Kelly, B.F.J. 2016. Spatial variability of cave-air carbon dioxide and methane concentrations and isotopic compositions in a semi-arid karst environment. Environ. Earth Sci. **75**(8): 700. doi:10.1007/s12665-016-5497-5.

Mohanty, S.R., Bodelier, P.L.E., and Conrad, R. 2007. Effect of temperature on composition of the methanotrophic community in rice field and forest soil: effect of temperature on soil methanotrophs. FEMS Microbiol. Ecol. **62**(1): 24–31. doi:10.1111/j.1574-6941.2007.00370.x. PMID: 17725622.

Morse, K.V., Richardson, D.R., Brown, T.L., Vangundy, R.D., and Cahoon, A.B. 2021. Longitudinal metabarcode analysis of karst bacterioplankton microbiomes provide evidence of epikarst to cave transport and community succession. PeerJ. **9**: e10757. doi:10.7717/peerj.10757. PMID: 33732542.

Nazaries, L., Karunaratne, S.B., Delgado-Baquerizo, M., Campbell, C.D., and Singh, B.K. 2018. Environmental drivers of the geographical distribution of methanotrophs: insights from a national survey. Soil Biol. Biochem. **127**: 264–279. doi:10.1016/j.soilbio.2018.08.014.

Nguyễn-Thuỳ, D., Schimmelmann, A., Nguyễn-Văn, H., Drobniak, A., Lennon, J., Tạ, P.H., and Nguyễn, N.T.A. 2017. Subterranean microbial oxidation of atmospheric methane in cavernous tropical karst. Chem. Geol. **466**: 229–238. doi:10.1016/j.chemgeo.2017.06.014.

Ojeda, L., Vadillo, I., Etiope, G., Benavente, J., Liñán, C. del Rosal, Y., et al. 2019. Methane sources and sinks in karst systems: the Nerja cave and its vadose environment (Spain). Geochim. Cosmochim. Acta **259**: 302–315. doi:10.1016/j.gca.2019.06.011.

Ortiz, M., Legatzki, A., Neilson, J.W., Fryslie, B., Nelson, W.M. Wing, R.A., et al. 2014. Making a living while starving in the dark: metagenomic

308

Environ. Rev. **31**: 298–309 (2023) | dx.doi.org/10.1139/er-2022-0034

insights into the energy dynamics of a carbonate cave. ISME J. **8**(2): 478–491. doi:10.1038/ismej.2013.159. PMID: 24030597.

Padilla, C.C., Bristow, L.A., Sarode, N., Garcia-Robledo, E., Gómez Ramírez, E. Benson, C.R., et al. 2016. NC10 bacteria in marine oxygen minimum zones. ISME J. **10**(8): 2067–2071. doi:10.1038/ismej.2015.262. PMID: 26918666.

Pol, A., Heijmans, K., Harhangi, H.R., Tedesco, D., Jetten, M.S.M., and Op den Camp, H.J.M. 2007. Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature **450**(7171): 874–878. doi:10.1038/nature06222. PMID: 18004305.

Ross, M.O., MacMillan, F., Wang, J., Nisthal, A., Lawton, T.J. Olafson, B.D., et al. 2019. Particulate methane monooxygenase contains only mononuclear copper centers. Science **364**(6440): 566–570. doi:10.1126/science.aav2572. PMID: 31073062.

Russell, M.J., and MacLean, V.L. 2008. Management issues in a Tasmanian tourist cave: potential microclimatic impacts of cave modifications. J. Environ. Manag. **87**(3): 474–483. doi:10.1016/j.jenvman.2007.01.012. PMID: 17368916.

Semrau, J.D., DiSpirito, A.A., and Yoon, S. 2010. Methanotrophs and copper. FEMS Microbiol. Rev. **34**(4): 496–531. doi:10.1111/j.1574-6976.2010.00212.x. PMID: 20236329.

Shrestha, P.M., Kammann, C., Lenhart, K., Dam, B., and Liesack, W. 2012. Linking activity, composition and seasonal dynamics of atmospheric methane oxidizers in a meadow soil. ISME J. **6**(6): 1115–1126. doi:10.1038/ismej.2011.179. PMID: 22189499.

Singleton, C.M., McCalley, C.K., Woodcroft, B.J., Boyd, J.A., Evans, P.N. Hodgkins, S.B., et al. 2018. Methanotrophy across a natural permafrost thaw environment. ISME J. **12**(10): 2544–2558. doi:10.1038/s41396-018-0065-5. PMID: 29955139.

Soberón, J. 2007. Grinnellian and Eltonian niches and geographic distributions of species. Ecol. Lett. **10**(12): 1115–1123. doi:10.1111/j.1461-0248.2007.01107.x. PMID: 17850335.

Söhngen, N. 1906. Über bakterien, welche methan als kohlenstoffnahrung und energiequelle gebrauchen. Zentrabl Bakteriol Parasitenk Infekt. **15**: 513–517.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W. Murray, C.J., et al. 2013. Quantifying community assembly processes and identifying features that impose them. ISME J. **7**(11): 2069–2079. doi:10.1038/ismej.2013.93. PMID: 23739053.

Stock, M., Hoefman, S., Kerckhof, F.M., Boon, N., De Vos, P. De Baets, B., et al. 2013. Exploration and prediction of interactions between methanotrophs and heterotrophs. Res. Microbiol. **164**(10): 1045–1054. doi:10.1016/j.resmic.2013.08.006. PMID: 24012541.

Timmers, P.H., Suarez-Zuluaga, D.A., van Rossem, M., Diender, M., Stams, A.J., and Plugge, C.M. 2016. Anaerobic oxidation of methane associated with sulfate reduction in a natural freshwater gas source. ISME J. **10**(6): 1400–1412. doi:10.1038/ismej.2015.213. PMID: 26636551.

Tollefson, J. 2022. Scientists raise alarm over "dangerously fast" growth in atmospheric methane. Naturedoi:10.1038/d41586-022-00312-2.

Veraart, A.J., Garbeva, P., van Beersum, F., Ho, A., Hordijk, C.A. Meima-Franke, M., et al. 2018. Living apart together—bacterial volatiles influence methanotrophic growth and activity. ISME J. **12**(4): 1163–1166. doi:10.1038/s41396-018-0055-7. PMID: 29382947.

Veraart, A.J., Steenbergh, A.K., Ho, A., Kim, S.Y., and Bodelier, P.L.E. 2015. Beyond nitrogen: the importance of phosphorus for CH_4 oxidation in soils and sediments. Geoderma **259–260**: 337–346. doi:10.1016/j.geoderma.2015.03.025.

Wang, S.J., Li, R.L., Sun, C.X., Zhang, D.F., Li, F.Q. Zhou, D.Q., et al. 2004. How types of carbonate rock assemblages constrain the distribution of karst rocky desertified land in Guizhou Province, PR China: phenomena and mechanisms. Land Degrad. Dev. **15**(2): 123–131. doi:10.1002/ldr.591.

Waring, C.L., Griffith, D.W.T., Wilson, S., and Hurry, S. 2009. Cave atmosphere: A guide to calcification and a methane sink. Geochim. Cosmochim. Acta **73**(13S): A1419.

Waring, C.L., Hankin, S.I., Griffith, D.W.T., Kertesz, M.A., Kobylski, V. Wilson, N.L., et al. 2017. Seasonal total methane depletion in limestone caves. Sci. Rep. **7**(1): 8314. doi:10.1038/s41598-017-07769-6. PMID: 28814720.

Webster, K.D., Drobniak, A., Etiope, G., Mastalerz, M., Sauer, P.E., and Schimmelmann, A. 2018. Subterranean karst environments as a global sink for atmospheric methane. Earth Planet. Sci. Lett. **485**: 9–18. doi:10.1016/j.epsl.2017.12.025.

Webster, K.D., Mirza, A., Deli, J.M., Sauer, P.E., and Schimmelmann, A. 2016. Consumption of atmospheric methane in a limestone cave in Indiana, USA. Chem. Geol. **443**: 1–9. doi:10.1016/j.chemgeo.2016.09.020.

Webster, K.D., Schimmelmann, A., Drobniak, A., Mastalerz, M., Rosales, L.L., Boston, P.J., and Lennon, J.T. 2022. Diversity and composition of methanotroph communities in caves. Microbiol. Spectr. **10**(4): e01566–e01521. American Society for Microbiology. doi:10.1128/spectrum.01566-21. PMID: 35943259.

Whiticar, M.J. 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chem. Geol. **161**(1-3): 291–314. doi:10.1016/S0009-2541(99)00092-3.

Whittenbury, R., Phillips, K.C., and Wilkinson, J.F. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. Microbiol. **61**(2): 205–218. doi:10.1099/00221287-61-2-205.

Yang, Q., Yang, Z., Filippelli, G.M., Ji, J., Ji, W. Liu, X., et al. 2021. Distribution and secondary enrichment of heavy metal elements in karstic soils with high geochemical background in Guangxi, China. Chem. Geol. **567**: 120 081. doi:10.1016/j.chemgeo.2021.120081.

Zengler, K., and Zaramela, L.S. 2018. The social network of microorganisms—how auxotrophies shape complex communities. Nat. Rev. Microbiol. **16**(6): 383–390. doi:10.1038/s41579-018-0004-5. PMID: 29599459.

Zhang, Z., Chen, X., Cheng, Q., Li, S., Yue, F. Peng, T., et al. 2020. Coupled hydrological and biogeochemical modelling of nitrogen transport in the karst critical zone. Sci. Total Environ. **732**: 138902. doi:10.1016/j.scitotenv.2020.138902. PMID: 32438160.

Zhao, R., Wang, H., Cheng, X., Yun, Y., and Qiu, X., 2018. Upland soil cluster γ dominates the methanotroph communities in the karst Heshang Cave. FEMS Microbiol. Ecol. **94**(12): fiy192. doi:10.1093/femsec/fiy192.

Zhou, J., and Ning, D. 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. **81**(4): e00002–e00017. doi:10.1128/MMBR.00002-17. PMID: 29021219.

Environ. Rev. **31**: 298–309 (2023) | dx.doi.org/10.1139/er-2022-0034

309