Diversity and composition of cave methanotrophic communities

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ABSTRACT Methane oxidizing bacteria (methanotrophs) are a ubiquitous group of microorganisms that represent a major sink for the greenhouse gas methane (CH_4). Recent studies have demonstrated that methanotrophs are abundant and contribute to CH_4 dynamics in caves. However, very little is known about what controls the distribution and abundance of methanotrophs in subterranean ecosystems. Here, we report a survey of sediments collected from >20 caves in North America to elucidate the factors shaping cave methanotroph communities. Using 16S rRNA sequencing, we recovered methanotrophs from nearly all (98 %) of the samples, including cave sites where CH_4 concentrations were at or below detection limits (≤ 0.3 ppmv). We identified a core microbiome among caves that was dominated by members of the USC-γ clade, which are recognized as high-affinity methanotrophs. Although methanotrophs were associated with local-scale mineralogy, their community composition did not systematically vary between the entrances and interior of caves, where CH_4 concentrations varied. However, we did detect a decay in compositional similarity of methanotrophic community composition with geographic distance. This biogeographic pattern is consistent with dispersal limitation perhaps due to the insular nature of cave ecosystems. Last, the relative abundance of methanotrophs was positively correlated with cave-air CH_4 concentrations—suggesting that these microorganisms contribute to CH_4 flux in subterranean ecosystems.

IMPORTANCE Recent observations have shown that the atmospheric greenhouse gas methane (CH_4) is consumed by microorganisms (methanotrophs) in caves at rates

comparable to CH_4 oxidation in surface soils. Caves are abundant in karst landscapes that comprise 14 % of Earth's land surface area, and therefore may be acting as a substantial CH_4 sink. A detailed ecological understanding of the forces that shape methanotrophic communities in caves is lacking. We sampled cave sediments to better understand the community composition and structure of cave methanotrophs. Our results show that the members of the USC- γ clade are dominant in cave communities, that the relative abundance of methanotrophs was positively correlated with CH_4 concentrations in cave air, and that methanotroph relative abundance was correlated with local scale mineralogy of soils.

KEYWORDS: Biogeography; Cave; Greenhouse Gas; Karst; Methane; Methanotroph

INTRODUCTION

Methane (CH_4) is a greenhouse gas in Earth's atmosphere, and an energy source for humanity (1, 2), inspiring research into the sources and sinks of CH_4 to and from the atmosphere. Two major processes are responsible for the removal of CH_4 from Earth's atmosphere, oxidation by hydroxyl radicals in the atmosphere, and by methane-consuming microorganisms (methanotrophs) in surface soils. Oxidation by hydroxyl radicals accounts for 90 % of atmospheric-CH_4 removal while methanotrophs consume roughly 5 % of the CH_4 that is removed from the atmosphere each year (3). Despite the influence that methanotrophs have on regulating Earth's climate, gaps remain in our understanding of

these organisms, like how they respond to changing CH_4 concentrations or how they disperse through the environment (4, 5).

Rocks that host caves are widespread on Earth, and are emerging as a global CH_4 sink (6–11) (Figure 1), but little is known about the methanotrophic communities in these environments. Recent studies of cave microbial communities have revealed the presence of atmospheric-CH_4 consuming or high-affinity methanotrophs in caves (8, 12–15). High-affinity methanotrophs have been previously observed from upland soils and these groups are referred to as the Upland Soil Cluster (USC) -α and -γ clades. Atmospheric CH_4 oxidation has also been observed by methanotrophs belonging to the Type II group (organisms in the *Methylocystaceae* and *Beijernickiaceae*), but it is not thought that these organisms can consume CH_4 at these concentrations indefinitely (16). Both high-affinity and Type II methanotrophs tend to be the most prominent members of the methanotrophic community in environments with low CH_4 concentrations and high O_2 concentrations. They are less common in environments with high CH_4 and low O_2 concentrations, like lakes or geological seeps, which are dominated by Type I methanotrophs (methanotrophs from the Methylococcaceae) (17, 18).

The factors that control the distribution and abundance of methanotrophs in caves are not well understood. Previous work has shown that methanotrophs are frequently related to CH_4 concentrations, sediment texture, and other microorganisms, but it is unclear how these factors, or the local mineralogy influences methanotroph communities in caves. Type I and Type II methanotrophs have been shown to exhibit niche partitioning between environments with high and low CH_4 concentrations (18, 19). However, it is

unknown if methanotrophic communities exhibit similar changes in community composition with atmospheric to subatmospheric CH_4 concentration gradients.

In addition, very little is known about the spatial distribution of methanotrophs, even though such information could shed light on the biogeography of microbes within and among cave ecosystems. While the community composition of methanotrophs may be influenced by environmental conditions of caves, their abundances may also be influenced by dispersal limitation. Caves are unique and insular habitats that have a patchy distribution in the terrestrial landscape. Similar methanotrophs have been observed in both Hawai'i and the Arctic (4) suggesting that the dispersal of these organisms is relatively unhindered. However, biota in caves are typically unique due to their isolation from other environments (20, 21). Thus, it may be possible that dispersal limitation is important in structuring the methanotrophic communities in caves. If no group of methanotrophs could consistently be found among caves, this may suggest that methanotrophs are dispersal limited.

In this study, we sampled 21 caves in North America to examine the factors regulating the community composition of cave methanotrophs (Figure 1). First, we hypothesized that the methanotroph community structure should be similar to the structure of methanotrophic communities in other low CH_4 environments, and that the relative abundance of methanotrophs should be correlated with cave-air CH_4 concentrations. Additionally, we hypothesized that methanotrophs should show relationships to sediment texture and the minerals present in the environment since these features are known to influence the diffusion of air and micronutrient abundances. Finally, we hypothesized that

high-affinity methanotroph communities in caves should exhibit signatures of dispersal-limitation owing to the insular nature of caves.

RESULTS

Methanotroph community structure. Methanotrophs were recovered in nearly all (98 %) of the cave-sediment samples, including locations where CH_4 concentrations were at or below the analytical detection limit (< 0.3 ppmv). The maximum relative abundance of methanotrophs in the cave sediment samples was 6.25 % and the median relative abundance was 0.52 %. The average methanotrophic community contained 3 ± 2 methanotroph OTUs. Methanotrophic communities in the sampled caves were dominated by the USC-*γ* clade, but members of the USC-*α* clade, Methylocystaceae, and Methylococcales were also present (Figure 2). Members of the USC clades comprised a median of 99.3 % of the total methanotrophic community at locations where methanotrophs were observed and members of the USC-*γ* clade represented 70.1 % of the community on average (median). Methanotrophs from the Type II and Type I functional groups represented 0.12 % of the observed methanotroph community on average (median). The observed diversity of the USC-*γ* and -*α* clades was lower than that of the Type II and Type I functional groups. Members of the USC-*γ* and -*α* were each represented by two unique OTUs, whereas Type II and Type I methanotrophs were represented by seven and 11 unique OTUs respectively.

Methanotroph core microbiome. Taxa that were present in at least 60 % of samples were defined as the core methanotroph community. Based on this definition, the

core microbiome was made up of only two OTUs. Both of the OTUs were from members of the USC-γ. These taxa were found together in 84 % of samples, and the most widely distributed OTU was observed in 93 % of samples. The core methanotroph community accounted for 59 % of the obtained methanotroph sequences.

Environmental influences on community structure. Methanotroph composition was related to the mineralogy of cave soils (Figure 4). The total methanotrophic community was related to the abundance of clinochlorite ($p = 0.008$), orthoclase ($p = 0.006$), muscovite ($p = 0.05$), and microcline ($p = 0.04$). The USC functional clade showed a relationship with the abundance of clinochlorite ($p = 0.02$) and the Type II and Type I methanotrophs were not related to the abundance of any observed mineral ($p > 0.05$). In contrast to mineralogical associations, the composition of the methanotrophic community was not related to soil texture (Table 1).

Biogeography. Biological communities are known to change with the geographic distance and the "environmental distance" between them—a pattern known as a distance decay relationship (DDR) (51, 52). At larger spatial scales, methanotrophs showed relationships with measures of geographic distance between the samples. Analysis of methanotroph DDRs revealed that the composition of high-affinity methanotrophs changed faster with respect to geographic than environmental conditions (m High-affinity methanotrophs = −0.14, m environmental conditions = −0.05, $p = 0.01$) (Figure 5). In contrast, low-affinity methanotroph community composition changed slower than environmental conditions with respect to the geographic distance between the samples (m

153 Low-affinity methanotrophs = 0.10, *m* environmental conditions = −0.06, $p = 0.004$). At
154 smaller spatial scales, however, the relative abundance of methanotrophs was not related to
155 the distance from a cave entrance (High-affinity: Spearman's rank correlations, $\rho = -0.05$,
156 S = 3854, $p = 0.78$; Type II: Spearman's rank correlations, $\rho = 0.21$, S = 2882, $p = 0.28$,
157 Type I: Spearman's rank correlations, $\rho = 0.13$, S = 2758, $p = 0.13$).
158
159 **Relationships with cave air CH_4 concentrations.** The CH_4 concentration in cave
160 air was positively correlated with the relative abundance of methanotrophs (Figure 3,
161 Spearman's Test, $\rho = 0.40$, S = 5290, $p = 0.01$). This relationship was largely driven by the
162 USC functional groups (Spearman's Test, $\rho = 0.40$, S = 5887, $p = 0.01$). The relative
163 abundances of Type II and Type I methanotrophs were not correlated with the CH_4
164 concentration (Type II: Spearman's rank correlations, $\rho = 0.04$, S = 9462, $p = 0.79$; Type I:
165 Spearman's rank correlations, $\rho = -0.01$, S = 10002, $p = 0.94$).
166
167 **DISCUSSION**
168 The process of life is fundamentally centered on obtaining energy from the
169 surrounding environment and methanotrophs are no exception. As such, methanotrophic
170 clades avoid competition with each other by partitioning the ranges across which they
171 consume CH_4. USC clades rely on CH_4 concentrations that are at or near atmospheric
172 levels, whereas Type II methanotrophs may only survive and metabolize atmospheric CH_4
173 for a limited time period under such conditions, while Type I methanotrophs are thought to
174 be unable to persist in environments with atmospheric CH_4 concentrations (22–24). While
175 energy availability is crucial, life is also dependent on the supply of specific nutrients and

176 micronutrients for biosynthetic processes. Our findings support these universal
177 requirements based on patterns of methanotrophic diversity in caves.
178
179 **Methanotroph *α*- and *β*-diversity in caves.** Methanotrophs are widespread in
180 cave environments. The dominance of the high-affinity methanotrophs from the USC
181 clades in the sampled caves is consistent with observations that have been made in other
182 low CH_4 environments. For example, high-affinity taxa dominate methanotroph
183 assemblages in forest soils, Arctic soils, and soils associated with basaltic rocks (4, 25, 26).
184 Additionally, the high relative abundance of methanotrophs in the sampled cave sediments
185 from this study, and in particular of the USC-*γ* clade, agrees with observations of high
186 relative abundances of USC methanotrophs from 2 to 12 % in other caves (8, 12, 14, 15).
187 The high relative abundances of methanotrophs observed in our study are higher than the
188 observations of methanotrophs from caves in Vietnam (7) and contrasts with the relatively
189 depauperate assemblage found in a Spanish cave, which reportedly consisted of only three
190 species (27)
191 Although less abundant, Type II and Type I methanotrophs were also commonly
192 observed from our cave survey. Type II methanotrophs are typically more abundant in
193 aerated soils with access to oxygen and Type I methanotrophs are typically observed in
194 environments with high CH_4 concentrations and low oxygen availability (17, 18). Cave
195 soils are typically nutrient poor (28, 29), implying that anoxic conditions created by the
196 decay of organic matter are not likely to be widespread. Thus, their presence in cave soils
197 may be limited to local pockets of anoxia which have been suggested based on
198 observations of limited methanogenesis in cave systems (10, 11).

199 We identified a core group of two OTUs that were present in the studied caves. The
200 core methanotrophic community was derived entirely of members from the USC-γ clade.
201 This suggests these methanotrophs are well attuned to cave environments and may be able
202 to disperse between them easily. The fact that these methanotrophs accounted for 59 % of
203 the observed methanotrophs suggests these organisms are reproducing in these
204 environments as well. Previous observations are also indicative of high-dispersal
205 capabilities for these organisms. For example, methanotrophs from a newly formed soil in
206 Hawai'i showed close taxonomic affiliations with Arctic methanotrophs (4). Additionally,
207 microbes are known to travel intercontinental distances on windblown dust (30), and
208 perhaps windblown dust may carry methanotrophs into caves.
209
210 **Microenvironmental influences on methanotroph communities.** The
211 relationship between the mineralogical assemblage and methanotrophs at the sample sites
212 is probably related to the abundance of trace metals. Copper is the trace metal responsible
213 for the oxidation of CH_4 in particulate methane monooxygenase enzyme of methanotrophs.
214 The methanotroph community showed relationships with the abundance of clinochlorite,
215 orthoclase, muscovite, and microcline. These minerals are associated with felsic
216 metamorphic and igneous rocks, and metals like copper are associated with these kinds of
217 rocks (31).

218 In contrast, the grain size of the cave sediments did not influence the methanotroph
219 community composition nor their abundance, even though it reflects important physical
220 and chemical properties. Previous research observed that methanotrophs tend to be more
221 abundant in silt and clay fractions of soils because these fractions made up the bulk of the

222 soils themselves, and potentially that these soils may have greater organic matter contents
223 (32, 33). The lack of relationships between methanotroph community composition and
224 methanotroph abundance agree with previous studies where no relationship was observed
225 between these variables (34).

226

227 **Biogeography of methanotroph communities.** Methanotrophic communities
228 showed relationships with regional assessments of geographic distance, but did not show
229 relationships with local assessments. The community composition of high affinity
230 methanotrophs changed faster than the environmental variables. This suggests that process
231 like community sorting are operating on this community (35). If community sorting is
232 operating on high-affinity methanotrophs, they may be actively adjusting to the cave
233 environment and preventing the establishment of other groups of organisms in this
234 environment. Conversely, the slope of the community composition of both Type II and
235 Type I (low affinity) methanotrophs, changed more slowly than the slope variables
236 comprising environmental similarity when compared to changes in the geographic
237 distance. This suggests that neutral processes are operating on these communities (35).
238 These processes are thought to operate more strongly on communities that are not strongly
239 influenced by the environment and are the strongest in small populations (36). This
240 suggests that these communities may be being passively dispersed into the caves or
241 awaiting favorable conditions to carryout active metabolism.

242 The composition and abundance of methanotrophic communities close to cave
243 entrances was not different compared to those in more interior locations. The most

probable explanation for this is that the distance from a cave entrance is a proxy variable for how interior a specific point is in a cave, but is not an absolute variable. Cave size and shape may prevent the distance from a cave entrance from being a useful variable for predicting cave methanotrophic community composition.

Relationships with cave air CH_4 concentrations. The relative abundance of methanotrophs was positively correlated with CH_4 concentrations in cave air. This pattern was driven by the high-affinity methanotrophs. High-affinity methanotrophs are thought to derive a significant portion of their energy from CH_4 at atmospheric concentrations and less energy is available for these organisms at locations in caves with sub-atmospheric CH_4 concentrations. Some of the variability observed in the relationship between high-affinity methanotrophs and cave air CH_4 concentrations may be explained by daily and seasonal variation in cave air flow patterns. Many caves are known to exhibit faster air flow when the external atmospheric temperature is lower than the temperature of cave air. This leads to higher CH_4 concentrations in caves during the winter (10, 37). Our samples were acquired during summer, so it is likely that the CH_4 concentration observed at the sample sites may fluctuate around our observations.

CONCLUSIONS

The structure of cave methanotroph communities appears to be best understood in terms of how cave methanotrophs access CH_4. Cave methanotrophic communities showed relationships with cave-air CH_4 concentrations suggesting these communities are actively

consuming CH_4 within the cave environment. Additionally, methanotroph communities showed relationships to minerals in the environment that are known to be related to the abundance of Cu, an essential trace metal for methane oxidation. Methanotrophs from the USC-γ clade were numerically dominant. This community structure mirrors that of other cave communities (8, 12, 14, 15). Members from this clade also comprised a core methanotrophic community with both members present together in 84 % of samples. The cave methanotrophic community assemblage appears to be undergoing the effects of community sorting and does not appear to be dispersal limited. This result also suggests that the dispersal barrier for high-affinity methanotrophs from cave to cave may be low.

MATERIALS AND METHODS

Microbial sampling. We sampled microbial communities from caves along the western front of the Appalachian fold and thrust belt, in intracratonic settings of the USA, and in the Sierra Madre of Mexico. We obtained 42 sediment samples from the caves along transects from their entrances to interiors. Cave sediment samples from walls and floors were collected using a spatula that had been sterilized with 70 volume % ethanol in water from locations that were 0.1 m^2 in area (38). Samples were stored on ice until they could be transferred to a −80 °C freezer.

Environmental variables. We measured multiple environmental variables to assess factors that potentially influence the composition of cave methanotrophic communities. We measured CH_4 concentrations *in-situ* with Fourier Transform Infrared

spectroscopy (FTIR) **(**Gasmet DX4030 – Milton Keynes, United Kingdom) or we collected discrete air samples collected in the field for measurement the laboratory with FTIR spectroscopy or gas-chromatography (Varian – Agilent Technologies, Palo Alto, California). Some of the CH_4 concentrations listed in this study have been previously published (11, 39), and new data were collected according to methods reported in the same publications. In cases where cave maps were available, the distance from the nearest entrance to the sampling location was calculated along the length of the cave passages. We measured cave sediment grain-size distributions with a Malvern Mastersizer 3000 (Malvern Instruments Inc., Westborough Massachusetts). Raw data from the Mastersizer were converted to % gravel, % sand, % silt, and % clay sized particles by volume using the GRADISTAT software package (40). Metadata used for statistical analyses are available in a public GitHub repository (https://github.com/websterkgd/CaveMethanotrophs)

Cave sediment mineralogy. We randomly selected 14 samples for mineralogical analysis using X-ray powder diffraction. Sediments were powdered to < 5 μm using a mortar, pestle, and acetylene. Following the methods of Furmann et al. (41), we used Bruker D8 Advance X-ray diffractometer with a Sol-X solid-state detector and a W X-ray tube operated at 30 mA and 40 kV to identify mineral phases. We placed cave sediment powders in an aluminum large frontpacked mount. Packed mounts were scanned from 2° to 70° using a count time of 2 s per 0.02° step. Rietveld refinements were used to determine the abundances of the minerals present in the sample and were quantified with TOPAS software.

311 **Molecular techniques.** We extracted Genomic DNA from cave sediment samples
312 with a MoBio PowerSoilTM extraction kit (MoBio, Carlsbad, California USA). About 10
313 ng of extracted DNA was used as a template for amplification by polymerase chain
314 reaction (PCR). The V4 region of the 16S rRNA gene was amplified using 515F and 806R
315 with barcoded primers designed to work with the Illumina MiSeq platform (42).
316 DNAamplification was performed using 50 µL reactions from each sample. PCR reactions
317 were carried out by a 3 min denaturing step at 94 °C, followed by 30 cycles of 94 °C for
318 45 s, 50 °C for 60 s, and 72 °C for 90 s. A final 10-minute extension was carried out at 72
319 °C. Quality of the PCR amplifications was checked by gel electrophoresis. Amplified
320 DNA was cleaned using a commercial kit (Beckman Coulter Agencourt AMPure XP,
321 Indianapolis, Indiana, USA). Once the DNA was cleaned, the DNA concentrations were
322 quantified using the QuanIt PicoGreen kit (Invitrogen) and were pooled at a final
323 concentration of 20 ng per sample.
324 We sequenced the cleaned PCR amplicons using Illumina MiSeq technology
325 (Illumina Reagent Kit v2, 500 reaction kit) at the Center for Bioinformatics and Genomics
326 at Indiana University. Data quality and unique sequences obtained from the PCR
327 amplifications were analyzed using mothur (43). DNA sequence data were aligned using
328 the Needleman algorithm and read lengths were limited to 197 base pairs. Sequences
329 matching chimeras were removed using VSEARCH (44). Sequences that matched
330 chloroplasts, Archaea, and other non-bacterial sequences were also removed. OTUs were
331 created by binning the data at 97 % sequence similarity using the opticlust algorithm (45).
332 OTUs were identified using the SILVA reference database (version 132).
333

334 **Identification of methanotrophs.** We identified methanotrophs from the 16S
335 rRNA sequences using a three step process. First, we compiled a database of organisms
336 known methanotrophs through literature surveys. Type I MOB were classified as the
337 known methanotrophic members of the Methylococcaceae. We defined Type II MOB as
338 known methanotrophic genera in the Methylocystaceae and Beijernickiaceae (23, 24). We
339 used the genera *Methylacidiphilum*, *Methylacidimicrobium* to identify methanotrophs
340 belonging to the Verrucomicrobia (24, 46). High-affinity MOB were defined as organisms
341 belonging to the USC-α and -γ clades (13, 23, 24). 16S rRNA sequences for USC-α and
342 USC-γ MOB were obtained using published 16S rRNA sequences for USC-α from (12)
343 and for USC-γ from (13). Second, organisms from this database were checked against the
344 identifications assigned to the OTUs during the initial analysis from the SILVA database.
345 Third, we compiled a FASTA file of the 16S rRNA gene of these organisms and this file
346 was compared against the FASTA file of all organisms in the dataset using the
347 *usearch_global* command to identify putative methanotrophs that may have been missed
348 through the SILVA analysis (usearch v.11.0.667) (47). This list of putative methanotophs
349 was then compared against the NCBI database. If the sequences did not match an organism
350 capable of the function of interest at 98 % similarity they were excluded from downstream
351 analysis. The compiled methanotroph FASTA file used in our analysis is available in a
352 public GitHub repository (https://github.com/websterkgd/CaveMethanotrophs).

353

354 **Statistical analyses.** We assessed the relationships between methanotrophs and
355 environmental conditions through a series of statistical analyses. First, to avoid
356 complication arising from unavoidable variation in the total number of reads between

357 samples, we calculated the relative abundance for each taxon within a sample. Next, to
358 quantify among cave variation, we calculated pairwise dissimilarities using the Bray-Curtis
359 metric. The methanotroph core microbiome was characterized by including taxa that were
360 found in 60 % or more of our samples. We used this cutoff because it is in the middle of
361 the range of what has been used in other studies (48, 49). We evaluated the effect of
362 sediment mineralogy and grain size on the multivariate composition of methanotroph
363 communities using canonical correspondence analysis (CCA) with the *vegan* package in R
364 (50).

365 To gain insight into the biogeography of cave methanotrophs, we constructed a
366 DDR while also comparing community composition along gradients from cave exteriors to
367 cave interiors. We analyzed DDR using Bray-Curtis measures of community similarity
368 and Euclidian measures of environmental similarity that had been scaled from 0 to 1.
369 Samples from Cave 38 (Cueva de Villa Luz) were excluded in DDR analyses since they
370 were roughly 2000 km away from the nearest sample. Slopes for geographic and
371 environmental DDR were calculated using ordinary least squares (52). We then tested for
372 differences in the slopes using the diffslope() function R package *simba*, which calculates
373 statistical significance through a permutation-based linear regression approach (53). We
374 used Spearman's correlation to examine how methanotrophic communities changed along
375 gradients from cave exteriors to interiors. Finally, we tested for a relationship between
376 methanotroph relative abundance and CH_4 concentrations using Spearman's correlation.
377

378 **Data and software availability.** This Targeted Locus Study project has been
379 deposited at DDBJ/EMBL/GenBank under the accession KCRG00000000. The version

described in this paper is the first version, KCRG00000000.1. All code and data used in this study can be found in a public GitHub repository (https://github.com/websterkgd/CaveMethanotrophs).

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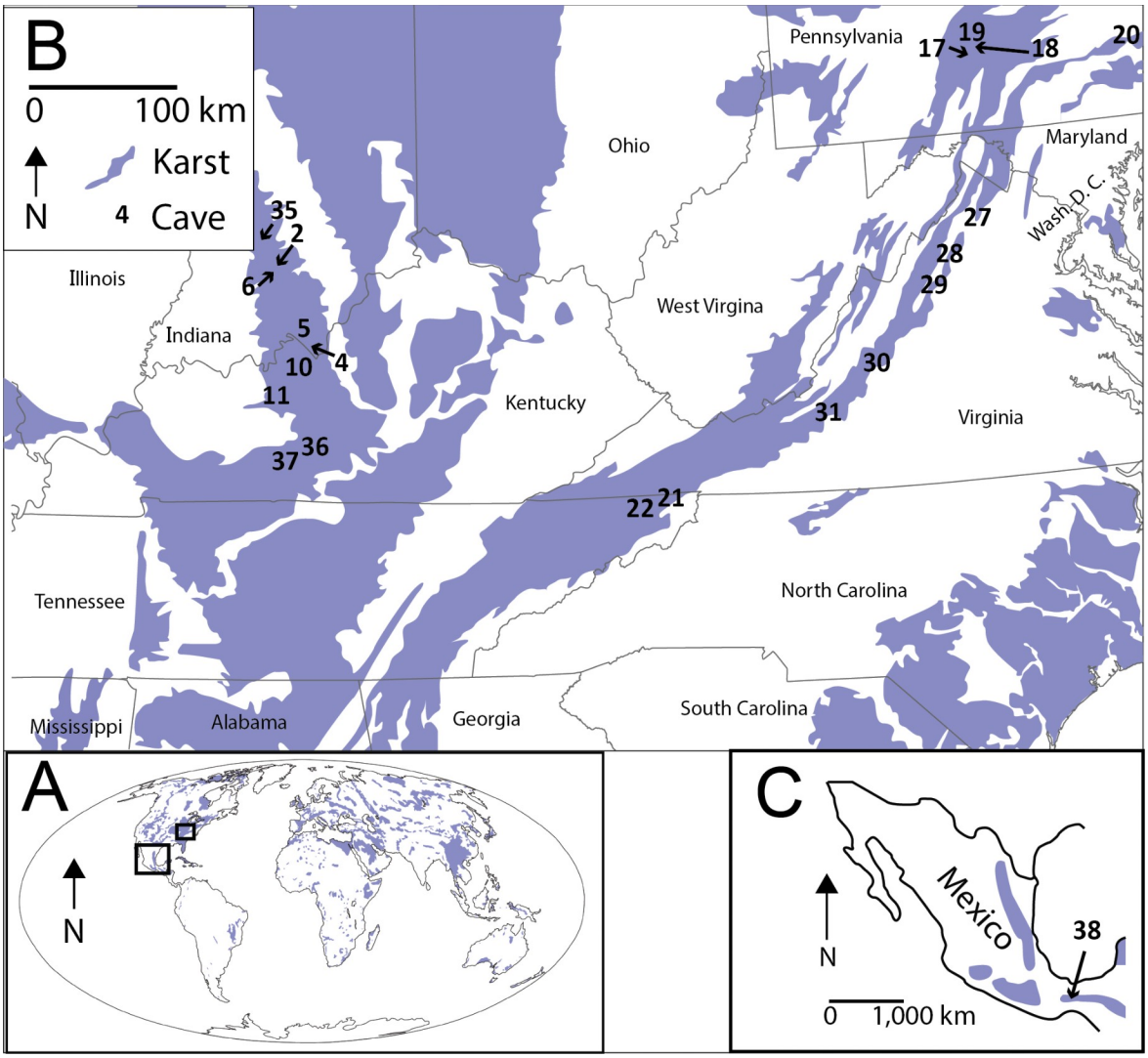

Figure 1: (A) The distributions of karst landscapes at global scale. Inserts show the occurrence of karst (B) in the eastern United States (C) and in Mexico. Numbers represent the locations of sampled caves in this study. Karst land cover data were obtained from (6, 54).

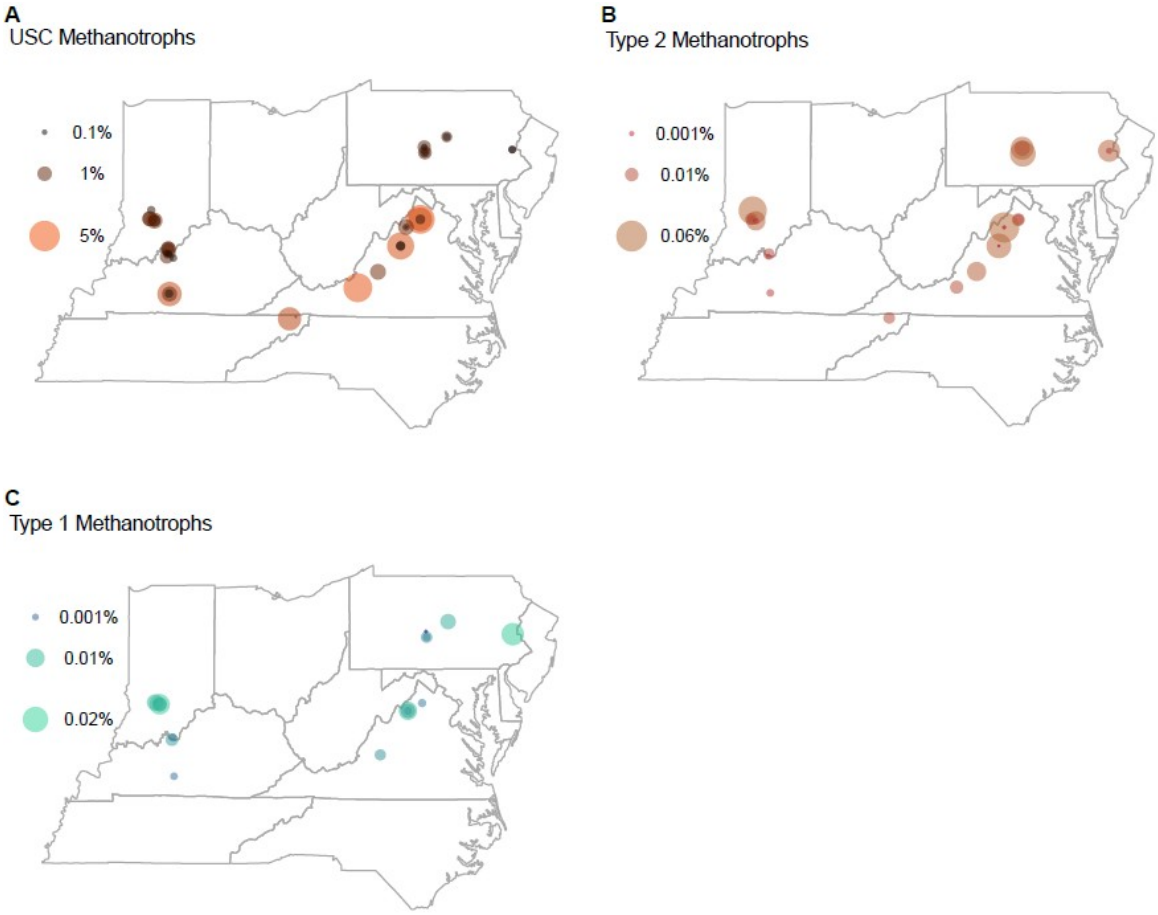

Figure 2: The relative abundance of A) USC methanotrophs, B) Type II methanotrophs, and C) Type I methanotrophs in the sampled caves.

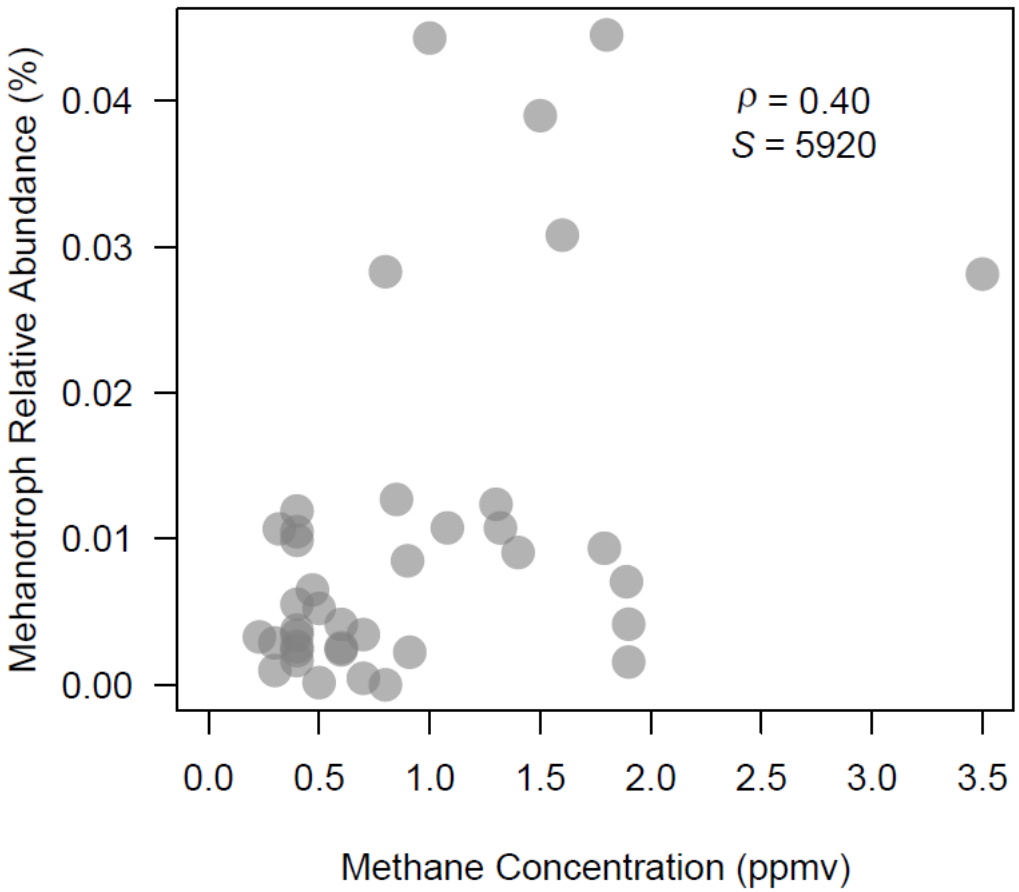

Figure 3: The relative abundance of members of the methanotrophic community plotted against the CH_4 concentration at each sample location (ρ = 0.40, S-statistic: S = 5290, p = 0.01).

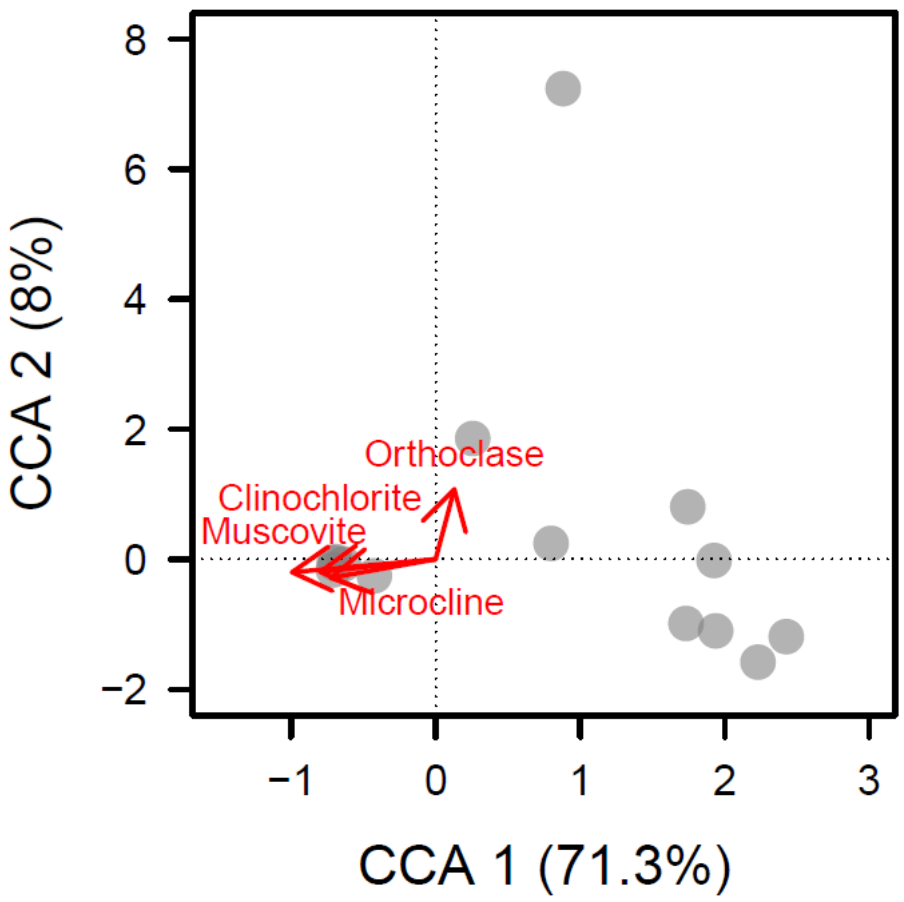

Figure 4: The total methanotrophic community showed relationships with the abundance of muscovite, clinochlorite, orthoclase, and microcline detected in the samples. The vectors show increasing abundances of minerals the samples.

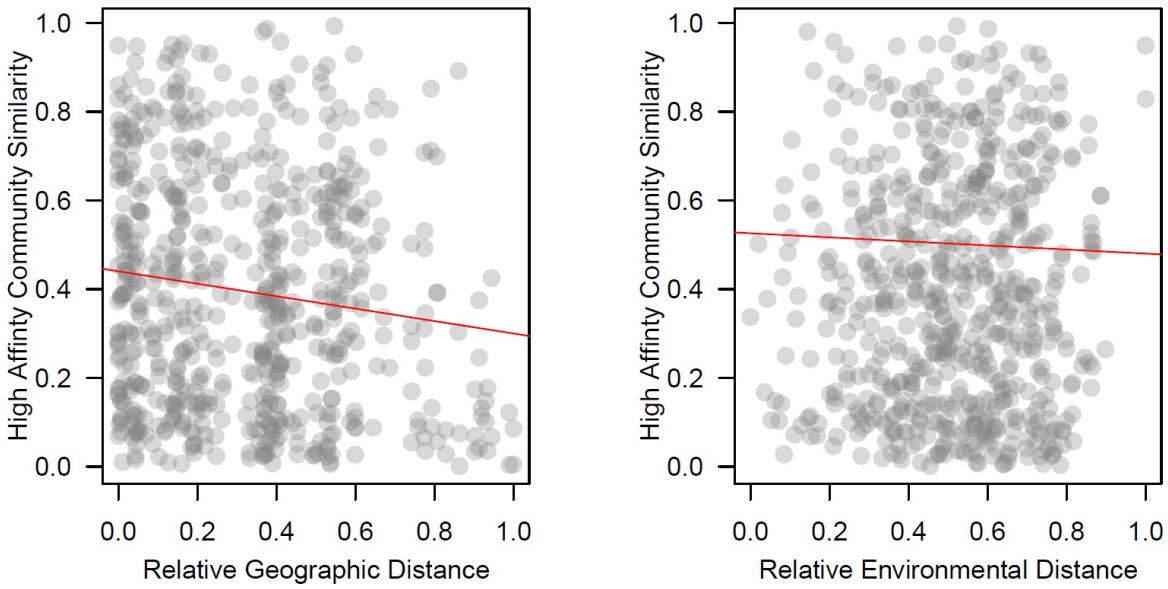

Figure 5: Distance decay analyses of the high affinity. The high-affinity community changed more quickly with changes in geographic distance than changes in environmental conditions. The samples from Cueva de Villa Luz have been removed from this analysis due to the large geographic separation of these samples from the rest of the samples.

Table 1: The *p*-values associated with the relationship between different methanotrophic communities and soil texture.

Particle Size	Total Community	USC	Type II	Type I
Gravel	0.39	0.42	0.34	0.65
Sand	0.48	0.47	0.33	0.39
Silt	0.29	0.44	0.19	0.83
Clay	0.55	0.54	0.51	0.53