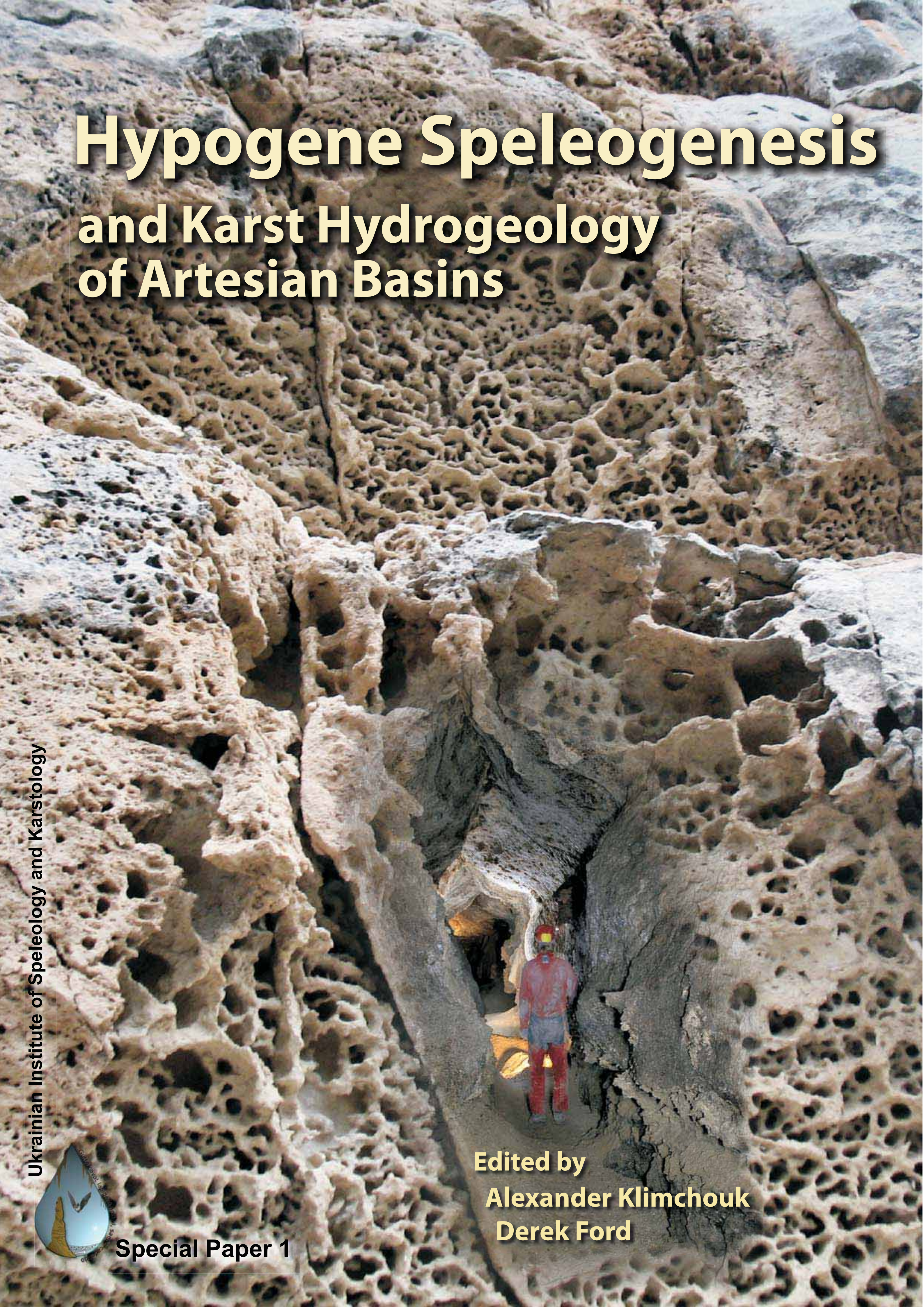


Hypogene Speleogenesis

and Karst Hydrogeology of Artesian Basins

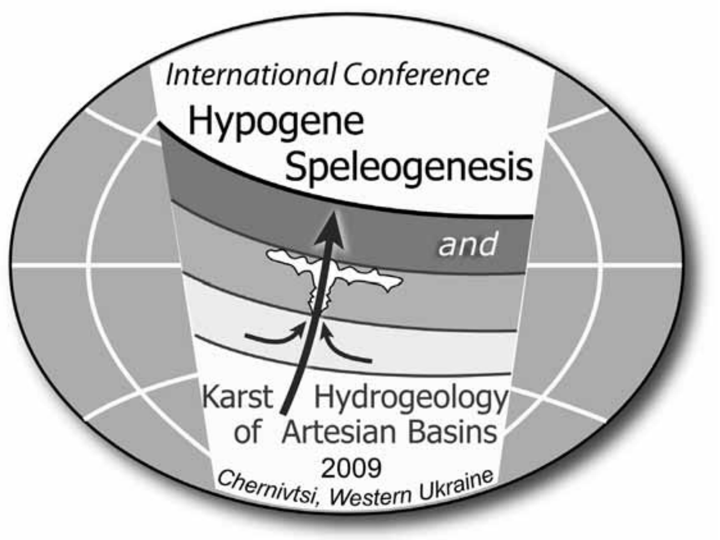
Edited by
Alexander Klimchouk
Derek Ford

Ukrainian Institute of Speleology and Karstology

Special Paper 1

Hypogene Speleogenesis and Karst Hydrogeology of Artesian Basins

Proceedings of the conference held May 13 through 17, 2009 in Chernivtsi, Ukraine



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and a passage in Slavka Cave, Western Ukraine (inset). Photos and collage by A.Klimchouk

Back cover: Hypogenic morphology in gypsum caves of the Western Ukraine.
Photos and collage by A.Klimchouk

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CONTENTS

MICROORGANISMS AS SPELEOGENETIC AGENTS: GEOCHEMICAL DIVERSITY BUT GEOMICROBIAL UNITY

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ABSTRACT

Caves provide habitats for a variety of native microorganisms. Many may be simply living in the subsurface, utilizing various sources of detrital organic matter without exerting much impact on their surroundings. However, some speleologically significant microbial communities appear to interact extensively with their rocky environments. These organisms can exert influence on the pace, extent, or nature of cave formation, contribute to precise forms that secondary cave decorations may take, potentially act as major agents of subsurface weathering, and sometimes produce a unique type of cave soil, speleosol. Sulfates and sulfides, iron and manganese, carbonates, copper minerals, or silica, may dominate the geochemistry and lithology of a site, but although the details of the chemistry and microbial diversity may differ, a suite of similar ecological themes with speleological consequences can be seen at work in each. These unifying geomicrobiological themes may be useful in assessing the potential biological interactions to be expected in new types of cave environments and may provide analytical tools in the future to help unravel the geochemistry, hydrology, and geology of such systems both on Earth and other Solar System bodies.

INTRODUCTION

It has been argued that microorganisms can exert influence on the pace, extent, or nature of cave formation (Northup and Lavoie, 2001; Boston *et al.*, 2001; Barton and Northup, 2007). Possible modes of action include enhancement of primary speleogenetic mechanisms (e.g. sulfuric acid speleogenesis, Hose *et al.*, 2000; Summers-Engel *et al.*, 2004; Boston *et al.*, 2006), secondary enlargement of primary cavities (e.g. enhancement of ongoing bedrock dissolution, Northup *et al.*, 2003; Spilde *et al.*, 2005, 2009), and significant contributions to the precipitation of a wide array of secondary minerals and speleothems (e.g., Vlasceanu *et al.*, 2000; Melim *et al.*, 2001, 2008; Gradzinski, 2003, *et al.*, 1997; Cacchio *et al.*, 2004; Curry *et al.*, 2009). These organisms may be major agents of subsurface weathering, not only in caves but also in the general rock fracture habitat producing a unique type of true cave soil dubbed *speleosol* (Spilde *et al.*, 2009). Although we have good evidence that many geomicrobial processes are at work (Table 1), we cannot yet precisely quantify the extent of microbial impact on any of them. The potential role of microorganisms in enhancing or retarding the specific suite of processes associated with hypogene speleogenesis is even less clear. However, the confined aquifer nature of such speleogenesis (Klimchouk, 2007) probably means that oligotrophic (low nutrient-adapted) microorganisms, often living off inorganic compounds rather than organic materials, may play a disproportionately large role in such systems. They may be at work long before such systems are breached and become known to us as caves with accessible natural entrances. Separating the effects of chemistry from physical processes in such systems is a major research task that awaits the speleological community and sorting out the role of geomicrobiology in these processes will be equally challenging.

UNIFYING THEMES BETWEEN DIFFERENT CHEMISTRIES AND COMMUNITIES

Although the details of chemistry and specific identities of organisms differ depending upon the geological setting of individual caves, we have noted that there are significant similarities in the interaction of biofilms, organisms, and geochemistry that can provide guidance for studies in a wide variety of subsurface environments (Boston *et al.*, 2007). These similarities include:

1. metal oxidation reactions as energy sources for organisms
2. ubiquity of biofilms and their abilities to transform minerals
3. indivisible multi-species communities, i.e. “consortia”, acting together as an ecosystem-level agent of change and facilitating mineral transformations

Table 1

Potential Influences of Microorganisms on Speleogenesis and Subsequent Cave Development

Speleological Process	Primary Mechanisms	Modes of Action	Geochemistries & Structures Thought to be Affected
Primary speleogenesis (creation of original passages)	1) Chemical dissolution via microbial action 2) Physical disaggregation	pH, acidification via organic or inorganic acids Tunneling, boring, leaching	Sulfide oxidation Silicates & opal mobilization Carbonates?
Secondary speleogenesis (enlargement of existing passages)	1) Chemical dissolution via microbial action 2) Physical disaggregation 3) pH changes	Organic or inorganic acid production or alkalinization	Iron and manganese Sulfate reduction
Speleothems (creation, enhancement, or directed patterning)	1) Concentration of elements 2) Production of crystallization 3) Altering physical properties, e.g. capillarity 4) Altering pH and affecting carbonate precipitation behavior	Tunneling, boring, mechanical breakage, providing biological substrate for subsequent precipitation	Copper sulfides to oxides Fe & Mn oxides Sulfates, elemental sulfur Carbonates, pearls, pool fingers Silica compounds, e.g. Opal-A
Speleosol formation (soils, pastes, clays)	1) Bedrock dissolution 2) Mineral reprecipitation 3) Mineral oxidation 4) Mineral phase progression	Leaching of oxidizable metals Organic acid production pH alteration Selective concentration, retention, or depletion of elements, especially REES (rare earth elements)	Carbonates, e.g. moonmilk Fe & Mn oxides, e.g. speleosol Authigenic clays

4. microbial pioneer species that move into and degrade bedrock providing conditions for subsequent species to exploit
5. precipitation by organisms of new mineral species from mobilized bedrock constituents
6. self-fossilization by living microbial communities often with exquisite morphological preservation
7. large number of unusual morphological microbial types
8. typically very slow growth rates of the most critically involved microorganisms
9. frequently very small cell sizes

Metal Oxidation Reactions as Energy Sources

The ubiquitous availability of metal compounds in rocky environments provides sources of metabolic energy for a very wide variety of organisms, from bacteria and archaea to fungi (e.g. Ehrlich, 2002; Rawlings *et al.*, 2003; Gadd, 2007). In many environmental circumstances, these organisms are biologically interesting but present in low numbers and living very marginal existences on limited energy sources. However, in certain special situations where geochemical or atmospheric conditions predispose it, they can take advantage of energy sources not readily available to many of their neighboring species. In these circumstances, such opportunistic microorganisms may make a major impact on their environment by processing these metal energy sources.

For example, microorganisms can affect the mobility and accumulation of iron and manganese. Microbial oxidation of reduced Fe and Mn is common in the terrestrial environment and can be found in deep marine, lake, and stream sediments, hot spring environments, and caves, where the end products, Mn- and Fe-oxides, may be present on cave surfaces and even on and in some speleothems. Iron and Mn-oxidation can serve as an energy source for a number of bacteria at both the acidic and neutral pH conditions found in various caves, which may promote primary or secondary speleogenesis by dissolution of Fe(II)- and Mn(II)-bearing carbonates. Our team's work has shown that Mn-oxidation by the microbial community can be a significant factor in the breakdown of bedrock carbonates in secondary speleogenesis (Spilde *et al.* 2005, 2009). Deposits of Fe and Mn minerals (ferromanganese deposits) have been found in a growing number of caves in the United States (New Mexico, Arizona, and South Dakota), in Turkmenistan, and in Spain. Manganese minerals, especially birnessite, todorokite, and lithiophorite, are often associated with Fe- and Al-(hydr)oxides including goethite, hematite and nordstrandite. Amorphous Mn and Fe oxyhydroxides are also present. We have shown that these deposits are microbially influenced (Boston *et al.*, 2001, Northup *et al.* 2000, 2003, Spilde *et al.* 2005), and DNA analysis yields a diverse community of microorganisms, including Mn-oxidizers (Northup *et al.* 2000, 2003). Bacteriogenic Mn-oxides are initially amorphous nanoparticulates or nanocrystalline aggregates that recrystallize into layered Mn-oxides (phyllomanganates).

MIC (microbially induced corrosion) of mild steel by SRB (sulfate reducing bacteria) has been extensively discussed in a series of papers that treat both biotic and abiotic processes of corrosion (Hamilton and Lee, 1995; Lee *et al.*, 1995; Lewandowski *et al.*, 1997; Hamilton, 2000a; Hamilton, 2000b), and consider the underlying physiology of SRBs (Hamilton, 1998; 1999). From these studies, it is clear that oxygen plays a central role as terminal electron acceptor. Strains within mixed microbial consortia are probably located at different individual sites within the biofilm that contributes to MIC (Hamilton, 2003). According to this author, the oxic/anoxic interface combined with ferric oxide or hydroxide provide separate focus areas of electrochemical activity, that result in iron dissolution and pit formation at the anodes of these tiny galvanic cells. Although this model was developed for microbial corrosion of mild steel, similar mechanisms can help to explain the ferromanganese acid processes that we see in both active sulfuric acid systems like Cueva de Villa Luz in Tabasco, MX, and in relict systems like Lechuguilla Cave, NM, that involve slower processes of bedrock breakdown but

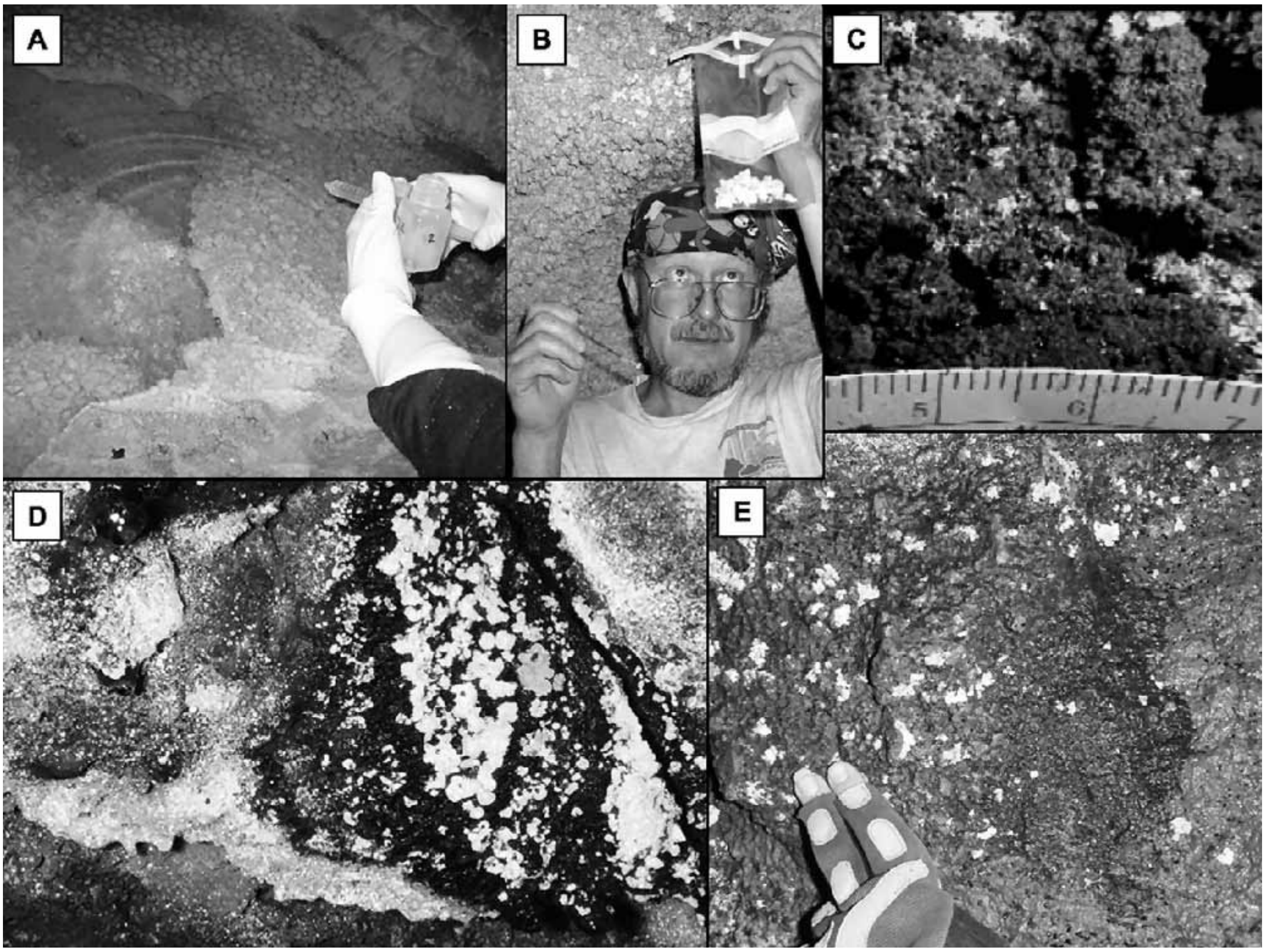

Figure 1. Cave microbial breakdown products (speleosols) and microbial mats. A. Subaqueous moonmilk known as "cottonballs" (Curry et al., 2009; image by A. Martin-Perez). B. Moonmilk coats the walls of Spider Cave, NM, USA (image by V. Hildreth-Werker). C. Iron and manganese rich speleosol on the walls of Lechuguilla Cave, NM, USA (Spilde et al., 2009; image by V. Hildreth-Werker). D. Highly patterned and colorful lavatube microbial colonies, Epperson's Cave, Hawaii, USA (image by K. Ingham). E. Actinobacteria-rich biomat patches on walls of granite cave in Gallicia, Northern Spain (image by P. Boston).

apparently over a much longer period of time. The precise age of the residual materials left by these processes has not yet been assessed.

Ubiquity of Biofilms and Mineral Transformation

Microorganisms are very small on an individual basis and have limited influence on their environments as single organisms. However, they apparently invented biofilm very early on in the history of life (Rosales and Ranero, 2007; Westall *et al.*, 2001, 2008) which has served to greatly extend their influence over their environment. In caves, the typically moist environment supports the growth and persistence of biofilms and the lack of surface weathering leaves them undisturbed to become fossilized for us to find, often as distinctive mineral coatings or biofabrics (Boston, 2004a). These complex layers of extracellullar polysaccharides (aka *slime*) can be seen in numerous caves and other habitats. From the point of view of the organisms, this coating of hydrated slime serves to protect them from desiccation or from toxic compounds in the environment and enables them to exchange materials and possibly information between individual cells via a process known as quorum sensing or efficiency sensing (Waters and Basler, 2005; Hense *et al.*, 2007). Biofilms enable organisms to store compounds and to create chemical, pH, and other conditions around themselves that differ from the overall environment in which they find themselves. For example, oxygen levels can be very different in different parts of a biofilm, allowing aerobes to thrive in one part of the biofilm while microaerophilic organisms live nearby. Organisms may use biofilms to transport and store nutrients and concentrate important compounds that are in low concentrations in the general milieu (Costerton *et al.*, 1994.)

The mechanism of biofilm or microbial mat formation is relatively simple. Bacteria at liquid/surface interfaces are attracted to the surface and adhere to it, producing a carbohydrate layer that binds proteins and lipids (Lappin-Scott and Costerton 1997). Small founder organism colonies form, which in turn attract further organic and inorganic matter. In environments where sediments are present periodically or continuously, these sediments are deposited and trapped by the biofilm or mat. In addition, mineral-precipitating microorganisms create mineral deposits from solutes in the liquid phase. In environments like caves, mineral precipitators abound and produce spectacular biomineralization deposits. Biofilms can be some of the most visually striking indications in caves that microorganisms are present (Figure 1).

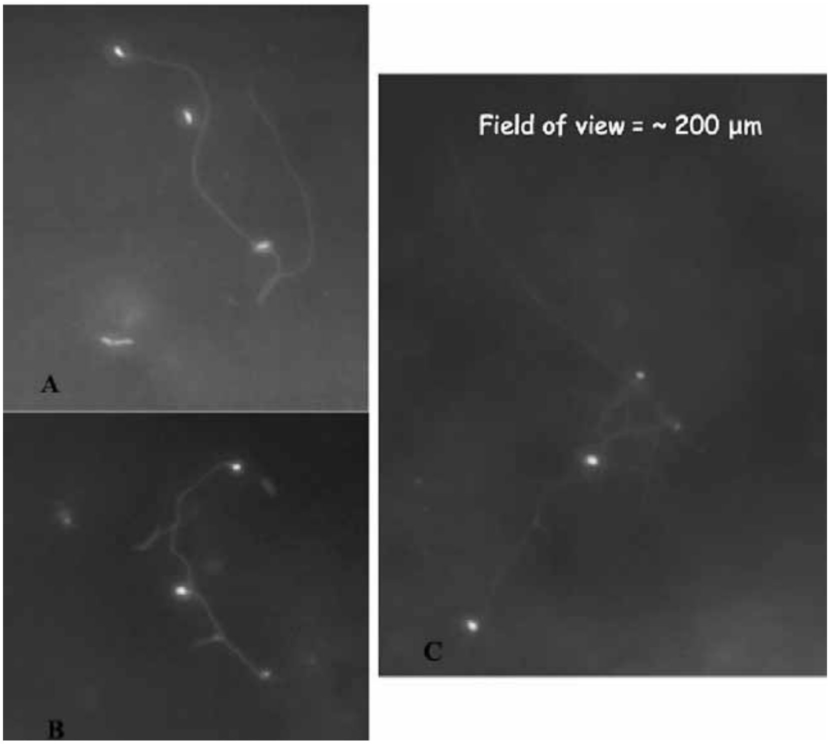

Figure 2. Epifluorescently dyed microbial cell bodies and processes from rock cores through the leached "punk rock" zone, Lechuguilla Cave, NM, USA. After Northup et al., 2000.

Multi-Species Consortia

In our experience, the number of organism strains found in cave microbial mats, biofilms, and pedogenic processes like speleosol or moonmilk are numerous and typically prefer to grow in complex mixed communities rather than tolerate isolation into single species in the laboratory. While there are exceptions, the microbial consortia of organisms also seem to be more effective at bedrock dissolution and certainly more effective at fully utilizing all of the mineral and metal nutrient sources in their environments. Acting together as miniature ecosystems, their interactions appear to facilitate mineral transformations. For example, microbial consortia rather than single organisms are invariably found in commercial-scale bioleaching operations (Johnson, 2001), and these organisms are typical of the variety we find in cave studies (Northup *et al.*, 2000, 2003). In our microbial culturing experiments, attempts to separate organism strains while still maintaining their abilities to manipulate environmental materials are often unsuccessful. Even if organisms continue to grow, they typically lose their abilities to precipitate various mineral phases.

Microbial Pioneer Species

As with large scale ecosystems above ground, microorganism communities in caves have strains representing the same ecological equivalents, namely pioneer species, primary producers, primary consumers, and secondary and tertiary consumers. However, all of this trophic complexity occurs in the space of a biofilm mat on a flowstone slope rather than in more ecologically familiar large scales like that of a tropical rainforest canopy. The role of pioneer microbial species in caves is often to move into and degrade bedrock, thus providing appropriate conditions for subsequent species to exploit. Examples of this from our team's work include the organisms that have facilitated the mineralogical leaching of bedrock limestone in Lechuguilla Cave (NM, USA). We see a zone of de-cemented bedrock (known as *punk rock*) that contains tiny cellular bodies and long filamentous processes that stain with biological dyes (Figure 2; Northup *et al.*, 2000; Spilde *et al.*, 2005). Such organisms produce organic acids which presumptively facilitate bedrock dissolution but the rate at which this occurs in nature is still unknown to us.

Mineral Re-precipitation

Mobilized bedrock constituents that are released from their original form by organism metabolism and other activities are free to re-precipitate into other mineral phases (Frankel and Bazylinski, 2003). A clear example of this re-precipitation mechanism can be seen in moonmilk, the white pasty material that is relatively uncommon but globally distributed in a wide variety of caves in the world (e.g. Hill and Forti, 1997; Gradzinski *et al.*, 1997: Curry *et al.*, 2009; Figure 1 A and B). This material has a similar appearance despite many mineralogical differences between occurrences.

Moonmilk is a general term that describes aggregates of microcrystalline substances that vary in composition and tend to be white, soft, pasty and resemble clots of cream cheese or cauliflowers (Hill and Forti, 1997). Moonmilk frequently has high water content, with accompanying high degree of plasticity (Hill and Forti, 1997). It has been suggested that the composition of moonmilk can vary depending on the parent rock; thus limestone caves generally consist of calcite moonmilk, while those with magnesium-rich dolomitic parent rock tend to form hydromagnesite, magnesite, huntite, and altered dolomite (Boston *et al.*, 2001). A typical moonmilk type is found in Spider Cave at Carlsbad Caverns National Park (New Mexico, USA) and commonly referred to as "Crisco." It is composed of filamentous calcium carbonate that is associated with microorganisms, and has a slippery, greasy feel similar to clay (Boston *et al.*, 2001). Our team has argued that mobilization of bedrock and re-precipitation onto microbial cells and filaments may be a major mechanism of the production of this material (Curry *et al.*, 2009, and unpublished results).

Microbial processes can also create a chemical environment that favors mineral precipitation. They can force mineral precipitation by changing the local pH, producing polysaccharide secretions for anchorage that provide sites for mineral nucleation, and forming mats of colonies that also provide sites for nucleation (Ehrlich, 1996). In addition, their surfaces can act as sites for mineral accumulation, and they themselves can precipitate minerals as a byproduct of their metabolic activities.

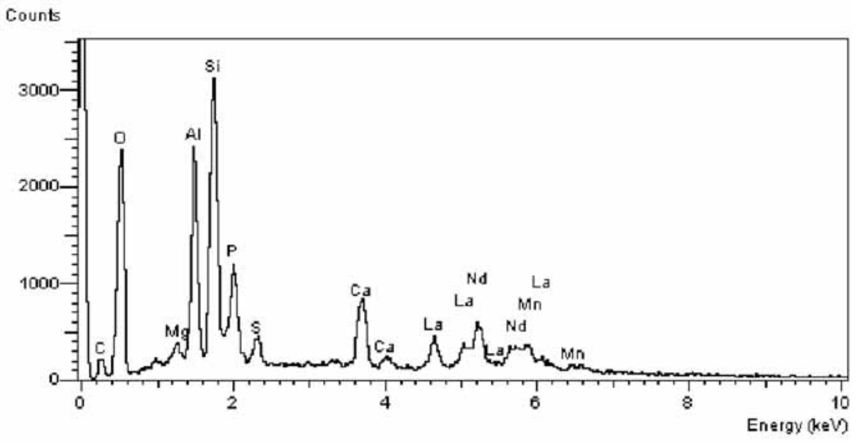

Figure 3. EDS (Energy Dispersive Spectroscopy) spectrum showing selective enrichment of REEs Lanthanum and Neodymium, along with major Mn enrichment that we have reported previously for these materials (Spilde et al., 2005). These metals are in trace amounts or undetectable in analysis of the bulk bedrock and are highly enriched compared to a simple carbonate dissolution residue analysis.

As a consequence both of microbial mobilization of materials and re-precipitation processes, extreme selectivity for certain elements can be seen. For example, rare earth elements (REE) appear to be selectively retained in higher concentration than is found in the bedrock in a number of caves (Spilde *et al.*, 2005, 2006; Northup *et al.*, 2003; Boston *et al.*, 2004). Figure 3 shows an example of selective enrichment of several REEs in speleosol from Lechuguilla Cave, NM, USA.

In situ Self-Fossilization by Living Microbial Communities

In the cave environment, the microbial community may produce both dissolution and re-precipitation . Extensive microbial growth in close proximity lends itself to preserving living materials with extreme morphological fidelity. The organisms are often coating themselves or impregnating their interiors with minerals. In many cases, these mineralization processes probably contribute to the organisms' demise, but it does confer a type of morphological immortality that most of the rest of us will not enjoy! In the absence of surface weathering, this preservation can remain undisturbed for geologically significant periods of time in spite of lacking the usual burial and replacement processes of ordinary fossilization in surface environments. Figure 4 B (seen below) shows copper-coated fungi and actinobacteria that have mobilized copper from copper sulfides and redeposited this on themselves as either copper oxides or elemental copper (Spilde *et al.*, 2006).

Unusual Morphological Types

One of the interesting aspects of subsurface microbiology is the array of unusual morphological forms of microorganisms present. Typically, bacteria come in a very small range of physical shapes, ranging from spheres (cocci), rods, and sometimes spirals. However in cave environments, multiple filaments and other stranded structures, fuzziness, spikiness, unusual holdfasts, stalks, mottled or crennulated surfaces, angular morphologies, knobs, bumps, and indescribable other protrusions frequently appear (Figure 4). Recently, we have reported on mysterious mesh-textured long strands of some type of organism or organism component that we have been unable to identify (Melim *et al.*, 2008). The presence of such unexplained apparent biological objects in cave samples points to our considerable lack of information about the speleomicrobial world and our strong need to increase the number of investigators working in this arena.

Very Slow Growth Rates of The Most Critically Involved Microorganisms

In our culturing studies, we find that the chemolithoautotrophic or chemoorganotrophic cave organisms typically grow extremely slowly compared to those in more nutrient rich, heterotrophic environments. Incubation periods of weeks to years is frequently necessary to observe growth. Beyond simple growth, maintaining cultures long enough to see mineral precipitation behavior can require many additional weeks to years. For example, Mn oxidation and precipitation can occur in as short a time as a few weeks for some organisms, but we have shown that initial amorphous Mn oxide formation is followed by an elaborate sequence of increasingly complex crystallization over the course of months to years in cultures maintained for long periods of time. Killed controls do not take the crystallization any further than it was at the time that the cultures were killed. We believe that this time frame for mineralization mimics what we find in nature (Boston *et al.*, 2001; Spilde *et al.*, 2005), and we have some long term experiments that demonstrate this over the course of 8 to 9 years as of this writing.

Very Small Cell Sizes

In the oligotrophic cave environments, very small cell sizes are common in the range of 300 - 500 nm diameter. This size range places them well within the middle range of what are being termed nanobacteria. The origin of nanobacteria-like particles in materials as diverse as travertines, human medical contexts, and Martian meteorites is very controversial (e.g. Martel and Ding-E Young, 2008) but little work has focused on the small cell sizes of clearly microbial forms that we have seen in electron micrographs and cultures. We have good evidence that our small bacteria are reproducing in the lab in normal microbial fashion.

CONCLUSIONS AND THE PROSPECTS FOR FUTURE WORK

The essence of scientific understanding lies in the ability to connect different manifestations of similar phenomena broadly across nature. In our work and that of other colleagues, we are seeing a wide range of biodiversity in the organisms involved in caves, a broad array of different geochemical settings and mineral products, and yet a distinct commonality of the major ecological processes and themes which we have discussed here. These unifying

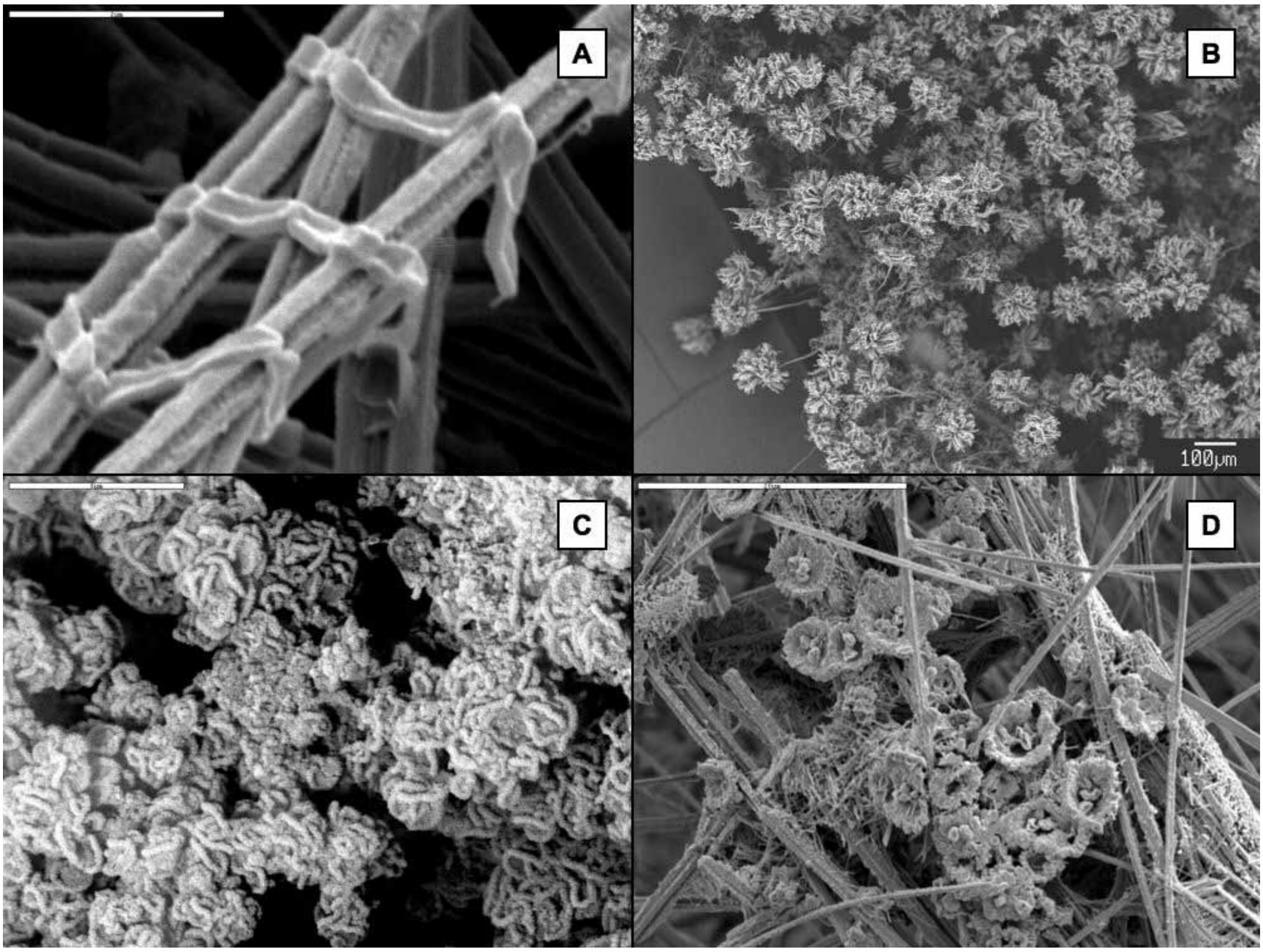

Figure 4. Unusual morphological types of microorganisms are frequently encountered in subsurface samples from caves and mines. A: "Silk stockings" draped across calcite filaments. Probably empty streptomycete sporophores (filamentous spore sacs). Spider Cave, NM, USA. B: Organisms coating themselves with copper oxides as a result of breaking down copper sulfide minerals. Specimen from the Harvard Mineral Museum collection, courtesy of Dr. Carl Francis. C. Fuzzy actinobacteria living amidst chrysocolla deposits, Maelstrom Lavatube, Hawaii. D. Microbial "nests" containing small numbers of cells amidst calcite rods in moonmilk, La Cueva de Las Barrancas, NM, USA.

geomicrobiological themes may be useful in assessing the potential biological interactions to be expected in new types of cave environments, for example, granite caves (Vidal-Romani and Vaquiero-Rodriguez, 2007) or quartzite (Gorbushina *et al.*, 2001), and may provide analytical tools in the future to help unravel the geochemistry, hydrology, and geology of such systems.

All of this brings us to consider the precise role of microorganisms in hypogene systems. We speculate that the primary role of microbial communities in such systems is by means of altering the chemistry of the waters and rock surface interfaces in confined aquifers. The efficacy of such interventions by organisms depends directly on the potential energy sources available in the environment. If there are metabolizable reduced sulfur compounds in a hypogene system, then microbial communities can make a major impact on limestone dissolution rates via enhancement of the overall acidity of the system. If there are oxidizable sources of iron or manganese compounds (or other metals like arsenic and uranium) then microorganisms exist which are quite capable of "mining" such metals from bedrock, consequently breaking it down and acting as weathering agents and enhancing speleogenetic rates. However, in a system that has little to offer to microorganisms in the way of nutrients, the role they can play in enhancing hypogene processes is similarly limited. On the other hand, the ability of microorganisms to produce biofilm, often in copious amounts, could conceivably act as a retardant of speleogenetic processes by actually protecting rock surfaces from chemical attack. Such film might even protect against some aspects of mechanical speleogenesis by serving as a lower friction surface that may control laminar versus turbulent flow regimes and fluid/rock interfaces. Of course, this is merely speculation and no work has been done on such topics in cave environments to our knowledge.

Besides the Earthly applications of the work discussed here, the consequences of these types of analyses include developing an ability to distinguish potential biological influences on subsurface environments on other planets, especially Mars (Boston, 2004b; Boston *et al.*, 1992; Gorbushina *et al.*, 2002; Grin *et al.*, 1998; Cushing *et al.*, 2007). The ability to recognize biological influence in the subsurface may have an important role to play in future life detection space missions.

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