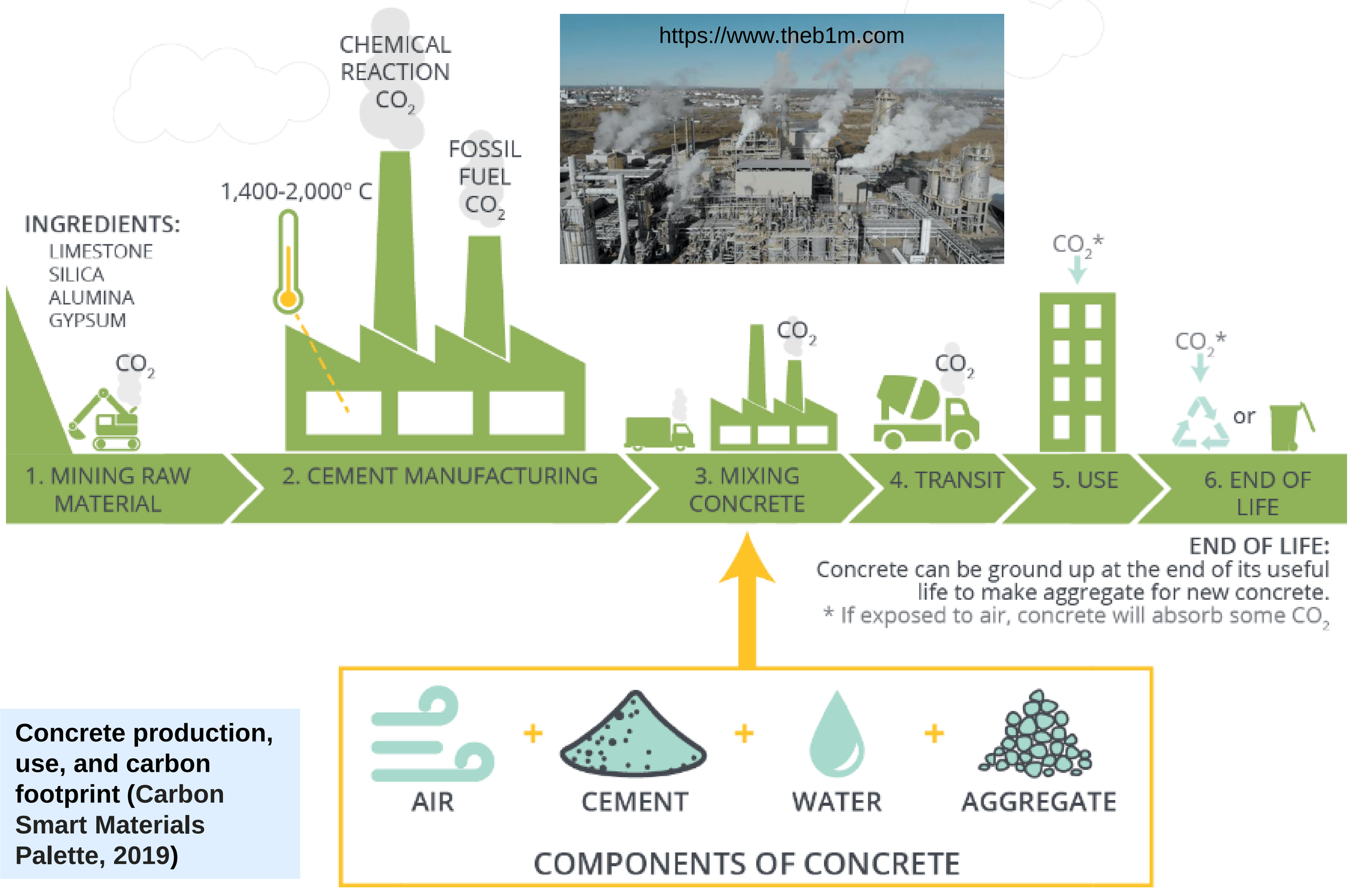
Understanding Incidental Microbial Communities Inside Ordinary Concrete Toward Decarbonization

Concrete Contributes to >8% of Global Greenhouse Gas Emissions

The need to decarbonize the concrete industry:

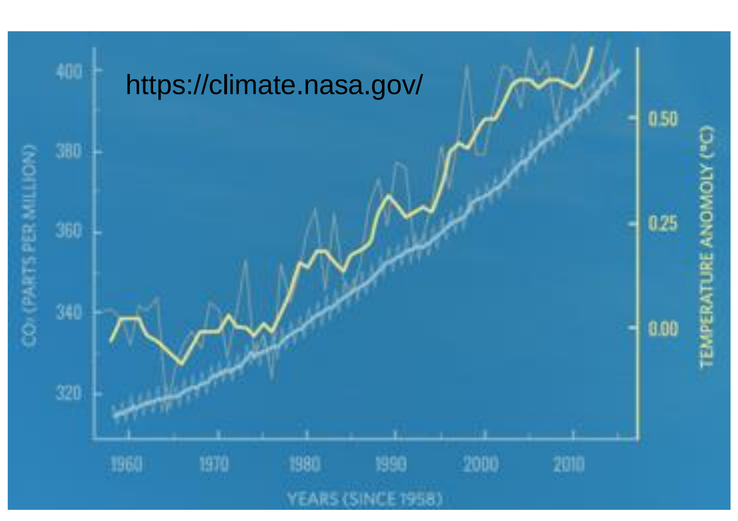
- **Annual usage of concrete worldwide is about 30 billion tons** (Nature Editorial, 2021) for constructing essential infrastructures
- Concrete production contributes to **>8% of global greenhouse gas emissions**, equivalent to the **3rd highest contributor** after China and the U.S.

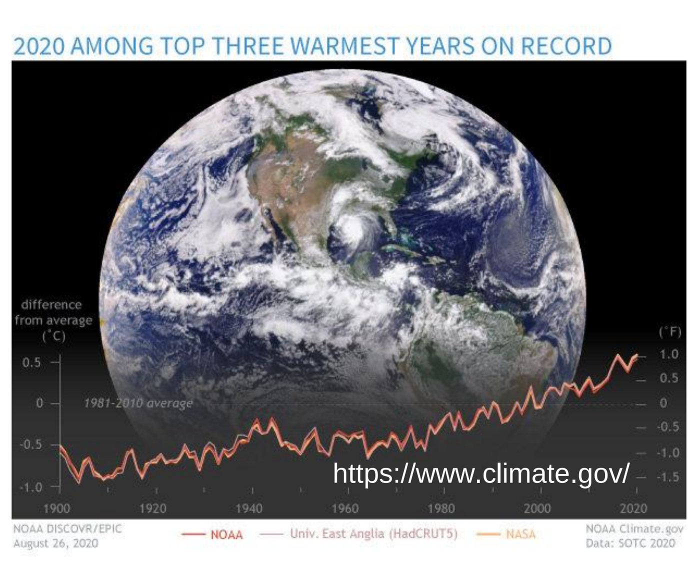


Concrete production, use, and carbon footprint (Carbon Smart Materials Palette, 2019)

Decarbonizing the concrete industry to meet two major societal needs:

- **Reduce global CO_2 emissions** to reach targets in 2016 Paris Agreement – limiting warming to 1.5°C, which means reducing global emissions by 45 percent from 2010 by 2030 (National Academies, 2022)
- **Make concrete safer**, given recent events of **infrastructure failure** (collapsed Miami condominium in 2021)





Potential approach and past research:

- Possible approach to **decarbonize the concrete industry**: understand microbes in concrete to maximize concrete durability and lifespan
- **No studies** have investigated the **ubiquity and community-level characteristics** of endoliths inside ordinary, **real-world concrete**, which is necessary to maximize potential applications

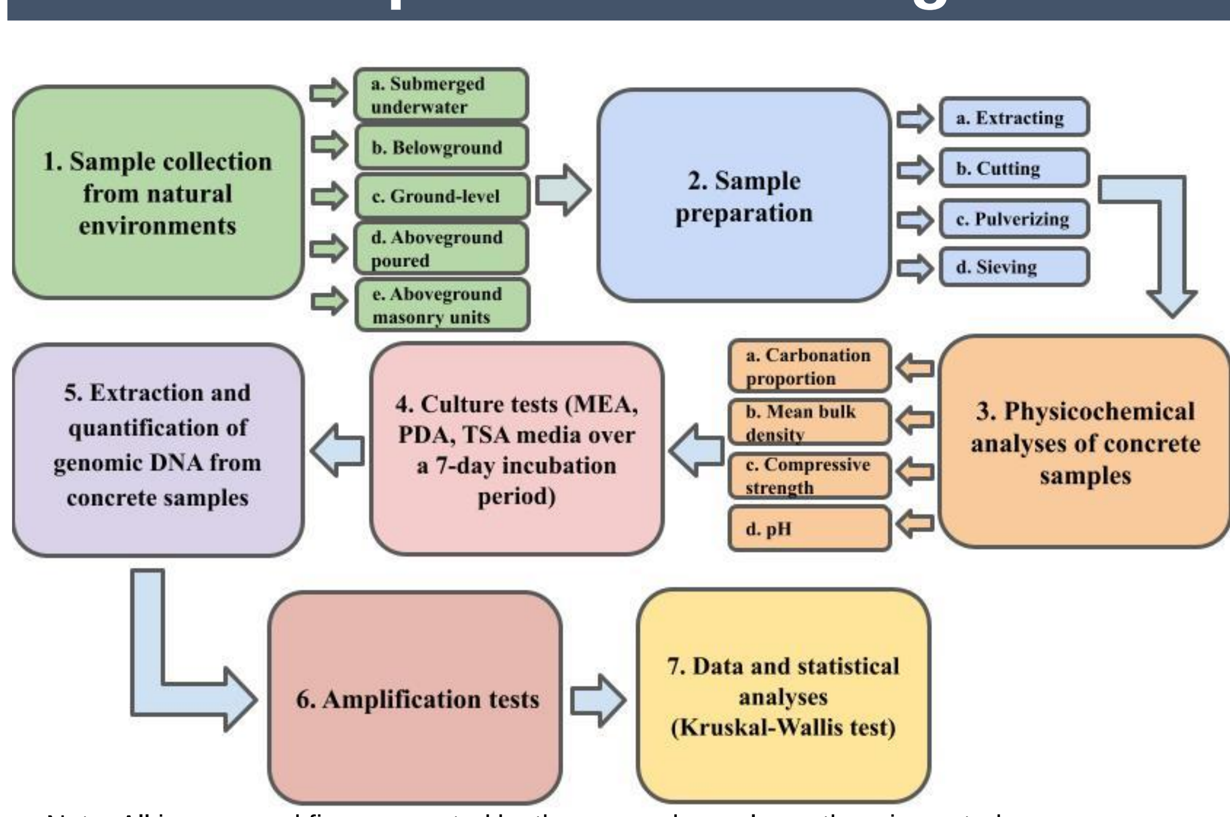
Objectives and Hypotheses

Objective 1: Complete endolithic life-detection survey of various forms of ordinary concrete gathered from various settings within a city environment.
Hypothesis 1: "Concrete-endolith hypothesis" – if the interior portions of concrete are a suitable habitat for microbes, then various internal concrete or "endo-concrete" samples will yield positive life-detection results.

Objective 2: Explore supplemental methods for detecting and describing concrete endoliths.
Hypothesis 2: "Endolith-concrete interaction hypothesis" – microbial community patterns inside concrete will vary by the physical and chemical characteristics of their respective concrete substrates as well as by general environments and forms in which the concrete exists.

Objective 3: Determine the type of species inside of concrete.
Hypothesis 3: After DNA extraction, successful PCR amplification tests will reveal the species of microbes that exist in concrete, including bacteria, archaea, and fungi.

Experimental Design and Methods



Note: All images and figures created by the researcher unless otherwise noted.

Living Microbes Exist Inside Ordinary Concrete

Culture test results by concrete bulk density

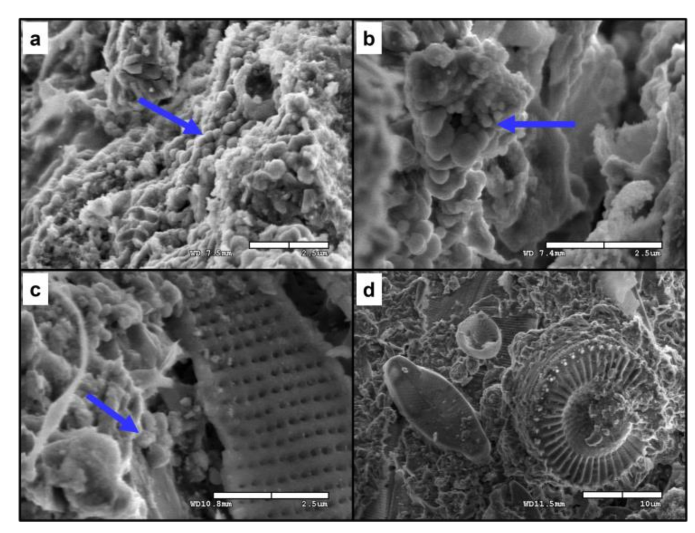
Sample ID	Bulk Density (g cm^{-3})	Media Type: MEA	PDA	TSA
Submerged-3	2.443			
Aboveground-3	2.367			
Submerged-5	2.361			
Ground-5	2.351			
Ground-2	2.331			
Belowground-4	2.326			
Belowground-1	2.323			
Ground-3	2.322			
Submerged-2	2.320			
Submerged-1	2.319			
Belowground-5	2.305			
Ground-4	2.304			
Belowground-2	2.300			
Belowground-3	2.285			
Aboveground-4	2.283			
Aboveground-5	2.241			
Ground-1	2.241			
Aboveground-2	2.223			
Submerged-4	2.185			
Aboveground-1	2.134			
CMU-5	1.876			
CMU-3	1.606			
CMU-1	1.586			
CMU-2	1.537			
CMU-4	1.318			

Culture test results by compressive strength

Sample ID	Compressive Strength (MPa)	Media Type: MEA	PDA	TSA
Belowground-1	11.74			
Aboveground-3	9.62			
Submerged-2	8.84			
Submerged-1	8.24			
Ground-5	7.74			
Belowground-2	7.64			
Ground-4	7.40			
Submerged-5	7.27			
Belowground-5	6.64			
Ground-1	6.38			
Ground-2	6.01			
Belowground-3	5.24			
Belowground-4	4.44			
Submerged-4	4.38			
Aboveground-4	4.21			
CMU-5	3.95			
Aboveground-5	3.87			
Ground-3	3.85			
CMU-1	3.59			
Submerged-3	3.45			
Aboveground-2	3.02			
CMU-3	2.96			
Aboveground-1	2.74			
CMU-4	2.60			
CMU-2	2.36			

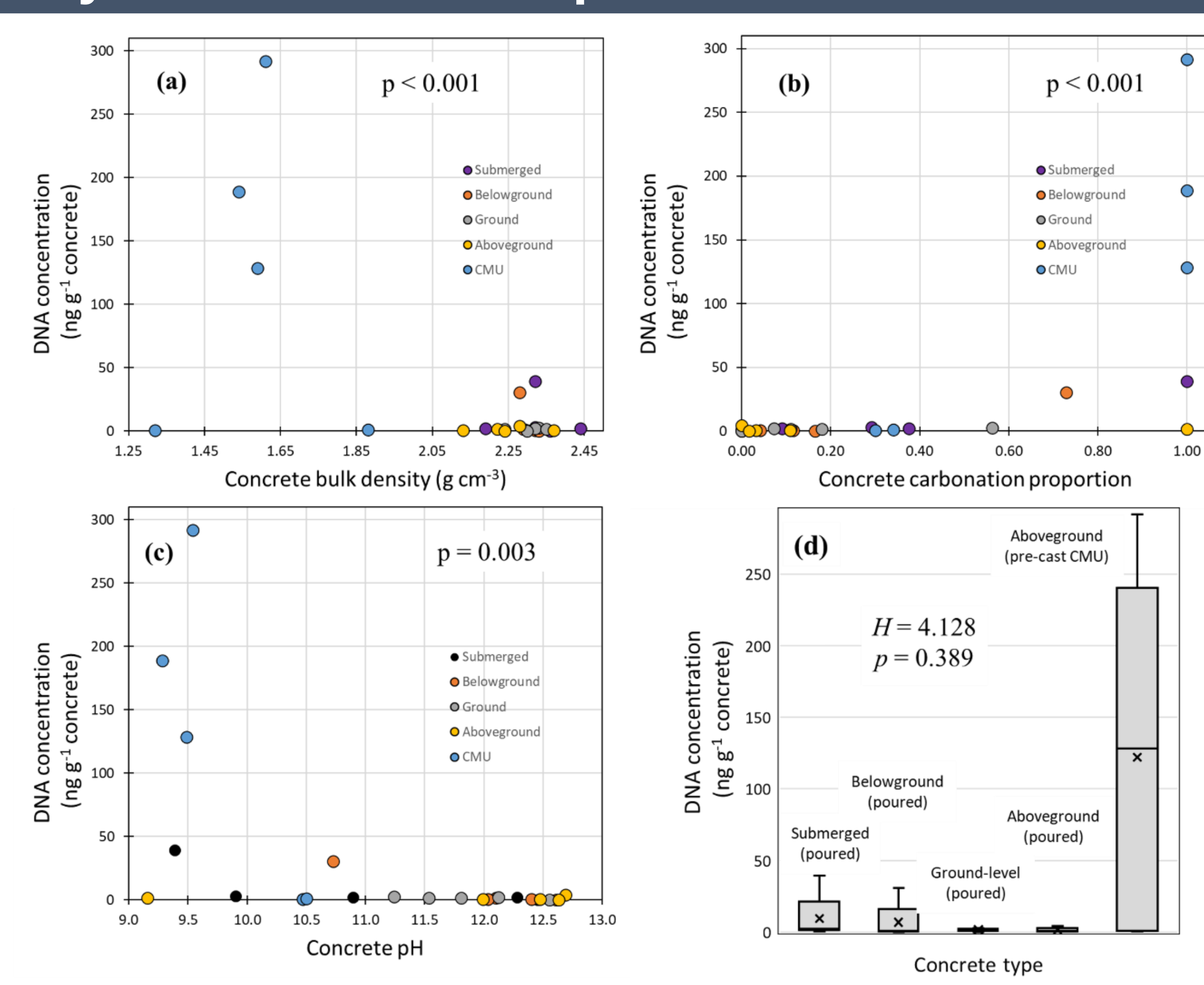
Culture test results by concrete pH

Sample ID	pH	Media Type: MEA	PDA	TSA
Aboveground-4	12.69			
Aboveground-5	12.63			
Belowground-4	12.61			
Ground-4	12.55			
Aboveground-1	12.47			
Submerged-5	12.45			
Belowground-1	12.40			
Submerged-3	12.28			
Ground-3	12.12			
Belowground-2	12.10			
Belowground-5	12.03			
Aboveground-3	11.99			
Ground-5	11.81			
Ground-1	11.54			
Ground-2	11.24			
Submerged-4	10.90			
Belowground-3	10.72			
CMU-5	10.50			
CMU-4	10.47			
Submerged-1	9.90			
CMU-3	9.54			
CMU-1	9.49			
Submerged-2	9.39			
CMU-2	9.28			
Aboveground-2	9.16			



- ESEM micrographs reveal **intact microbial cells** and **cell-like structures** embedded in the concrete substrates.
- **Growth patterns** were observed between culturability and **physicochemical variables**.

Concrete Microbes are Affected by Physicochemical Properties and Environment



- **Significant relationships ($p < 0.05$)** were observed between DNA concentrations and physicochemical properties (**density, carbonation, pH**).
- DNA counts are higher in lower-density, low-pH, and highly-carbonated concrete.
- There were **noteworthy variations** in DNA among different concrete samples.

Deciphering Concrete Endolithic Microbe Types

Concrete Category	Type of DNA targeted for amplification: Archaeal	Fungal	Bacterial	Any DNA amplification
Submerged fragments (poured)	20	20	80	80
Belowground structures (poured)	20	20	40	40
Ground-level slabs (poured)	0	0	40	40
Aboveground structures (poured)	0	0	40	40
Concrete Masonry Units (pre-cast)	60	40	80	80
All concrete samples	20	16	56	56

- **Bacterial DNA** was most-commonly detected; all samples containing archaeal/fungal DNA also contained bacterial DNA.
- Microbe type varied based on **physicochemical parameters**.

Sample ID	Description of closest genetic match: E value	Percent Identity	Accession	Phylum	Class	Order	Family	Genus	Species
Submerged-1	0.0	99.61	KX067847.1	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*(unassigned)*
Belowground-1	0.0	99.63	KX099664.1	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*alternata*
Ground-2	0.0	100	KM249084.1	Mucoromycota	Mucoromycetes	Mucorales	Rhizopodaceae	*Rhizopus*	*arrhizus*

- Fungal species cultured and isolated from three independent concrete samples suggest **isolates are distantly related members of different fungal classes**.

Conclusions and Broader Impacts

- Viable microbes are **ubiquitous in real-world concrete** samples – a hidden microbial biodiversity pool.
- Endolithic microbial communities inside concrete are **shaped by the environmental setting and physicochemistry** of the concrete – microbes are more abundant in lower-density, lower-pH, and highly carbonated concrete.
- Bacteria are the most common microbes, and viable fungal microbes were **newly discovered** to exist within ordinary concrete.
- The study opens a **new avenue for "concrete microbiology"** toward engineering sustainable concrete to decarbonize concrete industry and mitigate climate change.