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Research article

Endolithic microbes may alter the carbon profile of concrete

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Abstract: There is great interest to understand and reduce the massive carbon footprint of the concrete industry. Recent descriptions of microbes incidentally living inside concrete materials ("concrete endoliths") raised questions about how much carbon is either stored in or released from concrete by these microbes. We generated preliminary global estimates of how much organic carbon is stored within the living biomass of concrete endoliths (biomass-carbon) and much CO_2 is released from respiring concrete endoliths. Between 2020–2022, we collected widely varying samples of Portland cement-based concrete from Lubbock, Texas. After quantifying endolith DNA from 25 concrete samples and estimating the current global mass of concrete, we calculated that the global concrete endolith biomass-carbon as low as 5191.9 metric tons (suggesting that endoliths are a negligible part of concrete's carbon profile) or as high as 1141542.3 tons (suggesting that concrete endoliths are a pool of carbon that could equal or offset some smaller sources of concrete-related carbon emissions). Additionally, we incubated concrete samples in air-tight microcosms and measured changes in the CO_2 concentrations within those microcosms. Two out of the ten analyzed samples emitted small amounts of CO_2 due to the endoliths. Thus, "concrete respiration" is possible, at least from concrete materials with abundant endolithic microbes. However, the remaining samples showed no reliable respiration signals, indicating that concrete structures often do not harbor enough metabolically active endoliths to cause CO_2 emissions. These results are preliminary but show that endoliths may alter the carbon dynamics of solid concrete and, thus, the carbon footprint of the concrete industry.

Keywords: cement; carbon footprint; carbon storage; respiration; biomass; industrial ecology

1. Introduction

1.1. The popularity and ubiquity of concrete

Built environments such as cities and towns have rapidly expanded around the world since the 1950s, and concrete has emerged as the primary building material [1]. Now, concrete is the most abundant human-made material on Earth and a symbol of urbanization and modernity [2]. The total quantity of concrete worldwide cannot be directly ascertained (see Section 4.2); however, in 2020, it was estimated to be around 549 Gt, which is about half the dry weight of the Earth's living biomass [3]. This is astounding but unsurprising given that concrete production ranges from 20–30 Gt year^{-1}, which is much higher than the production rates of brick, asphalt, metals, plastics, and wood [4].

Concrete is popular because of its low cost, desirable mechanical properties, incredible versatility, and wide availability [5]. It is a composite material that can be modified and innovated upon to suit numerous applications [6]. The base materials include aggregate (usually sand and/or gravel which act as fillers and strength enhancers), cement (which eventually binds the aggregates together into solid structures), and water (which activates the cement's binding activity). The source, composition, proportion, and treatment of these ingredients widely varies, and these variations affect the workability, durability, and strength [7]. Concrete structures can be modified further by adding additional ingredients, performing mechanical treatments, and incorporating reinforcement elements. Concrete is used in various construction projects, poured on-site and pre-cast, dispensed by hand and with machinery, and placed in a wide variety of environments, including belowground and underwater. Unrivaled popularity, high production rates, and slow decomposition rates have made concrete a substantial part of the biosphere. Yet, while the utility of concrete is undeniable, it has several drawbacks.

1.2. The carbon footprint of concrete is its greatest drawback

Concrete has some undesirable mechanical properties, unique maintenance issues, problematic landscape functions, and negative aesthetic issues [8–10]; moreover, concrete has been most heavily scrutinized for its carbon profile. Many industrial life-cycle analyses show that the production and use of concrete generates between 5–10% of global greenhouse gas emissions [11,12]. Yet, concrete has some of the lowest embodied CO2 ratings of all construction materials, around 0.06–0.2 (meaning that 0.06–0.2 kg of CO2 is produced for every kg of concrete [13]). Much of the emissions associated with concrete are generated during the manufacture of one of concrete's basic ingredients: cement.

Ordinary Portland cement, the most popular type of cement, is typically manufactured by burning fossil fuels (often coal) to superheat raw minerals (mostly limestone and clay) in large kilns. The heating induces calcination reactions, which transform the minerals into the precursor material of cement and release CO2 as a byproduct. This pushes the embodied CO2 value for Portland cement alone to around 1 [14]. This is alarming, especially in the context of climate change; however, the embodied CO2 values of cement are still low compared to materials such as steel (0.4–6) and plastics (2–10) [13]. Furthermore, blending the cement with substances that have less embodied CO2 (e.g., coal fly ash, iron slag) further reduces the embodied CO2 of the final mixture [14]. In the end, the embodied CO2 of concrete or cement does not fully explain the massive carbon emissions attributed to these industries (which have been recently emitting around 3 billion metric tons of CO2 per

year [15]). Those emissions problems are better understood as a result of incredibly high production rates [13], that is, the emissions problems are directly tied to concrete's popularity and ubiquity.

The widespread recognition of concrete's massive carbon footprint has initiated a great deal of research. Much of this research has focused on cataloging the various carbon fluxes associated with concrete, from cradle to grave. We now know that carbon is released at many points throughout concrete's lifecycle [16] and that carbon is absorbed by solid concrete via carbonation (the gradual process by which CO2 migrates into concrete structures and reacts with the certain constituents of cement to form carbonate minerals). The long-term structural effects of carbonation are usually undesirable; however, in terms of carbon dynamics, carbonation represents a significant sequestration of atmospheric carbon into a highly stable mineral form [17]. The extent to which any concrete structure becomes carbonated depends on many factors, including the cement type, the structure shape, environmental conditions, and time [18]; moreover, this process may reabsorb between 7–57% of the CO2 emitted while making the necessary cement [19,20]. Carbonation is also conceptually important because it demonstrates that solidified concrete is not completely static or inert but instead is a dynamic system with cryptic properties.

1.3. Microbes may alter the carbon dynamics of rocks such as concrete

The fact that concrete structures are dynamic systems capable of chemistry-driven carbon flux (carbonation) raises questions about the possibilities of biology-driven carbon flux in concrete. This possibility is strengthened by relatively recent discoveries of microbes that live in and on concrete (e.g., [21]). While concrete seems an unlikely habitat, it is essentially synthetic rock, and it has long been known that microbes utilize natural rocks as habitats. The microbes that specifically live *inside* rocks are called endoliths [22]. The endolithic niche is interesting because it means that entire volumes of rocks (including concrete) are potential habitats for certain microbes, which, in turn, means that the inner volumes of rocks may be harboring carbon cycling ecosystems.

Endolithic microbial ecosystems are often sparse because life inside solid rock is often constrained by several factors, most notably the lack of light, nutrients, moisture, and space. Subsequently, endolithic systems generally contain little biomass and support little biological activity [23]. That said, levels of endolithy vary considerably among the wide variety of rocks and lithic formations found on Earth. Some endolithic communities beneath the ocean floor have cell densities as low or lower than 104 cell g^{-1} material [24,25], while some coral substrates hold over 100 mg of endolithic biomass per cubic centimeter [26] and some surface rock formations can hold up to 14 g of dry endolithic biomass per square meter [27]. Direct measures of carbon cycling and indirect measures of growth show that these organisms can incorporate and release carbon on either extremely slow, almost geological timescales (every 103–104 years) or on much more rapid decadal timescales [28,29]. Yet, as with concrete, appreciating the global carbon profile of endolithic microbes is primarily an issue of scale. Even if most rock material harbors only trace levels of endoliths, endolithic biomass and activity becomes significant when multiplied by the total volume of the potential endolithic habitat, which encompasses the top several kilometers of the Earth's crust (i.e., the upper geosphere, which is much more voluminous than the hydrosphere and pedosphere [30]).

The Earth's rock materials are increasingly being transferred into the novel rock type we call concrete; therefore, it is becoming important to study if and how endoliths utilize concrete. To date, few studies have documented the naturally occurring endoliths that inhabit ordinary concrete. It is currently known that microbial communities within concrete (in "endo-concrete" environments) can

include bacteria, archaea, and fungi [31], with endo-concrete bacteria currently being the most well described and perhaps encompassing the most taxonomic diversity [21]. At least two endolithic subtypes have been noted in concrete - cryptoendoliths (which were likely present in the original concrete ingredients, and then were entrapped in the solidified concrete [32]) and euendoliths (which colonize concrete from the outside by actively boring tunnels into the substrate [33]).

While perhaps less active and dynamic than other microbiomes, endo-concrete communities are not static and are subject to compositional shifts over time [32,34]. Furthermore, concrete endolith communities may be as variable as the concrete structures they inhabit, ranging from barely detectable in some concrete structures to surprisingly rich in other samples [31]. Across samples, community-level variables such as endolith viability and abundance often correlate with the basic physicochemical features of the concrete samples, with lower concrete alkalinities and densities seeming to favor the establishment of endoliths [31]. These *incidental* concrete endoliths are distinct from *intentional* concrete endoliths (which are seeded into experimental types of self-repairing concrete; see [35]). Given that concrete's role in the global environment is inextricably tied to carbon, it is fitting to study how widespread incidental endoliths might alter the carbon profile and carbon dynamics of concrete. As with the endoliths that inhabit natural rock formations, concrete endoliths represent quantities of organic matter and organic carbon. As viable organisms, concrete endoliths also represent the potential for microbially-driven carbon flux in or out of the concrete. Additionally, once again, determining if this facet of concrete is significant depends on scaling the findings up to match the global quantities of concrete.

1.4. Study objectives

Our first objective was to estimate the global biomass of concrete endoliths. Previous studies have indicated that, on a per gram basis, concrete usually contains very low levels of microbial biomass and, therefore, low levels of organic carbon. Yet, because there are hundreds of gigatons of concrete on the planet, it is conceivable that concrete endolithic ecosystems add up to a sizable pool of biomass and carbon. Also, being endolithic microbes that can potentially utilize the entire volumes of their host rock (as opposed to only the surfaces), concrete endolith biomass can be logically estimated from two currently obtainable pieces of data: the average concrete endolith biomass concentration (ng g^{-1} concrete) and the global estimate of concrete mass (Gt). However, there is considerable imprecision surrounding our understanding of concrete endolith biomass concentrations and global concrete quantities; therefore, we set out to compile a set of estimates that use different parameters and then evaluate the practical significance of these estimated quantities.

Our second objective was to determine if the metabolic activity of endoliths causes concrete structures to emit carbon as CO_2 gas. All cells, whether active or dormant, represent pools of organic carbon; however, only active cells produce CO_2 as they metabolize and respire. Unlike biomass, gaseous CO_2 can move and diffuse out of the system in which it was produced, and this can cause a system (including endo-concrete ecosystems) to release carbon. In a concrete structure, this biogenic release of carbon would occur relatively late in the concrete's lifecycle and would be in addition to carbon embodied in concrete during its production (Figure 1). Overall, concrete endolith respiration has the potential to enlarge the concrete's carbon footprint; therefore, we examined whether incidental concrete endolith communities release measurable amounts of carbon from their host concrete.

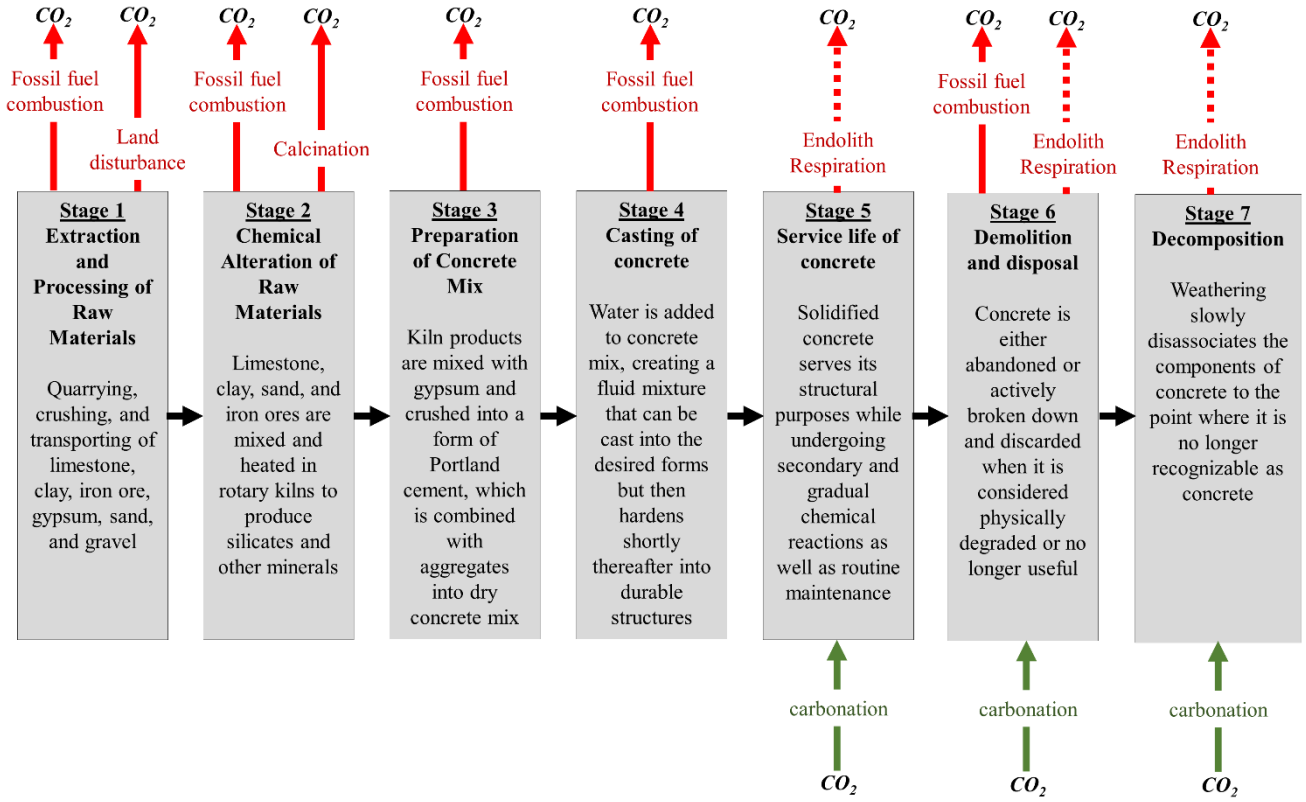

Figure 1. Typical life-cycle stages of Portland cement-based concrete with associated inputs and outputs of CO_2-C. Stages 1–4 and 6 involve known processes of carbon release (solid red arrows). At stages 5–7, after concrete solidification, some carbon capture occurs (solid green arrows). Hypothesized processes of carbon release resulting from concrete microbes are shown at stages 5–7 (dashed red arrows). This figure incorporates elements from other concrete studies: [16,36].

2. Materials and methods

2.1. Concrete sample collection

We collected two sets of concrete samples: one set for the biomass measurements (Objective 1) and one for the respiration experiment (Objective 2). We collected the concrete samples designated for biomass measurements during a previous study on concrete endoliths, as described in detail in Brown et al. [31]. Briefly, we procured 25 independent samples from Lubbock, Texas, USA (33.5° N, 101.8° W, ~978 m elevation) between June-August 2022. This city is semi-arid with a mean annual precipitation of 485 mm and a mean annual temperature of 15 °C [37]. We gathered concrete of various types and from various settings: poured concrete left submerged underwater, belowground poured structures surrounded by soil, ground-level poured slabs with exposed top surfaces and bottom surfaces contacting the soil, aboveground poured structures with no direct contact to the ground, and pre-cast concrete masonry units (CMUs), otherwise called cinder blocks.

We collected the concrete samples for the respiration experiment during August-October 2020. However, these samples (n = 10) also came from the city of Lubbock and were a mix of submerged, belowground, ground-level, aboveground, and CMU samples (Table 1). Samples for both experiments

were independent samples of “ordinary” concrete made with either Portland cement or a Portland cement blend. With permission, we collected samples that were abandoned, discarded, or leftover from demolition sites. Our bulk samples were between 10–25 kg with effective thicknesses of >60 mm, which enabled extraction of sub-samples of a sufficient size and quantity.

Table 1. Descriptions of ten concrete samples used for respiration analysis (Objective 2). Samples are sorted by the general type and source of concrete.

Sample ID	General type	Concrete sample description
R-Submrg-1	Submerged fragment (poured)	Fragment left underwater in a freshwater pond
R-Submrg-2	Submerged fragment (poured)	Remnant of an old footbridge in a perennial freshwater stream (with re-bar)
R-Blwgrnd-1	Belowground structure (poured)	Building footing excavated during a large-scale renovation (with re-bar)
R-Blwgrnd-2	Belowground structure (poured)	Post setting recently unearthed from an urban backyard
R-Ground-1	Ground-level slab (poured)	Sidewalk from within an apartment complex
R-Ground-2	Ground-level slab (poured)	Housing pad beneath a recently demolished house (with re-bar)
R-Abvgrnd-1	Aboveground structure (poured)	Indoor flooring from a multi-story commercial building (with re-bar)
R-Abvgrnd-2	Aboveground structure (poured)	Exposed flooring from the top story of a parking garage (with re-bar)
R-CMU-1	Aboveground CMU (pre-cast)	8-inch, 2-core cinder block left outside atop a pile of cinder blocks
R-CMU-2	Aboveground CMU (pre-cast)	4-inch, 3-core, hollow-style concrete block from an abandoned commercial building

Note: These samples are not the same samples used for microbial biomassing (Objective 1). Those are described in Brown et al. [31].

2.2. Extracting, cutting, and pulverizing of concrete sub-samples

We extracted sub-samples from each large bulk sample of concrete. For the concrete used for biomass measurements, the methods of sub-sample extraction and the procedures for cutting and pulverizing sub-samples are described in Brown et al. [31]; we prepared the sub-samples for the respiration analyses in the same manner. Briefly, we used a drill press to extract cylindrical sub-samples from the poured concrete bulk samples and a tile saw to extract cuboidal sub-samples from the CMU bulk samples. With a small tile saw, we removed the surface material from each concrete sub-sample to isolate the internal, endolithic material (which we defined as any internal material at least 5 mm from any surface of the original structure). The dimensions of the sub-samples were standardized as

much as possible in terms of the shape and volume (55 cm^3 ±1 cm^3). We surface sterilized and aseptically pulverized all sub-samples using a custom-made, mortar-and-pestle-type instrument [31]. These preparation methods and instruments reflected the fact that our field samples were of various shapes and sizes, our laboratory analyses required certain sample manipulations and volumes, and contamination risks were high. To the extent possible, our final sets of concrete samples represented a wide variety of urban concrete structures, though they only included the material and microbes that had existed within these structures.

2.3. Extraction and quantification of microbial DNA

We estimated the endolithic biomass in 25 concrete samples by first measuring the concentrations of double-stranded DNA (dsDNA). We used each pulverized sub-sample from each bulk sample as an independent replicate. The DNA extractions are described in Brown et al. [31], but we mostly followed the protocol by Kiledal and Maresca [38]. Briefly, we treated 10 g of each pulverized sub-sample with a lysis solution containing 0.5 M EDTA, 20 mg/mL Proteinase K solution, 20% SDS, and acetic acid. We followed this with an extended 24-hr incubation at 55 °C and then high-speed agitation and centrifugation to isolate the supernatant. For solubilization and binding, we combined the supernatant with Qiagen Buffer QG amended with a 25 mM NaCl solution and Triton X-100, a 1-mg/mL yeast RNA solution, and silica. We washed the silica-bound DNA pellets in 80% ethanol and air-dried. We resuspended and eluted the pellets three times using 10 mM Tris. We standardized the DNA quantifications by using consistent concrete sample inputs (10.0 g) and consistent extract volumes (50 µL). From the fluorometric DNA concentration readings (ng of dsDNA μL^{-1} extract), we calculated the total DNA recovery per sample (ng) and the DNA concentration of the concrete sample (ng DNA g^{-1} concrete).

2.4. Estimations of microbial biomass

We estimated the global mass of concrete endoliths using several different parameters and combinations of parameters. For each estimate, we either used the mean DNA concentration (0.0283 µg g^{-1} concrete) or the median (0.0016) calculated from this study's concrete samples (n = 25). We separately converted the two DNA concentration averages to either the microbial biomass-carbon (C_{mic}; µg g^{-1} concrete) using one of five microbial DNA-to-biomass conversion factors (Table 2). These conversion factors were developed by correlating DNA concentrations of soil samples with more direct measures of C_{mic}. We used DNA-to-biomass conversion factors because other preexisting methods for directly measuring C_{mic} were not suitable for concrete samples; moreover, we used conversion factors designed for soil microbes because no conversion factors for endolithic microbes were available (see Section 4.2).

Table 2. Selected DNA-to-biomass conversion factors shown in ascending order along with source information. Sample DNA concentrations are multiplied by the conversion factor to obtain an estimate of sample C_{mic} concentrations.

DNA-to-biomass conversion factor	Source	Study system	Relationship coefficient between DNA and biomass measurements
4.41	Semenov et al. [39]	Alkaline and carbonate soils	R^2 = 0.97
5.0	Anderson and Martens [40]	Arable and forest soils	R^2 = 0.95
12.0	Fornasier et al. (*low*) [41]*	Acidic arable soil	*R* = 0.96**
38.11	Gong et al. [42]	Arid and semi-arid soils	*R* = 0.99
63.5	Fornasier et al. (*high*) [41]*	Sandy arable soil	*R* = 0.96**

* Fornasier et al. [41] reported a range of conversion factors of which we used the lowest (*low*) and highest (*high*) factor.

**Shown is the overall correlation coefficient reported by Fornasier et al. [41] for multiple study systems. Correlation coefficients for individual study systems were not reported.

After multiplying an average DNA concentration (μg g^{-1} concrete) by a conversion factor to produce an estimate of C_{mic} concentration (μg g^{-1} concrete), we multiplied each C_{mic} concentration by the global mass of concrete as of 2023, which we estimated by assuming that the global annual cement production was 4.1 Gt [43] and that cement is typically one-seventh of the total concrete mass [4,13,44], meaning that 28.7 Gt of concrete has been annually produced for the last several years. Elhacham et al. [3] estimated the global mass of concrete to be 549 Gt in 2020; therefore, adding three years' worth of concrete production (86.1 Gt) amounted to a current global quantity of 635.1 Gt or 6.351 × 1015 g. Then, we converted the global Cmic quantities to metric tons (megagrams) by dividing by a fixed value (Eq 1). Additionally, we calculated the total microbial biomass of concrete endoliths (in metric tons) by dividing the Cmic estimates by 0.46 (the assumed proportion of organic carbon in total microbial biomass [40]).

$$Global\ biomass = \frac{(DNA\ concentration \times Conversion\ factor) \times (6.351\ \times 10^{15})}{1\ \times\ 10^{12}} \qquad (1)$$

2.5. Microbial respiration assays

We determined if CO_2 was released from concrete by respiring endoliths by analyzing gaseous changes within air-tight microcosms loaded with concrete. From each bulk sample of concrete, we extracted four 55-cm^3 sub-samples, which we aseptically pulverized for separate microcosms (Figure 2). Of the four sub-samples from a given bulk sample, we left two concrete sub-samples untreated

("fresh") and sterilized the other two sub-samples by autoclaving three times over five days. There is no established method of sterilizing concrete, but we partially validated this method with culture tests (culture plates inoculated with fresh endo-concrete consistently showed microbial growth within seven days, while plates inoculated with autoclaved concrete did not [31]). The sterilized sub-samples revealed whether concrete with no biological activity could cause gaseous changes within microcosms and prevented us from erroneously attributing abiotic gaseous changes to endolithic microbes.

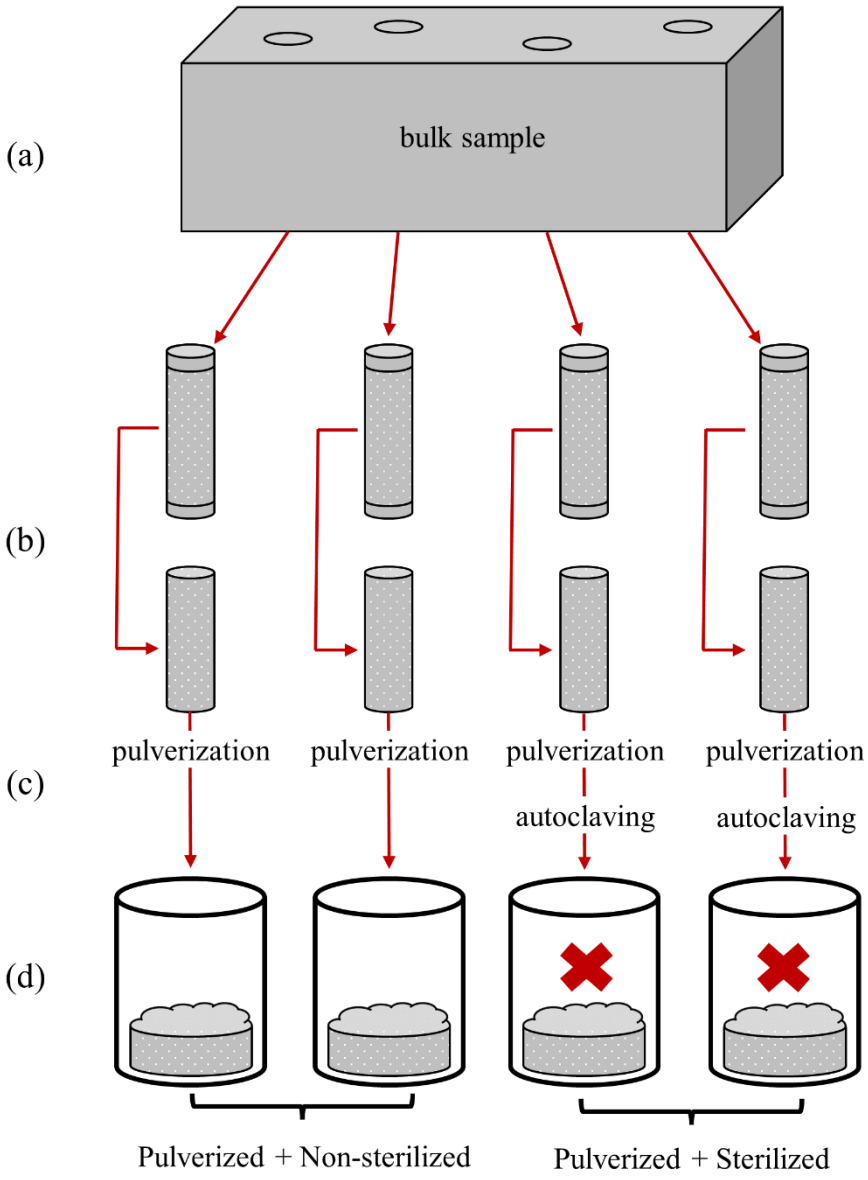

Figure 2. The extraction and treatment of concrete sub-samples used for respiration analysis. (a) From each bulk sample of concrete, we extracted four sub-samples, as cylindrical cores or cuboids. (b) We trimmed the surface portions off each sub-sample to isolate the endolithic portion. (c) We pulverized each sub-sample and autoclaved two of the four before (d) loading into microcosms.

We assembled the microcosms using 170-mL glass jars with modified twist-on metal lids (Figure 3). We fitted each plastisol-lined lid with one air inlet, one air outlet, and one septum. Each inlet was

a nickel-plated brass bulkhead set into a 6.35-mm hole drilled into the outer edge of the lid. We placed metal and silicone washers between both bulkhead tightening nuts and the lid. We attached a 15-mm silicone tube (3.175 mm ID × 6.35 mm OD) to the bottom bulkhead barb to enhance air mixing in the microcosm. To the top bulkhead barb, we attached tubing, a female Luer lock (3.2 mm, polypropylene), a one-way stopcock valve (polycarbonate, female-to-male Luer lock), and a male Luer plug (polypropylene). We installed each outlet on the edge of the lid directly opposite of the inlet. The outlets were identical to the inlets except that these neither had tubing on the bottom bulkhead barb nor a stopcock valve. We drilled 10.6-mm holes into the center of each lid and inserted a 20-mm butyl rubber septum. We autoclaved and dried the partially assembled microcosms prior to sample loading.

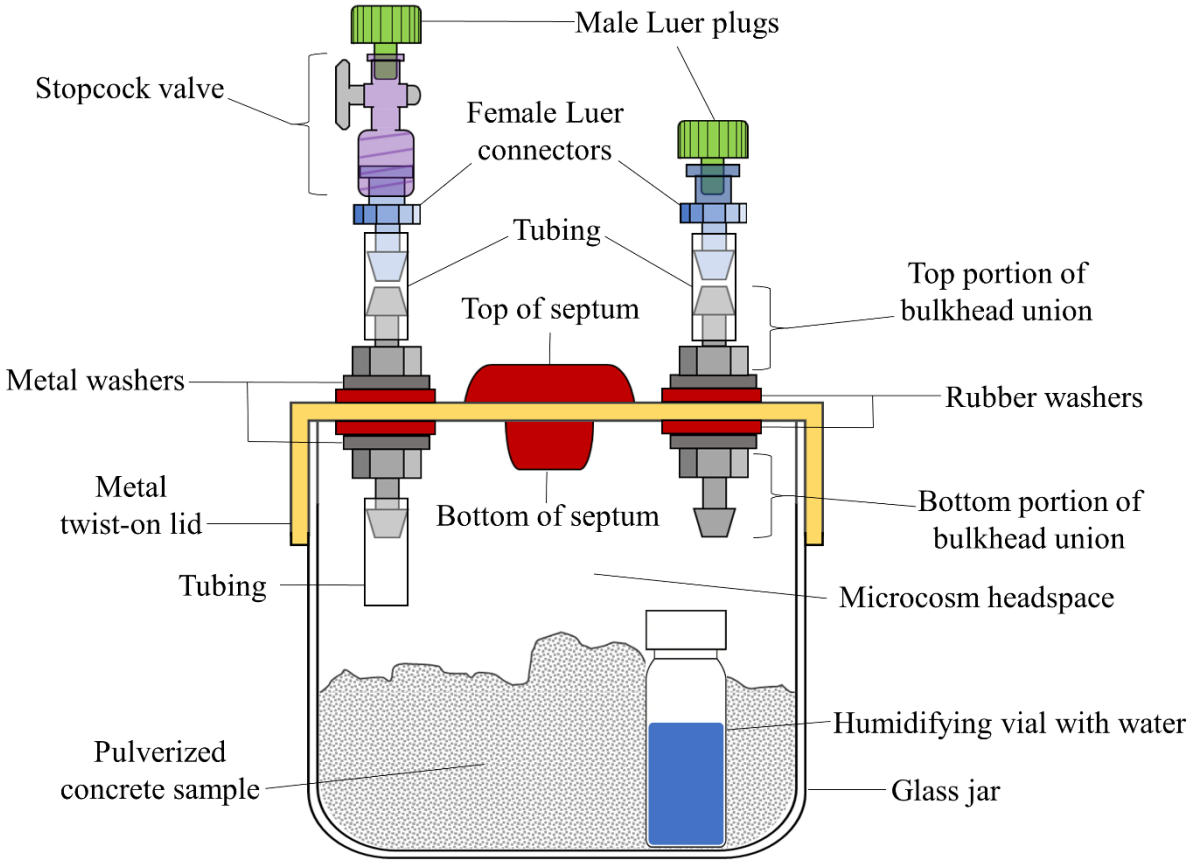

Figure 3. Schematic side-view/cross-section of a partially sealed microcosm.

In a laminar flow hood and using sterile technique, we transferred each concrete sub-sample to a microcosm along with a humidifying vial (8-mL, pre-sterilized, glass scintillation vial containing 4 mL of sterile, degassed water and a perforated screw-on cap; Figure 3). Each vial released water vapor via diffusion through the perforation in the vial cap, thereby humidifying the microcosm headspace without wetting the concrete. We temporarily sealed the microcosms immediately after sample loading and vial placement by screwing on the lid, pressing the plumbing putty around the bases of the inlets, outlets, and septa, covering the putty with plastic wrap, then wrapping laboratory film around the junction between the jar and lid and around the upper portions of the inlet and outlet.

Immediately after this, we filled the microcosms with CO_2-free air (Airgas® Ultra Zero Grade Air) to make any later CO_2 increases more apparent (but see Section 4.4). We connected the pressurized cylinder of CO_2-free air to the inlet of a microcosm via sterilized silicone tubing fitted with

a 0.22-µm membrane filter (to limit airborne contamination of the microcosm). Once the inlet and outlet were open and unplugged, we flushed the microcosm with CO_2-free air for 90 seconds at a rate of one liter per minute (LPM). Then, we plugged the outlet, immediately closed the stopcock valve on the inlet, detached the line connected to the inlet, and plugged the inlet. This prevented the backflow of atmospheric air into the microcosms, though it sometimes left the microcosms slightly pressurized. We depressurized the microcosms by inserting a sterile syringe into the septum of every flushed microcosm and allowing the syringe to backfill with microcosm air until a pressure equilibrium was reached between the syringe and microcosm. Thus, the excess microcosm air was trapped in the syringe and removed from the microcosm. We wrapped the ends of the inlets and outlets with laboratory film to finalize the sealing. We dark incubated the microcosms for 90 days at 23 °C.

After incubation, we analyzed the headspace gas of each microcosm for CO_2 enrichment. First, we gently shook the microcosm to disperse the air trapped among the concrete fragments. Then, we inserted the needle of a gas-tight syringe into a microcosm's septum and further homogenized the headspace air by filling and emptying the syringe twice before finally withdrawing 10 mL of headspace air. Then, we injected this microcosm air sample into the injection chamber of our gas analysis system (Figure 4). We installed the injection chamber (an air-tight chamber built similarly to the sample microcosms but with a smaller, 57-mL jar) between the same CO_2-free air source used to fill the microcosms and an infrared gas analyzer (FMS-1601-14, Sable Systems International). The CO_2-free air continuously flowed through the injection chamber and gas analyzer so that baselines of 0.0012% CO_2 (± 0.0005%) and 20.8% O_2 (± 0.0005%) read consistently on the data logging software (ExpeData, v.1.9.13, Sable Systems International). We maintained these baselines with an incoming air flow rate of 1 LPM, an internal analyzer flow read between 195–199 ml per minute, an internal pump rate at 11.4%, and an ambient temperature at 23 °C. Preliminary testing showed that we did not need additional scrubbing columns and filters to maintain consistent analytical baselines.

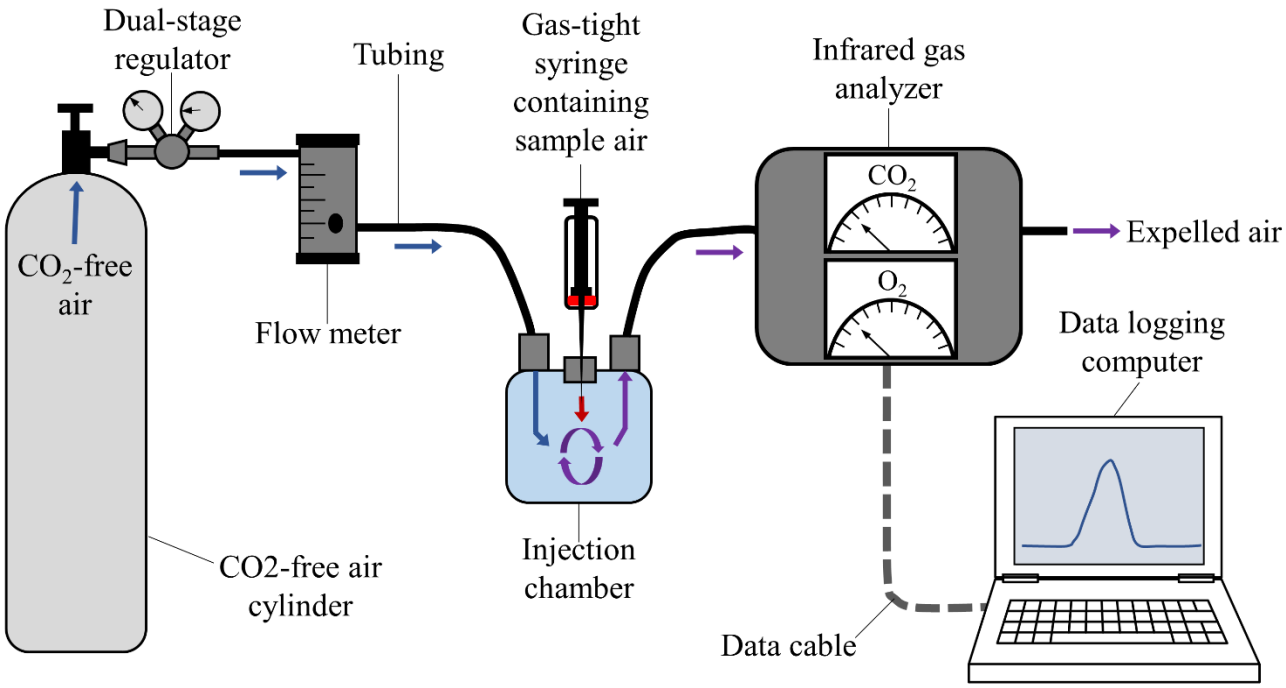

Figure 4. Schematic diagram of the gas analysis system in which CO_2-free air carries air from experimental microcosms to a gas analyzer. Blue arrows indicate the direction of CO_2-free air flow, red arrows represent sample air withdrawn from an incubated

microcosm then injected into the analysis system, and purple arrows indicate the flow of CO_2-free air combined with sample air.

After inserting the syringe needle into the injection chamber, we stabilized all components of the analysis system and initiated data logging for the sample. Then, we waited 15 seconds before quickly but steadily injecting the sample air. We waited 60 seconds after injection before removing the syringe and ending the logging cycle. Any injections containing CO_2-enriched air registered as brief peaks on the otherwise flat CO_2 baseline, while injections of non-enriched air registered without any peaks.

The microcosms that were filled with sterilized concrete and paired with microcosms filled with fresh concrete were the primary control microcosms. Additionally, we included a negative control group consisting of five microcosms loaded with 120 g of sterilized glass beads. The beads imitated the approximate mass and volume of the concrete samples in their microcosms while presumably eliminating the possibility of microbial activity. Any respiration signals from these microcosms would indicate gas leaks or other systemic problems. For positive controls, we incubated five microcosms loaded with of 120 g of soil (a silty-loam agricultural topsoil sample that we sieved and air-dried for approximately 30 days). We presumed this soil to be of low biomass but with enough microbial activity to easily register on our analysis system.

We noted CO_2 enrichment when the air sampled from a microcosm produced a visible peak in the logged CO_2 concentration data. Therefore, the area under the graphed peak was proportional to the level of CO_2 enrichment that occurred in the corresponding microcosm. We calculated the area under the discernible peaks using the area integration function in the Expedata logging software. We integrated by seconds (the frequency at which the gas analyzer logged CO_2 concentrations) and set the baseline at 0.0012 (the approximate mean CO_2 concentration of the CO_2-free carrier gas). Then, we standardized the peak areas for all concrete sub-samples by dividing each peak area value by the mass of concrete in its corresponding microcosm and multiplying that by 4.05 (to represent the 90-day incubation period as one year's worth of CO_2 enrichment). We calculated relative and dimensionless measures of microbial respiration, which we refer to as "respiration values" because we could not confidently convert the peak areas to absolute molar units of CO_2 efflux from the sample (e.g., μmol CO_2 g^{-1} concrete s^{-1}); we were unable to account for all the variables that affect peak development in the logged data (microcosm volume, sample injection volume, internal gas analyzer settings, etc.). However, these respiration values were suitable to determine *if* concrete endolith activity occurred and the *relative* levels of respiration among the samples (see Section 4.4 for further discussion).

For the concrete microcosms, we calculated the mean respiration value of duplicate microcosms (which had sub-samples from same bulk sample and were treated the same way in terms of sterilization). Then, we calculated the *net* respiration value of a concrete sample by subtracting the mean respiration value of its sterilized concrete microcosms from the mean respiration value of its associated fresh concrete microcosms (so that the net respiration value reflects only CO_2 enrichment caused by live microbes in the sample).

3. Results

3.1. Possible quantities of endolithic biomass in concrete

We produced ten preliminary estimates of how much organic carbon is contained in the microbes living inside the global stock of concrete (Table 3). The estimates ranged from 4,579–1.14 million

metric tons of C_{mic}. The final estimates were greatly influenced by differences between the mean and median DNA concentration and among the various conversion factors. Estimates based on our mean concentration of endolithic DNA were much higher than estimates based on the median concentration, even though these averages only differed by 0.027 µg g-1 concrete. The highest conversion factor (from Fornasier et al. [41]) produced estimates that were 14 times higher than estimates produced using the lowest conversion factor (Semenov et al. [39]).

Table 3. Estimates of endolithic C_{mic} contained in the global stock of concrete. We based these measurements on average microbial DNA concentrations from 25 concrete samples. We converted DNA concentrations to C_{mic} using one of five conversion factors and assumed the current global stock of concrete is 635.1 Gt.

DNA concentration average statistic	Conversion Factor Source	Conversion Factor	C_{mic} (µg g^{-1} concrete)	Global quantity of C_{mic} in concrete (metric tons)
Mean	Semenov et al. [39]	4.41	0.124829	79278.8
Mean	Anderson and Martens [40]	5.00	0.141529	89885.2
Mean	Fornasier et al. (*low*) [41]	12.00	0.339670	215724.5
Mean	Gong et al. [42]	38.11	1.078736	685105.1
Mean	Fornasier et al. (*high*) [42]	63.50	1.797421	1141542.3
Median	Semenov et al. [39]	4.41	0.007210	4579.3
Median	Anderson and Martens [40]	5.00	0.008175	5191.9
Median	Fornasier et al. (*low*) [41]	12.00	0.019620	12460.7
Median	Gong et al. [42]	38.11	0.062310	39573.0
Median	Fornasier et al. (*high*) [41]	63.50	0.103823	65937.7

For this study, we assumed that every microgram of C_{mic} represented 2.174 micrograms of total microbial biomass (which includes carbon and other elements). As such, our total biomass estimates were larger than our C_{mic} estimates but followed the same patterns of variation (Table 4). The global estimates ranged from 9955–2.48 million tons. Additionally, these estimations indicate that the "average" gram of concrete contains between 0.01–4 µg of endolithic biomass, depending on the assumptions of the calculations.

Table 4. Estimates of total endolithic biomass contained in the global stock of concrete. We based these measurements on average microbial DNA concentrations from 25 concrete samples. We converted DNA concentrations to C_{mic} using one of five conversion factors and assumed the global stock of concrete is 635.1 Gt. We converted C_{mic} to total microbial biomass by multiplying by 0.46.

DNA concentration average statistic	Conversion Factor Source	Conversion Factor	Total endolithic biomass (μg g^{-1} concrete)	Global quantity of endolithic biomass in concrete (metric tons)
Mean	Semenov et al. [39]	4.41	0.271367	172345.1
Mean	Anderson and Martens [40]	5.00	0.307672	195402.7
Mean	Fornasier et al. (*low*) [41]	12.00	0.738413	468966.4
Mean	Gong et al. [42]	38.11	2.345078	1489359.0
Mean	Fornasier et al. (*high*) [42]	63.50	3.907438	2481613.7
Median	Semenov et al. [39]	4.41	0.015675	9955.0
Median	Anderson and Martens [40]	5.00	0.017772	11286.8
Median	Fornasier et al. (*low*) [41]	12.00	0.042652	27088.4
Median	Gong et al. [42]	38.11	0.135456	86028.2
Median	Fornasier et al. (*high*) [41]	63.50	0.225701	143342.8

3.2. Respiration readings from endo-concrete samples

After 90 days, seven out of the ten concrete samples did not emit measurable amounts of CO_2 via microbial endolith respiration; however, two samples showed convincing (albeit low) respiration signals (Table 5). The air in duplicate microcosms containing one of the cinder block samples (R-CMU-1) became slightly enriched with CO_2 over time (i.e., produced small peaks in the gas analysis outputs); meanwhile, their associated control microcosms, which contained sterilized portions of the same concrete, did not. These fresh sub-samples of R-CMU-1 had a mean respiration value of 0.0079 g^{-1} concrete $year^{-1}$, which is about 10% of the mean respiration value calculated for the low-biomass soil samples (respiration value = 0.076 g^{-1} concrete $year^{-1}$). The microcosms holding concrete from a belowground sample of (R-Blwgrnd-2) also became enriched with CO_2, though only to about half the level of the microcosms holding the cinder block sample (having a mean respiration value about 5% of the soil's mean value). Additionally, the associated control microcosms behaved as expected, showing no signs of CO_2 enrichment.

One microcosm that contained a fresh sub-sample of underwater concrete (R-Submrg-1) showed a faint respiration signal, similar to that of the belowground sample, but its own duplicate sample did not. Furthermore, one of the associated control samples also showed a very faint respiration signal, which we noted as a sign of either abiotic CO_2 enrichment or an analysis error. In any case, the net respiration value for this sample was above zero but very low (0.0005 g^{-1} concrete $year^{-1}$). The negative and positive control microcosm behaved as expected. The five negative control microcosms containing sterilized glass showed no CO_2 enrichments, while all positive control microcosms containing low-biomass soil became enriched with CO_2 (respiration values ranged from of 0.063 to 0.084 g^{-1} soil $year^{-1}$).

Table 5. Relative measures of CO_2 enrichment by concrete samples. The ten bulk samples of concrete are sorted by the general type of concrete and the relative CO_2 enrichment values are shown for 40 microcosms (20 fresh sub-samples and 20 sterilized sub-samples). We duplicated each sub-sample treatment into sub-replicates which we averaged into mean enrichment values. The net mean enrichment for each bulk sample equals the sample's respiration value (the level the endolithic respiration per g concrete per year).

Bulk sample ID	Sub-replicate number	Enrichment by fresh sub-replicate	Mean enrichment by fresh sub-replicates	Enrichment by sterilized sub-replicate	Mean enrichment by sterilized sub-replicates	Mean net enrichment (respiration value)
R-Submrg-1	1	0.0031	0.0015	0.0021	0.0009	0.0007*
	2	0		0		
R-Submrg-2	1	0	0	0	0	0
	2	0		0		
R-Blwgrnd-1	1	0	0	0	0	0
	2	0		0		
R-Blwgrnd-2	1	0.0034	0.0035	0	0	0.0035
	2	0.00.35		0		
R-Ground-1	1	0	0	0	0	0
	2	0		0		
R-Ground-2	1	0	0	0	0	0
	2	0		0		
R-Abvgrnd-1	1	0	0	0	0	0
	2	0		0		
R-Abvgrnd-2	1	0	0	0	0	0
	2	0		0		
R-CMU-1	1	0.0086	0.0079	0	0	0.0079
	2	0.0072		0		
R-CMU-2	1	0	0	0	0	0
	2	0		0		

*The lack of enrichment for all the corresponding fresh sub-replicates and the presence of an enrichment signal in one of the sterilized sub-replicates suggests that this is an erroneous measurement

4. Discussion

4.1. Concrete microbes as an overlooked pool of organic carbon

Without knowledge of incidental concrete endoliths, it would be difficult to imagine the interior volumes of concrete harboring life of any kind; even with knowledge of concrete endoliths, it would be difficult to imagine these microbes as a significant portion of the concrete's mass. Previous studies (and the difficulties thereof) indicated that concrete typically contains very little biomass, similar to many other endolithic and subsurface ecosystems. For example, Maresca et al. [21] found that conventional DNA extractions did not yield measurable amounts of DNA from concrete, and the

precursor study to this one found that many sensitive tests were often unable to detect life within concrete [31]. Still, *any* microbial biomass inside concrete represents a hidden, yet globally distributed pool of organic carbon. We formally estimated the size of this pool so that we could begin deciding if this pool was consequential to concrete's overall carbon profile.

We relied on microbial DNA to estimate the C_{mic} concentrations. Several assumptions and tradeoffs were embedded in these estimates (discussed in Section 4.2), but we accounted for some uncertainty by reporting several estimates, each utilizing a unique set of parameters. Among individual concrete samples, we calculated widely varying estimates of C_{mic}. Both the low and high estimates are interesting, but we thought it appropriate to derive global estimates from the "average" levels of sample biomass [45]. We extrapolated upon the mean Cmic concentration (28.3 μg g^{-1} concrete) and the median (1.6). Both statistics indicated that most of our samples had low DNA concentrations, though these summarized our samples in slightly different ways, and these slight differences resulted in noticeably different global estimations.

Our most conservative estimate of the C_{mic} contained in the world's supply of concrete came to about 4579 metric tons (Table 2). This means that endolithic C_{mic} represents 0.000000721% of the total mass of concrete. If manufacturing one kg of concrete emits between 60–200 g of CO_2 [13], then it follows that manufacturing one kg of concrete releases between 0.016–0.054 kg of carbon (carbon is 27.27% of the molar mass of CO_2). Scaling up, this means that producing the world's current stock of concrete (635.1 Gt) released between 10.4 and 34.6 Gt of carbon. If our lowest C_{mic} estimate was considered as a quantity of carbon that was sequestered in the cells of concrete endoliths, then the presence of concrete endoliths could only offset between 0.000044% and 0.000013% of the carbon emitted thus far by the concrete industry. Therefore, our lowest global estimate of global C_{mic} depicts concrete endoliths as a miniscule carbon pool or sink that is inconsequential for most practical purposes. This estimate aligns with the general idea that concrete is not greatly affected by biology, and it was produced with what *may* be the most appropriate DNA-to-biomass conversion factor (4.41). This factor was developed by Semenov et al. [39] specifically for alkaline and carbonaceous soils, which may be the substrate that most closely resembles concrete (of all substrates that have been studied for microbial biomass).

Our highest estimate of C_{mic} residing in the global stock of concrete was about 1.1 million tons (Table 3). This implies that about 0.000179% of our concrete's mass is attributable to the organic carbon content of the endolithic microbes. With the same assumptions as before, this means that the biomass of concrete endoliths offsets between 0.011% and 0.0033% of cumulative carbon emissions from concrete. This brings concrete endoliths much closer to being an important carbon pool and carbon sink (assuming the endoliths can fix and stabilize carbon). This microbial carbon sink would not rival the sink capacity of abiotic cement carbonation, which might have allowed solidified concrete to cumulatively re-absorb as much as 6.5 billion tons of CO_2-carbon [17] (see Section 1.2). Still, the endolithic Cmic pool might be large enough to offset some of the smaller sources of concrete-related emissions, such as the emissions associated with concrete disposal [46,47]. Moreover, while our estimate of 1.1 million tons might be inappropriate because it was based on a DNA-to-biomass conversion factor developed for microbe-rich arable soils [41], this estimate may reflect the ultimate potential and eventual condition of concrete. In other words, concrete may become more microbe-rich over time as it weathers and as its lithology changes to become more hospitable to microbes (sensu [48,49]). As this happens, the biomass levels and substrate conditions inside concrete may become more similar to those of soils. This would have global consequences because the world

continues to accumulate old and discarded concrete [50].

Additionally, we estimated the global quantities of C_{mic} inside concrete using intermediate DNA-to-biomass conversion coefficients (Table 3). These coefficients and the global estimates they produced (which ranged from 5191–685105 metric tons) demonstrate that the true value of global C_{mic} inside concrete could fall anywhere in between the highest and lowest estimates we discussed above. Furthermore, the various conditions under which the five coefficients were developed could provide clues as to how microbial biomass levels can be influenced by substrate pH [39,41], texture [41], mineral content [39], and moisture [40,42].

We focused on microbial carbon because, in the era of anthropogenic climate change, it is concrete's carbon profile that demands the most attention. However, we also estimated the total biomass of the concrete endoliths (which includes other elements besides carbon). In the context of concrete, the total microbial biomass is also an interesting metric because it represents the biological material fraction of concrete (a substrate that is often viewed as a purely mineral and abiotic). Because we assumed that carbon is roughly half the weight of microbial biomass [40], our total biomass estimates were roughly twice that of our Cmic estimates, ranging from 9995 tons to 2.48 million tons (Table 4). The lower estimate suggests that the endolithic biomass is unlikely to affect the global accounting of concrete material inflows and outflows, even when all biological matter is considered. The higher estimate suggests that concrete endoliths are on track to become a significant ecological guild that only exists because of human activity. Studies have documented how some isolated ecosystems acquired new functional guilds upon the arrival of exotic species [51,52]; however, the concrete endolith guild would have a global distribution, possibly occurring almost anywhere concrete is installed.

4.2. The uncertainty surrounding carbon and biomass estimates

Every term in our C_{mic} estimation equation (Eq 1) is associated with a unique set of uncertainties; however, to promote more refined and more robust studies in the future, we discuss some of the major sources of uncertainty and possible improvements. First, our estimates were based on the average DNA concentrations of our concrete samples. Although we sampled a wide variety of concrete structures, more reliable averages could be calculated from a sample set that is larger and encompasses more of the incredible variety seen in concrete structures around the world [53] but still includes old and discarded concrete, as we did.

We removed the top 5 mm from our concrete sub-samples. These surface ("epilithic") portions of concrete are interesting because many organisms can live and grow *on* concrete [54,55], but these were outside our study's scope. Furthermore, we could not estimate global concrete surface area as we did global concrete mass. Still, samples somehow trimmed closer to the concrete's surface (e.g., at 1–2 mm below the surface) or otherwise include more of the available endolithic material would provide a more complete picture of the endo-concrete environment. Additionally, we had no basis for weighting our DNA concentration averages according to the relative abundances of the various types of concrete (i.e., we found no information about how much concrete is cast in place versus pre-cast, nor how much concrete is positioned underwater, underground, at ground-level, or aboveground). However, if this information becomes available or if another way of meaningfully classifying concrete structures is developed, then more sophisticated weighted averages could be used.

We extracted DNA from concrete using a protocol that prioritizes the isolation of amplifiable, PCR-quality DNA instead of DNA quantification [38]. Future studies focused on biomassing should

consider omitting late-stage purification steps because these may unnecessarily reduce extraction yields and lead to critical underestimates of biomass [41]. Future studies should also standardize the volume of DNA extracts, as done here, to make the DNA concentrations readings comparable among various samples (e.g., [56]). We vacuum centrifuged all our final extracts down to 50 µL, though other methods, such as more tightly controlled elutions, may be better for standardizing extract volumes.

We converted the average DNA concentrations to C_{mic} concentrations using established conversion factors after attempts to measure biomass more directly with chloroform fumigation-extractions (CFE) [57] were unsuccessful. We found no discernible differences in extractable carbon between fumigated and non-fumigated concrete samples, as well improbably high concentrations of carbon (data not shown). We anticipated this because concrete has several features that sometimes inhibit chloroform-based assays, including unconventionally low biomass levels [58], high proportions of dormant microbes [59], high substrate particle densities [60], and high substrate alkalinity and carbonate content [39]. Therefore, while we encourage future studies to explore other more direct biomassing methods for concrete samples, we utilized soil-based DNA-to-biomass conversion factors knowing that DNA data were obtainable (and useful for other experiments). The soil-based conversion coefficients varied widely, and we were unsure which coefficient was most appropriate for concrete, so we mitigated these problems by reporting several biomass estimates derived using several conversion factors.

Lastly, we assumed that 635.1 Gt of concrete is scattered across Earth. We figured this straightforwardly using a recent global estimate of concrete mass, a recent rate of global cement production, and an average cement-to-concrete ratio, but there is uncertainty associated with each of these parameters. The 2020 estimate of global concrete mass was compiled by Elhacham et al. [3] using information from prior studies concerning global socioeconomic material stocks [61], industrial metabolism [62,63], and mineral resource usage [7]. Yet, there are no complete national statistics of concrete production, primarily because of the sheer scale and decentralized manner of production and because concrete is a composite material assembled in various ways [7,13]. Thus, concrete amounts are estimated based on cement, a commodity whose production is tracked more closely (e.g., by the US Geological Survey and the Global Cement and Concrete Association). Yet, tracking cement production is not equivalent to tracking cement use. We can only assume that *most* cement ends up in concrete, though cement is also used to make mortar, stucco, and grouts. Additionally, we assumed that cement comprises between 10–15% of concrete's final mass based on the world's most common concrete mixture formulas [4,13,44], though there are numerous concrete mix types [7]. Another matter is concrete recycling, which involves breaking down concrete for use in new concrete mixtures [64]. Concrete recycling is feasible [65] and very common in some regions [66], but it is unclear how this slows global concrete accumulation.

4.3. Endolithic respiration can sometimes cause concrete to release carbon

It would be one matter if microbes simply comprised a static, carbon-based component of concrete; however, it would be another matter if these microbes actively cycled carbon in or out of solid concrete. Therefore, we examined the release/efflux/outputting of CO2 from solidified concrete by respiring concrete endoliths. Respiration is key to understanding the functioning and activity of individual microbes, microbial communities, and the ecosystems that microbes inhabit [67,68]. Prior to this study, very few studies have mentioned the in-situ activity of concrete endoliths; Coombes et al. [33] described euendoliths boring into concrete substrates and Kiledal et al. [32] suggested that

certain populations of concrete endoliths shrink and swell over time. However, we were specifically interested in the community-level metabolic activity because it relates to endolithic carbon cycling [69,70]. When we tested the hypothesis that small amounts of CO2 are released from concrete by respiring microbes, we found that most of the concrete endolith communities sampled did not cause concrete structures to release significant amounts of CO_2, but there were notable exceptions.

The mean respiration value for our concrete samples was exceedingly low (0.0012) because seven out of ten samples had respiration values of zero. Zero values do not necessarily mean that the microbial communities within these samples were entirely inactive, but it does suggest that these communities were not active enough to cause their concrete ecosystems to be net sources of CO2. This fits the assumption that endolithic ecosystems within concrete generally function at slow rates and at low levels, like other endolithic ecosystems [28,71]. Even if the endoliths within concrete were metabolically active, it is possible (given our own biomass estimates) that there were too few concrete endoliths microbes to produce measurable respiration signals. Yet, two concrete samples, from a belowground structure and a pre-cast CMU, produced respiration signatures that we could attribute to microbial activity (as opposed to analytical error or abiotic processes; Table 5). We did not explicitly design our respiration tests to identify the factors that affect "concrete respiration," but the respiration signals from these two concrete samples suggests that a combination of three interrelated factors allowed these samples to develop microbial communities capable of respiring at significant levels. First, being positioned outdoors likely allowed these samples to receive more life-sustaining moisture and nutrients from rainwater, soil particles, etc., as well as additional microbes [21]. Second, these samples appeared very weathered and porous, and the presence of many large and interconnected pore spaces is known to promote processes such as nutrient diffusion and light penetration, which would in turn promote microbial colonization and overall metabolism [72]. Third, these samples came from structures that appeared several decades old. Thus, the concrete had probably carbonated and became less alkaline by the time of sampling; this change towards a more neutral pH could have allowed more microbes to survive inside these concrete structures [31].

Additionally, while the amount of carbon that respired from these sample was small (between one-tenth and one-twentieth of the carbon released by our low-biomass soil sample), these samples demonstrate the microbial carbon emissions from within concrete do occur in some instances. Future studies should attempt to quantify this phenomenon similar to how we attempted to quantify global concrete endolith biomass. We should also keep in mind that, as much of the world's concrete weathers and ages, that concrete may eventually come to harbor endolithic communities that are active enough to generate net outflows of carbon.

4.4. Some experimental concerns surrounding endolithic respiration tests

Several aspects of our respiration tests were experimental compromises between maximizing the detection of respiration signatures and minimizing sample manipulation and contamination. We opted to measure respiration ex situ in long-incubated microcosms because we presumed that the respiration rates would be too low to measure in the field in real time. Moreover, we loaded our microcosms with relatively large concrete samples (>70 g); we suspected that samples <50 g may not contain enough endolithic cells to produce detectable respiration signals. We half-filled the microcosms with concrete to reduce the headspace volume and increase the chances that the headspace air would become measurably enriched with CO2 [73]. Yet, given the preponderance of negative results here, we would now recommend filling the microcosms more.

To further maximize the chances of detecting CO2 enrichment, we initially filled our microcosms with CO2-free air. There are some analytical advantages associated with using CO2-free air during respiration experiments [74]. We avoided over-enrichment of CO2, which sometimes constrains microbial metabolism (in a type of negative feedback) and confounds respiration analyses [75]. Yet, it is possible that the lack of CO_2 in the microcosms inhibited the functioning of any microbes that rely on CO_2 assimilation (and the microbes that rely on these microbes). This could have reduced respiration levels below what would have otherwise been detectable. Repeating this experiment with microcosms filled with air containing CO_2 may produce different results.

During the respiration assays, we used pulverized concrete samples instead non-pulverized samples because we decided that the benefits of such sample manipulation outweighed the costs. We suspected that intense grinding or powderization would signficantly alter the microbial communities. Yet, because of concrete's high density and particle arrangement, we also suspected that leaving the concrete samples intact would slow the diffusion of respired CO_2 from the inside of the concrete to the surrounding headspace air (just as CO2 respired in soil is slower to diffuse when the soil is compacted; [76,77]). Therefore, to facilitate the detection of respired CO2, we pulverized the concrete but stopped short of intense grinding. Yet, even this may have caused the concrete to excessively dry out. The insides of concrete can contain water leftover from the initial mixing process and/or water that has seeped in from the external environment [78]. The microscopic sources of water usually amount to very little water, which is why our samples appeared dry, but this moisture can affect the functioning of concrete structures [79]. To counteract any moisture loss without adding too much excess water to the concrete, we included humidifying vials in each microcosm. Additionally, we decided to not induce respiration by adding nutrients. Adding nutrient solutions to a substrate can activate or stimulate otherwise dormant and slow-growing microbes, thus causing their respiration to reach measurable levels. In this way, nutrient amendment is an effective life-detection strategy (e.g., [80,81]); however, our goal was not life detection – our goal was to determine if typical endolithic activity causes carbon emissions from concrete.

Concrete respiration analyses may also be confounded by carbonation. It is conceivable that some of the CO2 respired by concrete endoliths was reabsorbed by the cement in our concrete samples, thus preventing CO2 enrichment in the microcosm headspaces. A similar process can happen in soil when the CO2 respired by soil organisms is absorbed by soil chemicals before it can flow out of the soil [82]. Yet, this would not be an issue when the cement within the concrete is already fully carbonated and unable to absorb more CO_2 (as is usually the case for old, damaged, or pervious concrete; [83]). Also, the CO2 respired by some concrete endoliths may have been assimilated by other concrete endoliths, which sometimes happens among soil microbes [84,85]. We have no evidence that CO2 assimilating microbes occur in the dark environments within concrete, but biological CO2 absorption within ordinary concrete deserves further investigation. Regardless of the many processes that produce or capture CO_2 within concrete, our tests determined whether our concrete samples were net emitters of carbon, which is arguably the more important point in the context of global greenhouse gas emissions.

Finally, while we successfully tested our concrete respiration hypothesis, our tests did not produce absolute measures of carbon efflux (see Section 2.5). Therefore, we could not estimate the mass of carbon being emitted globally from concrete endoliths. Future studies should explore gas analyses that can measure concrete respiration in molar units, such as gas chromatography, or use gas standards to help convert respiration signals into molar masses.

5. Conclusions

In summary, we investigated endoliths as a global pool of organic carbon within concrete (Objective 1) and as agents of carbon flux from concrete (Objective 2), thus finding another connection between concrete microbiology and sustainability. Regarding the first objective, we demonstrated that trace levels of microbial biomass within concrete (>4 μg g^{-1} concrete) could add up to sizable global quantities of carbon (4500–1150000 metric tons). Our lower estimates implied that the amount of carbon locked up within concrete endolith biomass was too small to significantly alter carbon budget of concrete or the concrete industry, while the higher estimates implied that the C_{mic} of concrete endoliths may be enough to offset smaller sources of concrete-related carbon emissions.

Regarding our second objective, we tested the idea that carbon can be mobilized and emitted by the metabolic activities of concrete endoliths. We found that most of our concrete samples did not emit CO_2 because of respiring endolithic microbes, which suggests that concrete endolith communities often comprise relatively few microbes and/or largely inactive microbes. Yet, we observed small but clear respiration signals from two concrete samples, suggesting that "concrete respiration" occurs under certain circumstances.

Our results are preliminary, but this study can serve as a template for future investigations. Moving forward, we suggest that concrete studies maintain some focus on microbial carbon storage and cycling because the carbon footprint of the concrete industry is expected to remain high in the coming decades [86], and Earth will continue accumulating concrete materials [87]. The current study was conducted in a piecemeal fashion, but an obvious improvement would be to measure biomass, respiration, and other carbon-related measurements in the same set of samples, as well as to correlate those biological measurements to other variables like concrete pH, density, and carbonation levels. This would allow researchers to identify which factors affect concrete endolith biomass and respiration rates. Additionally, future studies should use larger sample sizes, concrete from other regions, and more types of concrete, including new types of concrete that have been explicitly developed to have smaller carbon footprints [88,89]. Future studies should refine and explore other methods of measuring concrete endolith biomass and respiration. Global rates of concrete respiration may even be estimated once this process is further verified and quantified. Individual concrete samples should also be analyzed more closely to determine if microbes and microbial activity are concentrated in certain portions of concrete structures (e.g., near the surface, within carbonated zones, or within pore spaces). Additionally, there is a need to elucidate and quantify other ways carbon can be stored or released by microbes (e.g., the carbon contained in non-living organic matter, the carbon fluxes associated with microbial mineral precipitation and dissolution; [35,90]), as well as examine the fate of concrete as it decomposes in the environment and how this corresponds to microbial activity.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

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