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Meteorites as Food Source on Early Earth: Growth, Selection, and Inhibition of a Microbial Community on a Carbonaceous Chondrite

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Abstract

Meteoritic material accumulated on the surface of anoxic early Earth during the Late Heavy Bombardment around 4.0 Gya and may have provided Earth's surface with extraterrestrial nutrients and energy sources. This research investigates the growth of an anaerobic microbial community from pond sediment on native and pyrolyzed (heat-treated) carbonaceous chondrite, Cold Bokkeveld. The community was grown anaerobically in liquid media. Native Cold Bokkeveld supported the growth of a phylogenetically clustered subset of the original pond community by habitat filtering. The anaerobic community on meteorite was dominated by the Deltaproteobacteria Geobacteraceae and Desulfuromonadaceae. Members of these taxa are known to use elemental sulfur and ferric iron as electron acceptors, and organic compounds as electron donors. Pyrolyzed Cold Bokkeveld, however, was inhibitory to the growth of the microbial community. These results show that carbonaceous chondrites can support and select for a specific anaerobic microbial community, but that pyrolysis, for example, by geothermal activity, could inhibit microbial growth and toxify the material. This research shows that extraterrestrial meteoritic material can shape the abundance and composition of anaerobic microbial ecosystems with implications for early Earth. These results also provide a basis to design anaerobic material processing of asteroidal material for future human settlement. Key Words: Carbonaceous chondrite—Anaerobic microbial community—Early Earth—Iron reduction—Sulfur reduction—Habitat filtering. Astrobiology 22, xxx–xxx.

1. Introduction

During the Late Heavy Bombardment, asteroidal material in the inner Solar System was scattered, and early Earth was bombarded with approximately 200 times more mass of extraterrestrial material than in the present (Chyba and Sagan, 1992; Love and Brownlee, 1993; Jenniskens *et al.,* 2000). This resulted in the arrival of around 1.2 * 10^9 kg of organic carbon per year, assuming that all organic carbon survived entry through the atmosphere (Jenniskens *et al.,* 2000). Evenly distributed over the surface of Earth, this would have covered Earth with around 2 mg per m^2 per year, although in reality it was probably localized to locations of meteoritic infall.

From the point of view of biological use, meteoritic material can be rich in nutrients and organics, with carbonaceous chondrites containing up to 2 wt % organics (for a review, see Sephton, 2002). The carbon in carbonaceous chondrites consists of 70% insoluble, macromolecular, kerogen-like material (Hayes, 1967; Mautner, 1997; Sephton, 2002). The soluble organic material, which is biologically available, includes amino acids, carboxylic acids, hydrocarbons, nucleobases, sulfonic acids, and phosphonic acids (Pasek *et al.,* 2007; Martins, 2011). Additionally, carbonaceous chondrites contain biologically available phosphorus, iron, sulfur, calcium, magnesium, sodium and potassium, as well as hydrogen and oxygen (Kerridge, 1985; Mautner, 1997; Watson *et al.,* 2010).

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The composition of carbonaceous chondrites seems beneficial for life. However, it remains a question whether the infall of this material would have influenced the habitability of early Earth and the structure of early anaerobic microbial ecosystems growing in the presence of it. This question extends beyond Earth and applies to any young planet potentially harboring life.

On the other hand, this material could have been toxic for life. Mautner *et al.* (1995) showed that under certain conditions pyrolyzed meteorite could inhibit aerobic microbial growth. Meteorite pyrolysis is the heat-treating of meteorite and can occur through heating processes on the planet's surface, such as geothermal heating and volcanic activity, which could induce chemical alterations (Bryant *et al.,* 2013). This is different from the natural process of atmospheric entry. Meteoritic entry through Earth's atmosphere causes a meteorite's fusion crust (*i.e.,* a silicate glass layer surrounding the meteorite caused by compressing atmospheric gasses during atmospheric entry), which differs from pyrolyzed meteoritic material. Because of the high volcanic activity and geothermal heating on early Earth, it is of considerable interest to determine whether pyrolysis by natural thermal alteration on a hotter young Earth could transform the biological potential of recently delivered extraterrestrial meteoritic material.

Research on the effects of carbonaceous chondrites on microbial growth has primarily focused on the growth response of single microbial species, rather than a microbial community, even though the latter represents a more natural analogue (Mautner *et al.,* 1995, 1997; Mautner, 1997; Gronstal *et al.,* 2009). Furthermore, the microbial species previously investigated were mainly aerobic organisms. Aerobic growth enhancement and growth limitation were both observed by the addition of powdered carbonaceous chondrite and extracts of hydrous pyrolyzed carbonaceous chondrites to growth media (Mautner *et al.,* 1995; Gronstal *et al.,* 2009). Extracts from the meteorite had a growth enhancing effect on several bacterial species (Mautner, 1997; Mautner *et al.,* 1997). Mautner (2002) demonstrated microbial growth enhancement on liquid extracts from carbonaceous chondrites under microoxic conditions. As the microbial community was not identified, it remains unknown how meteoritic material and the geochemical environments it created, influenced the community composition of the community supported by it. As early Earth had very low atmospheric oxygen concentrations during the Late Heavy Bombardment (<0.001% of the present atmospheric oxygen concentration; Kump, 2008), a key question is what effect meteoritic material would have had on anaerobic communities.

In this paper, we test the hypothesis that anaerobic communities can be sustained on carbonaceous chondritic material and that this material would select for specific communities of organisms associated with the nutrients and redox couples associated with the meteorite. Additionally, we investigate the effect of pyrolysis of the meteoritic material on anaerobic communities. We show that carbonaceous chondritic material allows for the growth of a specific set of anaerobic microbial organisms but that pyrolyzed carbonaceous chondritic material does not. We discuss the implications of the presence of meteoritic material and environmental conditions on Earth and beyond for life.

2. Materials and Methods

2.1. Microbial community sampling

Sediment samples were collected from Blackford Pond in Edinburgh, UK (55°55′31″N, 3°11′47″W) in December 2018 from the top 5 cm of sediments, approximately 1.5 m from the edge of the pond, where the water depth was approximately 1 m at the time of collection. Blackford Pond was artificially created around the year 1900 and has little flow of water. The pond is eutrophic, caused by water inflow from allotments nearby, as well as bird feces, leaf litter, and food remnants from feeding waterfowl, resulting in a variety of complex organics in the pond sediment (*Hermitage of Braid and Blackford Hill Local Nature Reserve Management Plan 2011–2021,* nd; Pringle and Beale, 1960; Boyd, 1995). Eutrophication of water bodies can cause hypoxia in the water column and anoxic conditions in the sediment (Smolders *et al.,* 2006; Waajen *et al.,* 2014). Samples were taken from the top layer of the sediment, as this layer usually contains the highest concentration of organic matter (Munsiri *et al.,* 1995). The top sediment layer can have a thin, oxic surface layer above a thicker, anoxic subsurface layer (Munsiri *et al.,* 1995). We therefore sampled both the potentially oxic surface layer and the thicker anoxic subsurface layer, collecting organisms capable of degrading a wide variety of simple and complex organic compounds.

Although the pond contains a wide variety of organics associated with the largely oxic, metazoan-inhabited Earth (as for example plant material was observed on top of the pond sediment), we wanted to obtain sediment that would possess a wide diversity of organics in anaerobic conditions so that we could examine whether the carbonaceous meteorite would downselect a subset of organisms from this putatively diverse anaerobic organic-degrading community.

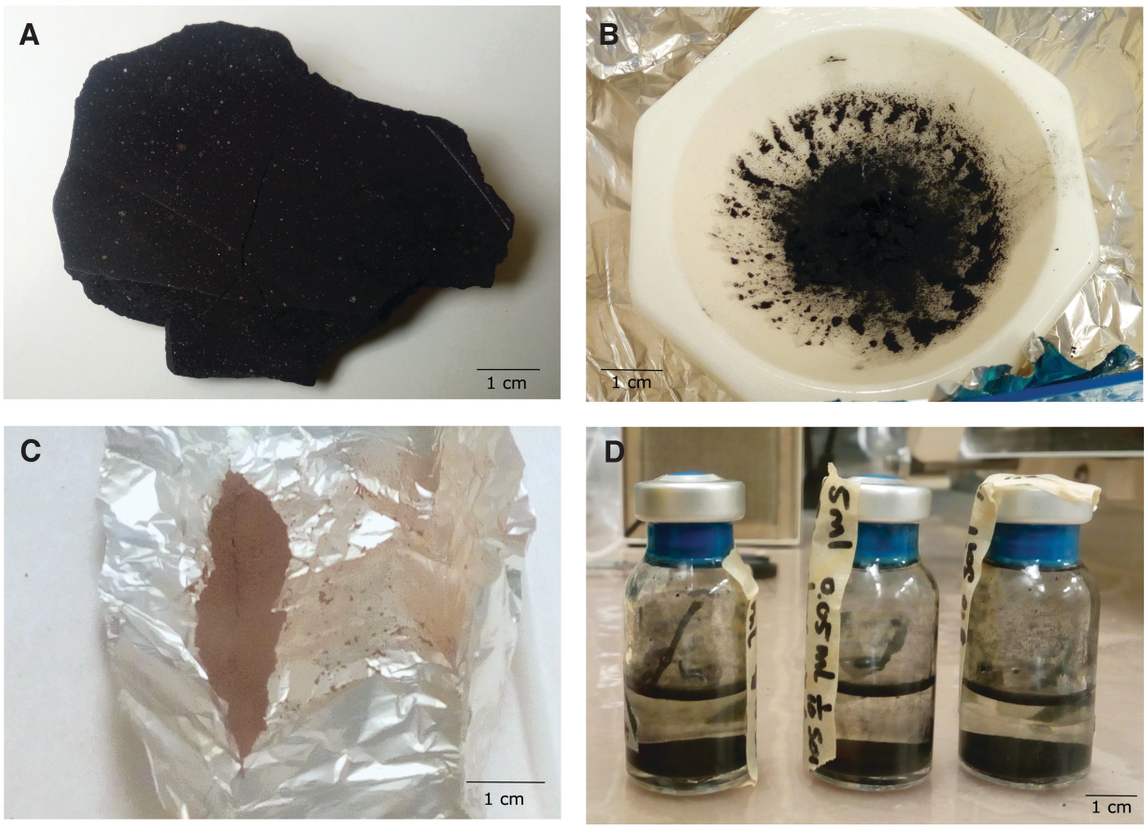
2.2. Meteoritic material

The CM2 carbonaceous chondrite (a Mighei-like carbonaceous chondrite that is petrologic type 2) Cold Bokkeveld was provided by the National Museum of Scotland (Fig. 1A). Cold Bokkeveld contains 2.16 ± 0.35 wt % carbon ◀F1 (Pearson *et al.,* 2006). In the parent body, the celestial body from which the meteorite originates, Cold Bokkeveld had not undergone thermal metamorphism but had undergone extensive aqueous alterations (McSween, 1979; Browning *et al.,* 1996; Kitajima *et al.,* 2002). As a result, less labile organic matter and more graphitic, stable matter is found in this meteorite when compared to other carbonaceous chondrites (Komiya *et al.,* 1993; Naraoka *et al.,* 1997; Kitajima *et al.,* 2002).

2.3. Preparation of anaerobic cultures

Glassware was made organic-free following Eaton *et al.* (2005). First, glassware was washed with common detergent. Then glassware was rinsed three times with distilled water before submerging the glassware overnight in 0.1 M HCl. Thereafter, glassware was rinsed three times with distilled water and was air-dried. Glassware was then capped with aluminum foil and heated to 550°C for 6 h in a Carbolite 1100°C Chamber Furnace.

Butyl rubber stoppers were washed with common detergent and rinsed three times with distilled water before



4C ▶

FIG. 1. Cold Bokkeveld and microcosms. (**A**) The carbonaceous chondrite Cold Bokkeveld. (**B**) Powdered, native (untreated) Cold Bokkeveld. (**C**) Powdered, pyrolyzed Cold Bokkeveld in aluminum foil. (**D**) Microcosms containing water, powdered native Cold Bokkeveld, and the microbial community.

boiling the stoppers three times for 5 min in distilled water. Afterward, the stoppers were rinsed three times with distilled water, and air-dried.

Prior to making a powder from the meteoritic material, the exterior of the meteorite was disinfected by wiping with 70% ethanol. The meteorite was then broken into pieces with a heat-sterilized chisel and powdered with a mortar and pestle (Fig. 1B), which had been ashed by heating to 550°C for 6 h in a Carbolite 1100°C Chamber Furnace. The meteoritic interior was not sterilized in order to prevent chemical alteration of the material. Pyrolysis was performed by dry-heating the powdered Cold Bokkeveld to 550°C for 6 h in a Carbolite 1100°C Chamber Furnace and allowing the material to cool down prior to preparation of the microcosms. Meteorite powder was weighed prior to and after pyrolysis. Pyrolyzed meteorite can be seen in Fig. 1C.

Inside an anaerobic chamber (Coy Laboratory Products), anoxic microcosms were created in 12.5 mL glass bottles by the addition of 0.5 g powdered, native (untreated, non-pyrolyzed) Cold Bokkeveld to 5 mL anaerobic molecular grade water. The inoculum was prepared by centrifuging the sampled sediment, discarding the supernatant, homogenizing the sediment in the anaerobic chamber, and diluting the sediment 10 times in molecular grade water. Anoxic microcosms in glass bottles (Fig. 1D) were then inoculated with 50 µL of the inoculum. Inoculated anoxic microcosms, that is, batch cultures, were then incubated under 1 atm, 2% H_2, 98% N_2 at room temperature for approximately 2 months. Then 50 µL of the community was transferred to fresh anoxic microcosms, incubated for approximately 2 months again, after which 50 µL of this microcosm was transferred to fresh anoxic microcosms again to establish a stable anaerobic community.

This stable anaerobic community was inoculated to fresh microcosms containing 5 mL molecular grade water and either 0.5 g powdered native Cold Bokkeveld (Fig. 1B and 1D) or 0.5 g powdered, pyrolyzed Cold Bokkeveld (Fig. 1C) or control microcosms as explained below.

2.4. Microbial growth measurements

Growth of the anaerobic microbial community on Cold Bokkeveld in the anoxic batch cultures at room temperature was measured by colony-forming unit (CFU) counts on LB agar plates (adapted from DSM 381: 10.0 g/L tryptone; 5.0 g/L yeast extract; 5.0 g/L NaCl; 20 g/L Agar; pH 7.0). Plates were incubated anaerobically at room temperature. CFU estimations were made by single plate-serial dilution spotting (SP-SDS) (Thomas *et al.,* 2015). SP-SDS of the community in batch cultures was done daily for 10 days, an incubation time based on earlier test runs.

Three controls were tested: a biological control without meteorite but with glass beads (control A), a biological control without meteorite (control B), and a nonbiological

control with meteorite (control C). The microcosms of control A contained 5 mL molecular grade water and 38 glass beads with 3 mm diameter and was performed to test whether the growth of the community was enhanced on a surface and with nutrients potentially carried over with the inoculum, for example, intracellularly. Control B contained 5 mL molecular grade water to test whether microbial growth occurred in the absence of meteorite. Control C contained 5 mL molecular grade water and 0.5 g powdered Cold Bokkeveld and was not inoculated. Control C tested whether any observed microbial growth occurred by microorganisms that could have potentially been present on the meteorite. SP-SDS on control A and B was done at the starting and endpoint. SP-SDS on control C was performed four times over the course of 11 days. All experiments were conducted in anoxic conditions and in triplicates.

The pH of the microcosms prior to inoculation and at the end of the growth experiment was measured in triplicate with a Jenway 3510 pH meter (Cole-Parmer, Staffordshire, UK) and InLab Semi-Micro-L pH electrode (Mettler Toledo Ltd, Leicester, UK). A single pH measurement was taken of the inoculum by using the above equipment. The pH of the centrifuged soil was measured according to Hendershot *et al.* (1993). Triplicate samples of 2.0 g air-dried soil were weighed; 4.0 g DI H_2O was added to each sample. Samples were stirred intermittently for 30 min and let settle for 1.5 h prior to pH measurements.

A one-sample *t*-test was used to assess the difference between the final cell concentration in the pyrolyzed meteorite condition and zero, and between the final cell concentration in control B and zero. A one-way ANOVA was performed to assess the difference between the final cell concentrations in the different conditions. To ensure the assumptions for an ANOVA were not violated, the Shapiro-Wilk normality test and Levene's test for homogeneity of variance were executed. A *post hoc* Tukey test assessed pairwise comparisons of the final cell concentrations of the different conditions. Paired, two-sample Student's *t*-tests were carried out to assess the difference in pH before and after inoculation in each of the conditions.

An unpaired, two-sample Student's *t*-test was conducted to assess the difference in pH in the microcosms containing the native and the pyrolyzed meteorite prior to inoculation. To ensure the assumption of normality for both the paired and unpaired, two-sample Student's *t*-test were not violated, Shapiro-Wilk normality tests were carried out. To ensure the assumption for homogeneity of variance for the unpaired, two-sample Student's *t*-test was not violated, an *F*-test was performed.

2.5. 16S rRNA gene amplicon sequencing and analysis

DNA was extracted from the following: (1) three cultures with native Cold Bokkeveld containing a stable anaerobic community in late exponential phase, (2) three samples of homogenized sediment from Blackford Pond in order to determine the composition of the starting community, (3) one sample of control C. The DNeasy PowerSoil Kit was used for DNA extractions (QIAGEN GmbH, Germany). Extracted DNA samples were then stored at -20°C until they were shipped to the Research and Testing Laboratories (RTLGenomics, Texas, USA) for amplicon sequencing. The primers 28F (GAGTTTGATCNTGGCTCAG) and 388R (TGCTGCCTCCCGTAGGAGT) were used to amplify the 16S rRNA gene. PCR amplification was completed by using 25 μL reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 μL of each primer (5 μM concentration), and 1 μL of template DNA. ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, California, USA) were used for reactions with the following thermal profile: 95°C for 5 min, then 35 cycles of 94°C for 30 s, 54°C for 40 s and 72°C for 1 min, followed by 1 cycle of 72°C for 10 min, and hold at 4°C. A second PCR using first stage amplicons was run to add barcodes to the samples. Primers for secondary PCR were Illumina Nextera PCR primers (Forward AATGATACGGCGACCACCGAGAT CTACAC[i5index]TCGTCGGCAGCGTC and Reverse CAAGCAGAAGACGGCATACGAGAT[i7index]GTCTC GTGGGCTCGG). The same thermal profile was used for the second PCR, except from a reduction of 25 cycles in the thermal profile of the second PCR.

The products of the amplification were visualized with eGels (Life Technologies, Grand Island, New York, USA). Products were pooled to equal molar concentrations, and pools were then selected by size in two rounds by using SPRIselect Reagent (BeckmanCoulter, Indianapolis, Indiana, USA) in a 0.75 ratio for both rounds. Quantification of the size selected pools was performed with the Qubit 4 Fluorometer (Life Technologies). Libraries were then sequenced with Illumina MiSeq (Illumina Inc., San Diego, California, USA) 2×300 flow cell at 10 pM.

FASTQ files from RTLGenomics were analyzed from the cultures on the native meteorite and sediment from Blackford Pond using the EDGE Bioinformatics Web-based platform (Li *et al.,* 2017), which utilizes QIIME2 version 2019.10.0 automated scripts (Bolyen *et al.,* 2019). The quality control method selected was operational taxonomic units (OTUs), with a binned OTU representing a sequence similarity of 97%. A quality score cutoff of 19 was set, therefore retaining sequences with a Q-score of 20 or above. The minimum number of consecutive high-quality base calls to include a read (per single end read) as a fraction of the input read length was set at 0.75. One ambiguous nucleotide per sequence was allowed. Sequences were demultiplexed and joined using QIIME2 vsearch methods and commands (Rognes *et al.,* 2016).

Retained sequences were clustered into OTUs by using qiime vsearch cluster-features-open-reference command and the SILVA-132-99 database. Chimeric sequences were removed with UCHIME (qiime vsearch uchime-ref command). Taxonomic identity of OTUs was determined by using the q2-feature-classifier plugin (Bokulich *et al.,* 2018).

A rooted phylogenetic tree was created from the raw OTU feature table with 10,469 OTUs (qiime phylogeny align-to-tree-mafft-fasttree command) by using MAFFT alignment tool (Katoh *et al.,* 2002) and FastTree method (Price *et al.,* 2009). Qiime diversity core-metrics-phylogenetic command was applied to the rarefied OTU table with a sampling depth of 14,885 sequences (7856 OTUs) to analyze alpha diversity, generating the species diversity indices Faith's phylogenetic diversity (PD) and Shannon-Weaver diversity. A community composition barplot and Venn diagram were constructed from the rarefied OTU table excluding families containing less than 10 sequences per condition.

Beta diversity was also examined with a weighted Unifrac distance matrix, which evaluates phylogenetic diversity and abundance (Lozupone and Knight, 2005). A weighted Unifrac distance matrix generated in QIIME2 (qiime diversity core-metrics-phylogenetic) was imported in PRIMER-e v.7 and analyzed using non-metric multidimensional scaling (nMDS).

Further evaluation of the 16S rRNA data was completed to assess phylogenetic community structure and test for phylogenetic clustering (*i.e.,* taxa are more closely related than expected by chance) or overdispersion (*i.e.,* taxa are less closely related than expected by chance). This was performed by using a null model approach (Webb *et al.,* 2008; Miller *et al.,* 2017) between sample types by calculating the average taxonomic distinctness (avgTD or Delta+) and variation around the mean taxonomic distinctness (varTD or Lambda+; Clarke and Warwick, 1998, 1999, 2001). Taxonomic distinctness is a univariate biodiversity index that calculates the average distance between all pairs of species in a sample, where that distance is defined as the path length through a standard Linnean or phylogenetic tree connecting these species. AvgTD and varTD were calculated from a rooted phylogenetic tree of all OTUs recovered from all samples by using the patristic distance [sum of branch lengths between pairs of species (*ij*)] and providing a calculation for relatedness between two species pairs (completed in Geneious Prime 2021.0.3). The patristic distance matrix was then imported to PRIMER-e v.7. The algorithm TAXDTEST was applied to generate expected values of avgTD and varTD under the null model that all OTUs in the phylogenetic tree from all samples have equal chance of being represented in any one random sample, suggesting that community assemblages were constructed at random from a regional pool of possible bacterial community members (*i.e.,* no phylogenetic clustering or overdispersion due to competition). TAXDTEST runs permutation tests of random subset of OTUs at various sample sizes, thereby testing for a departure from the null model where all OTUs in the phylogeny have equal probability of being included in any random sample draw (without replacement), and evaluates the phylogenetic relatedness and breadth of the community (Clarke and Warwick, 1998). In the TAXDTEST routine, the number of paths calculated was limited to 10,000 due to the large number of OTUs in the Blackford Pond samples (10,469 OTUs). Along with generating histograms for each sample of the permutation tests completed, we generated a 95% probability funnel that illustrates the avgTD and varTD across the range of OTUs [proxy for species richness, (S) found in the actual samples (minimum $S = 74$; maximum $S = 5668$)]. This procedure illustrates whether OTUs in a sample present a higher- or lower-than-expected phylogenetic relatedness (avgTD). If a sample falls inside the 95% probability funnel, the null hypothesis cannot be rejected, and communities appear to be assembled at random. If it falls either above (overdispersion) or below (phylogenetic clustering), the null model is rejected. VarTD illustrates whether OTUs in a sample present a higher- or lower-than-expected variation around the mean taxonomic distinctness. If a sample falls inside the 95% probability funnel, the null hypothesis cannot be rejected, and communities appear to be assembled at random. If it falls either above (phylogenetic clustering) or below (overdispersion), the null model is rejected.

3. Results

3.1. Microbial growth on native and pyrolyzed meteorite

A stable anaerobic microbial community was obtained, growing in the presence of the powdered, native carbonaceous chondrite Cold Bokkeveld in molecular grade water. Microbial growth was determined on the native and pyrolyzed meteorite, and control A and B by CFU counts (Fig. 2). Exponential growth was observed in the batch cultures containing native meteorite. The stationary phase was reached after 7 days with a final cell concentration of $3 * 10^7$ CFU/mL. No growth was observed in the batch cultures containing pyrolyzed meteorite. On the contrary, the microbial community concentration declined during the exposure to pyrolyzed meteorite. After 10 days, the final concentration of the microbial community in the presence of pyrolyzed meteorite (83 ± 76 CFU/mL) was not significantly different from 0 CFU/mL [t-test $(2) = 1.89$, p-value $= 0.20$]. Cell concentrations are shown as mean ± standard deviation. Physical changes of the meteorite were also observed by pyrolysis. Pyrolysis resulted in an 8% weight loss and in a color change from black (Fig. 1B) to light brown (Fig. 1C) of the meteoritic material. ◀F2

No growth was observed in the presence of glass beads instead of meteorite (control A). Some growth was observed in the microbial community in the absence of meteorite with a final concentration of $4.5 * 10^6 \pm 1.6 * 10^6$ CFU/mL ($n = 3$), being significantly different from 0 CFU/mL [t-test $(2) = 4.75$, p-value $= 0.042$]. In control C, no growth was observed (data not shown). The absence of viable cells in control C on the final day shows that the meteorite contained no indigenous microbial contamination that was able to grow in the experimental conditions. Final cell concentrations on the native meteorite, pyrolyzed meteorite, control A, and control B were compared using a one-way ANOVA and *post hoc* Tukey test. As there were no viable cells found on the final day for control C, control C was omitted from the statistical tests. The Shapiro-Wilk normality test showed normality of the final cell concentrations (p-value $= 1$, 1, 0.98, and 0.54 respectively for native meteorite, pyrolyzed meteorite, control A, and control B). The Levene's test for homogeneity of variance showed that the homogeneity of variance was not violated (p-value $= 0.10$). There was a significant difference in the final cell concentrations between the different conditions [ANOVA: F-statistic $(3,8) = 209.8$; p-value < 0.001]. The *post hoc* Tukey test showed a significant difference between the final cell concentrations in the native and pyrolyzed meteorite (p-value < 0.001); between the native meteorite and control A (p-value < 0.001); and between the native meteorite and control B (p-value < 0.001). A marginally nonsignificant difference was observed between the final cell concentrations in the presence of pyrolyzed meteorite and control B (p-value $= 0.052$).

3.2. Microbial usage of meteoritic carbon

Assuming an average wet weight per bacterial cell of 10^{-12} g, an average dry weight of 40% of the wet weight (Bratbak and Dundas, 1984), and a carbon content of 50% of the dry weight (Loferer-Krößbacher *et al.,* 1998), each bacterial cell contains $2 * 10^{-13}$ g carbon. As the final cell

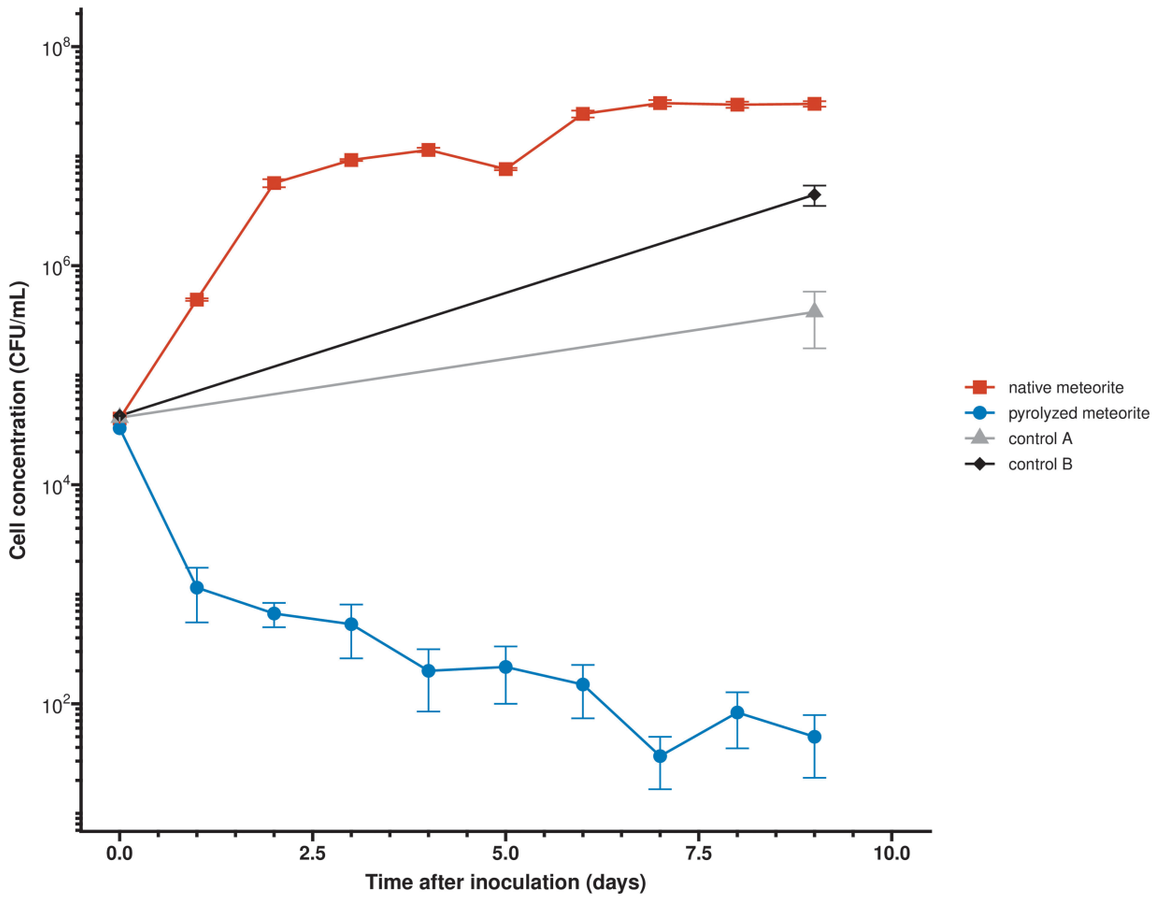


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FIG. 2. Microbial growth on Cold Bokkeveld. Cell concentration of the microbial community concentration (CFU/mL) over time (days) of microcosms containing native meteorite with water, pyrolyzed meteorite with water, glass beads with water (control A), or water (control B). Points are means, and error bars are standard error. The standard errors of the native meteorite are within the markers.

concentration on the native meteorite was 3 * 10^7 CFU/mL and each batch culture contained 5 mL water, the total carbon in biomass per batch culture was estimated to be 3 * 10^{-5} g. Each batch culture contained 10^{-2} g organic matter from Cold Bokkeveld, which implies that potentially, assuming the meteorite was the sole carbon source, 0.3 wt % of the meteoritic organic matter was used by the microorganisms.

3.3. pH measurements

T1 ▶ pH results are shown in Table 1. The pH of the inoculum and the soil was circumneutral. All pH microcosms were also circumneutral prior to inoculation, apart from a slightly alkaline pH (9.09 ± 0.02) of the microcosms containing pyrolyzed meteorite. Prior to inoculation, the pH of the microcosms containing pyrolyzed meteorite was significantly higher than that of the microcosms containing native meteorite [*t*-test (2) = -26.4, *p*-value <0.001]. Shapiro-Wilk normality tests showed normality of the pH measurements prior to inoculation (*p*-value = 0.52 and 0.46 respectively for native meteorite and pyrolyzed meteorite). An *F*-test showed homogeneity of variance between the native and pyrolyzed pH measurements prior to inoculation [*F*-statistic (2,2) = 19.9; *p*-value = 0.10].

The pH of the pyrolyzed meteorite decreased significantly during the experiment [*t*-test (2) = 60.5, *p*-value <0.001], while the pH of the native meteorite, control A, and control B did not change significantly [*t*-test (2) = 1.91, *p*-value = 0.20; *t*-test (2) = 2.69, *p*-value = 0.11; *t*-test (2) = -1.14,

TABLE 1. pH RESULTS

Table 1A. pH results of Blackford Pond soil sediment and the inoculum. The soil measurement was made in triplicate; the inoculum was measured once.

Sample	*pH*
Soil	7.91 ± 0.02
Inoculum	7.69

Table 1B. pH results of the microcosms prior to inoculation (start) and at the end of the growth experiment (end). All measurements were made in triplicate.

Sample	*pH start*	*pH end*
Non-pyrolyzed meteorite	7.64 ± 0.09	7.50 ± 0.04
Pyrolyzed meteorite	9.09 ± 0.02	8.36 ± 0.04
Control A	7.52 ± 0.29	7.06 ± 0.03
Control B	6.61 ± 0.06	6.67 ± 0.04

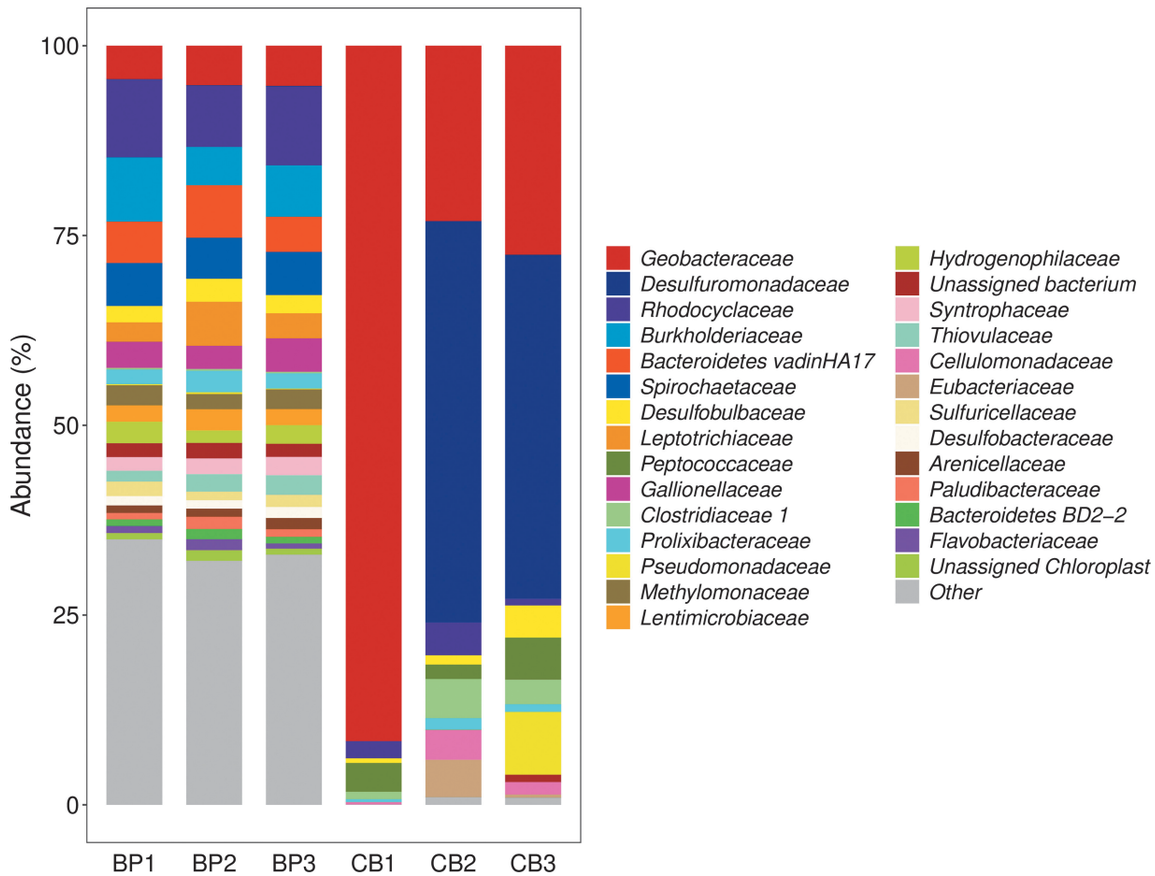

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FIG. 3. Microbial community composition on native Cold Bokkeveld. Bar graph of the relative abundance of microbial communities in batch cultures containing native meteorite at the family taxonomic level. Taxonomic classifications are based on the SILVA 132.99 database. Sample BP1, BP2, and BP3 are triplicate sediment samples from Blackford Pond. CB1, CB2, and CB3 are triplicate batch cultures of the microbial community in the presence of native Cold Bokkeveld. ''Other'' contains families at an abundance smaller than 1%.

p-value = 0.37 respectively]. Shapiro-Wilk normality tests showed normality of the difference in pH measurements before and after inoculation in each of the conditions (*p*-value = 0.08, 0.46, 0.70, and 0.68 respectively for native meteorite, pyrolyzed meteorite, control A, and control B).

3.4. 16S rRNA amplicon sequencing of microcosms

16S rRNA amplicon sequencing results revealed the community composition of the bacterial community on native Cold Bokkeveld and the sediment from Blackford Pond were considerably different (Fig. 3). No DNA was detected in control C, the nonbiological control containing powdered native Cold Bokkeveld. Sequences in the presence of native meteorite were mainly the family Geobacteraceae (92%) in CB1. In CB2 and CB3, Desulfuromonadaceae (53% and 45% respectively) and Geobacteraceae (23% and 28% respectively) were mainly observed. The high abundance of Geobacteraceae in CB1 was mainly due to one OTU (JQ086796.1.1478), accounting for 80% of all sequences in CB1.

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The sequences in the pond sediment contained a much higher variety of families, with 253 families identified in the

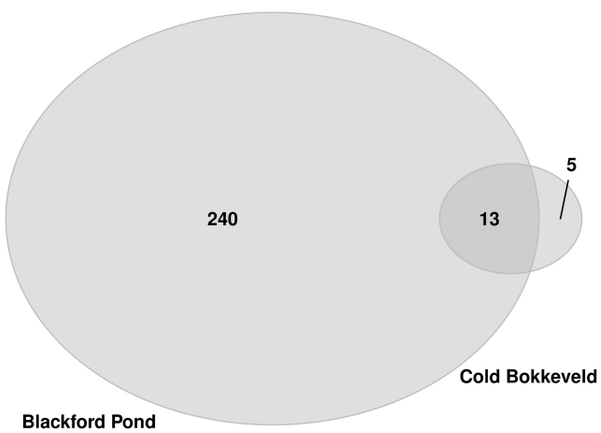
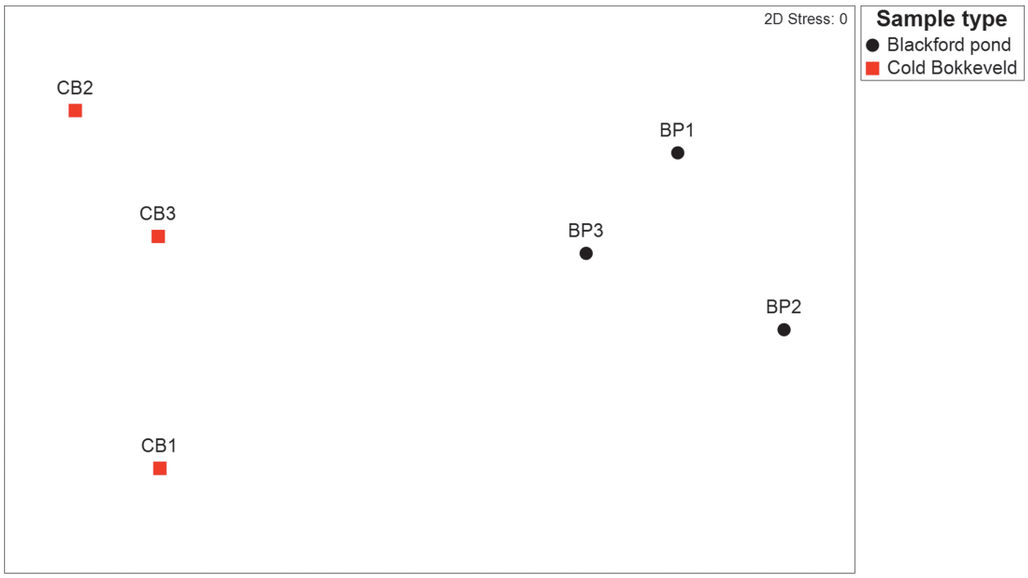


FIG. 4. Venn diagram of community richness. Venn diagram of number of families found in the sediment of Blackford Pond and in the batch cultures in the presence of native Cold Bokkeveld.



4C ▶

FIG. 5. Non-metric multidimensional scaling (nMDS) plot of community composition in sediment of Blackford Pond replicates (BP1, BP2, and BP3) and in the batch cultures in the presence of native Cold Bokkeveld replicates (CB1, CB2, and CB3).

pond sediment, whereas only 18 families were found in presence of native meteorite (Fig. 4). Sixty-seven OTUs from five families were identified in the meteorite samples that were not found in the pond sediment samples. F4 ▶

Based on weighted Unifrac distances, the compositional difference between the pond and meteorite communities are highlighted by an nMDS plot (Stress = 0.0; Fig. 5). Although some natural variability is seen between the starting samples and meteorite communities, distinct clustering is visible, separating the sample types along the *x*-axis. F5 ▶

Alpha diversity of the Blackford Pond and meteorite samples was calculated for Faith's PD and Shannon-Weaver diversity (Fig. 6). Blackford pond samples have higher alpha diversity measures, with a Faith's PD of 239.46 ± 2.82 and Shannon-Weaver diversity 10.36 ± 0.08. Native meteorite samples have a Faith's PD of 12.52 ± 3.30 and Shannon-Weaver diversity of 2.92 ± 1.31, considerably lower than the source community. F6 ▶

Phylogenetic community structure and relatedness of the Blackford Pond and meteorite samples was analyzed further, testing the null hypothesis that communities were assembled at random, with a given OTU having an equal probability to occur in any one sample. Phylogenetic relatedness (avgTD and varTD) would then not differ from what would be expected by chance.

Figure 7 illustrates that BP1 and BP3 have a significantly lower than expected value of avgTD (Delta+), suggesting that these samples have OTUs that are more closely related ◀ F7

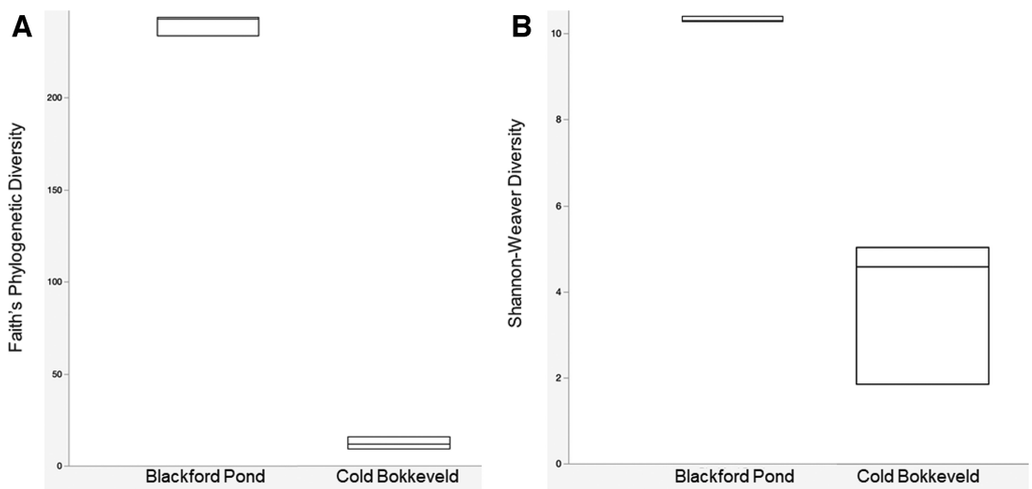
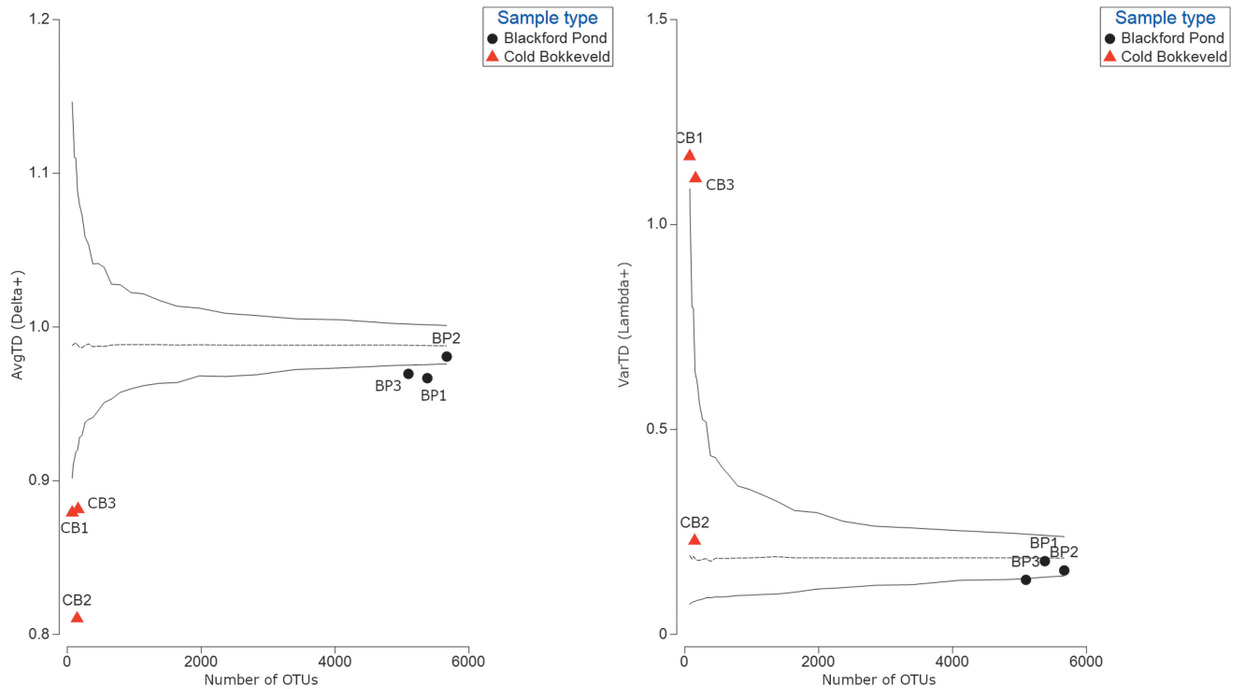


FIG. 6. Alpha diversity. (**A**) Faith's phylogenetic diversity and (**B**) Shannon-Weaver diversity of community composition samples in sediment of Blackford Pond and in the batch cultures in the presence of native Cold Bokkeveld.



4C ▶

FIG. 7. Funnel plots of the 95% confidence intervals (black lines) of the avgTD (Delta+) and varTD (Lambda+) expected by chance, and the observed avgTD and varTD of Blackford Pond replicates (BP1, BP2, and BP3) and native Cold Bokkeveld replicates (CB1, CB2, and CB3).

to each other than would be expected by chance, and that some phylogenetic clustering is taking place in the pond. Cold Bokkeveld samples all had statistically lower than expected avgTD (Delta+), illustrating that these samples are all strongly phylogenetically clustered. The OTUs that occur in these samples are statistically more closely related when compared to random draws of species from the entire phylogeny. Blackford Pond samples 1 and 3 are not significantly different from the expected varTD (Lambda+), while BP2 has a statistically lower varTD. This suggests that the range of taxonomic relatedness of BP1 and 3 is similar to what would be expected by chance but that there is less variation in BP2. varTD (Lambda+) was higher than expected for two of the three meteorite samples, and suggests that they contain a few groups of closely related OTUs, but those groups are quite distinct (distantly related) from one another. This strong phylogenetic clustering with a broad variation was less prominent in CB2, which had a variation which was not significantly different to what would be expected by chance. AvgTD and varTD values and their two-sided *p*-values are shown in Table 2. The *p*-value is the proportion of random values that are less than or equal to the observed value of avgTD or varTD for a given sample, multiplied by 2 for a two-sided *p*-value. Supplementary Figure S1 shows the histograms of the permutation tests, and where the observed avgTD and varTD lie compared to the permutation test results.

T2 ▶

SF1 ▶

4. Discussion

Meteoritic material is known to have bombarded early Earth, potentially providing organic material and other nutrients for early life. We hypothesized that this material would have influenced the presence of microbial metabolisms that could access and use this material as a nutrient or energy source. If this is the case, then meteoritic material could have shaped the diversity and biomass of early microbial communities in the presence of this material. In this study, we investigated the influence of carbonaceous chondritic material on the growth and community composition of an anaerobic microbial community.

Our experiments showed that the carbonaceous chondrite Cold Bokkeveld could sustain a stable anaerobic microbial community, with final cell concentrations reaching $3 * 10^7$ CFU/mL, a significantly higher concentration than microcosms in the absence of the meteoritic material. As the liquid growth media only consisted of molecular grade water and the powdered Cold Bokkeveld, the meteorite was

Table 2. Taxonomic Diversity

Sample	*avgTD*	p-*value avgTD*	*varTD*	p-*value varTD*
BP1	0.97	0.004	0.16	0.284
BP2	0.99	0.985	0.13	0.008
BP3	0.96	0.002	0.15	0.156
CB1	0.88	0.018	1.17	0.012
CB2	0.81	0.002	0.23	0.462
CB3	0.86	0.002	1.09	0.002

Average taxonomic diversity (avgTD) and variation around the mean taxonomic diversity (varTD) and their *p*-values of original microbial communities in Blackford Pond (BP1, BP2, BP3) and of the microbial communities in the presence of Cold Bokkeveld (CB1, CB2, CB3).

the sole external nutrient and energy source to the community. This shows that meteoritic infall of carbonaceous chondrite can support stable anaerobic microbial communities by providing the community with a nutrient and energy source.

Growth was monitored on LB agar plates, as minimal M9 media plates were found not to support growth. Given the richness of the LB medium, the cell counts are likely to be an underestimation. Additionally, we investigated the use of light microscopy and fluorescence microscopy as a tool for visualization of the cells. However, the meteoritic material obscured the microbial cells in light microscopy, and due to autofluorescence of the meteoritic material, we were unable to distinguish the microorganisms from the meteoritic powder.

To verify the observed microbial growth in the presence of native meteorite, several controls were performed. They showed that growth was significantly less in the absence of meteorite and that the mere presence of a surface (*i.e.,* glass beads) did not stimulate growth. The presence of some growth in the absence of meteorite could be explained by the fact that the inoculum was taken from a microbial community growing on Cold Bokkeveld at late exponential phase. The inoculum was not washed prior to inoculation to prevent cell damage. This could have resulted in the carryover of some meteoritic and biological nutrients to the control.

Our controls also demonstrated that the meteorite contained no indigenous microorganisms capable of growth under the conditions in the experiment, showing that the community selection effects we observed are caused by the meteorite acting on the original microbial community without exogenous microbial input.

In the microcosms containing native meteorite, the estimated biomass contained 0.3 wt % of the carbon present in the meteorite in the batch cultures, whereas previous research had shown a usage of up to 3% of meteoritic carbon (Mautner *et al.,* 1997). If we assume that this carbon came from the indigenous carbon-containing compounds in the meteorite, then this demonstrates that a small amount of carbon (1.5 mg) from carbonaceous chondrites can sustain significant cell numbers in an anaerobic microbial community.

The fact that the microbial community uses only 0.3% of the meteoritic organic matter raises the question of why only such a small percentage was used. One reason could be that the other 99.7% of the organic matter could not be accessed by the microorganisms, for example if it is not metabolizable. Alternatively, competition for surface area or space or a buildup of toxic waste products in the batch cultures could have limited further microbial usage of the organics. As opposed to the closed system in the batch cultures, the open, continuous environments during early Earth could have diffused soluble nutrients and waste products, which could have resulted in the use of a higher fraction of meteoritic organics.

Microbial growth in the presence of pyrolyzed meteorite did not occur, and the cell concentrations decreased over time. The final cell concentration was significantly lower than the final cell concentration in the presence of native meteorite and was statistically not different from zero. Although the final cell concentration in the presence of pyrolyzed meteorite was not statistically different from the concentration in absence of the meteorite (p-value = 0.20), the results indicate that the pyrolyzed meteorite had a negative impact on microbial growth. This is based on the overall decrease in microbial cell concentration in the presence of pyrolyzed meteorite and the increase in cell concentration in the absence of meteorite. Moreover, the final cell concentration in the absence of meteorite was statistically different from zero (p-value = 0.042). These results indicate that the presence of pyrolyzed meteorite is inhibitory to the growth of the microbial community. The inhibitory effect of dry and hydrous pyrolyzed carbonaceous chondrites has previously been shown in *Flavobacterium orzihabitans,* and of hydrous pyrolysis in *Pseudomonas maltophilia* (Mautner *et al.,* 1995). The inhibitory effect has been hypothesized to be caused by chemical inhibition, or by trapping the microorganisms on the large surface area of the powdered meteorite (Mautner *et al.,* 1995). In the present study, the surface area of pyrolyzed meteorite was presumed to be the same as that of the native meteorite, as the meteoritic material of both conditions had been ground by using the same procedure. As we do not observe an inhibitory effect of the native meteorite, we suggest that the surface area was not causing the inhibitory effect observed in the pyrolyzed meteorite. Therefore, we can focus on the hypothesis of chemical inhibition.

Chemical inhibition could occur by chemical changes caused by the pyrolysis. Pyrolysis caused a visual change in the color of the meteoritic material from black to light brown, accompanied by an increase in pH and an 8% weight loss in the powdered meteorite, all indicating chemical changes during pyrolysis. Weight loss by pyrolysis had previously been observed by Remusat *et al.* (2005), who observed 26 and 30 wt % weight loss in the carbonaceous chondrites Murchison and Orgueil respectively by vacuum pyrolysis at 600°C. The significantly higher pH in the microcosms containing pyrolyzed than native meteorite—which had a similar pH to the inoculum and the pond soil—could have induced stress to the microbial community and inhibited microbial growth. However, the pH in the pyrolyzed meteorite containing microcosms decreased significantly over the course of the experiment, whereas the pH in the other conditions did not change significantly. Further research on the elemental composition of the native and pyrolyzed meteorite could reveal more regarding leaching of the material into the liquid, potentially causing these pH changes. When investigating chemical changes by pyrolysis, different essential nutrients for microbial life should be taken into account. First, pyrolysis will have changed the chemical composition of the organics. The pyrolysis will have evaporated the thermally labile fraction of the organic matter, which is potentially the most biologically available, while the thermally stable fraction may have been altered to graphite-like matter (Komiya *et al.,* 1993). Next to carbon, this stable organic matter is a source of hydrogen, oxygen, nitrogen, and sulfur, whose microbial accessibility will also have changed by pyrolysis (Pizzarello *et al.,* 2006). Second, pyrolysis will have chemically changed some of the sulfur- and nitrogen-containing molecules in the meteorite. During vacuum pyrolysis at 600°C of the Murchison and Orgueil meteorites, some meteoritic sulfur was converted to H_2S and SO_2, while 75% of the sulfur remained in the insoluble

residue (Remusat *et al.,* 2005). To detect nitrogen-containing gases during vacuum pyrolysis, the temperature had to be increased from 600°C to 900°C (Remusat *et al.,* 2005). This indicates that the pyrolysis in the present study could have been of low enough temperature to keep the nitrogen present in the residue. The majority of meteoritic sulfur and nitrogen will thus still be present in Cold Bokkeveld after the pyrolysis of the present study, although sulfur- and nitrogen-containing molecules may have been chemically altered. Third, iron-containing molecules will have been chemically altered by pyrolysis. This was visually observed by the change in color of the meteorite powder from black to light brown after pyrolysis, indicating the removal of organic material and the formation of iron oxides. Although iron oxides can be tolerated in millimolar concentrations by iron-reducing organisms (Bird *et al.,* 2013), the formation of iron oxides may indicate the occurrence of chemical reactions with oxygen during pyrolysis. We cannot rule out the production of free radicals and oxygenated species by pyrolysis under atmospheric conditions, although these could also be produced during anoxic pyrolysis. Free radicals form for example during the production process of biochar, where biomass residues are pyrolyzed under oxygen-limiting conditions (Odinga *et al.,* 2020). Additionally, trace compounds could have an accumulating toxic effect over time. Further analysis on the chemical composition of the meteorite would be necessary to explain the chemical changes of pyrolysis resulting in the inhibitory effect of pyrolyzed meteorite. The inhibitory effect of pyrolyzed material indicates that geothermal activity on early Earth could have chemically altered meteoritic material, thereby transforming the material from a resource to inhibitory material. This indicates that the environmental conditions on early Earth's surface could have determined whether meteoritic material was beneficial or deleterious to early life.

The stable microbial community was established by incubating a microbial community from Blackford Pond for 2 months in microcosms containing water and powdered Cold Bokkeveld. After 2 months, the community was transferred to a fresh microcosm, and this was repeated after a further 2 months. In total, the stable community was formed over 6 months. Despite time limitations preventing us incubating the community for a longer period, we believe that this time period is long enough to establish a community containing microorganisms that can survive and grow in the presence of Cold Bokkeveld.

4.1. Cold Bokkeveld filtered the microbial community

We were unable to extract DNA directly from the meteorite, which suggests that DNA was either absent or below detection. This implies the lack of significant bacterial contamination. As microorganisms are known to colonize meteorites (*e.g.,* Steele *et al.,* 2000; Tait *et al.,* 2017), this indicates that the meteorite used in the present study has been well preserved and curated, thereby minimizing microbial contamination. This was confirmed by the lack of microbial growth in the nonbiological microcosms containing native meteorite (control C).

DNA extraction of the microbial community at late exponential phase on Cold Bokkeveld allowed us to investigate the community composition after colonization by microbes. This study focused on the bacterial community, and we would recommend further research to investigate archaea and fungi as well. Phylogenetic and diversity analyses showed a distinct difference between composition of the original community in Blackford Pond and the cultured community in the presence of native meteorite. Only a subset of the Blackford pond bacterial community was able to grow in the presence of the carbonaceous chondrite, and that subset was of closely related OTUs in distinct groups that were more distantly related from each other than expected by chance. This suggests that the community from Blackford Pond was filtered by the environment of Cold Bokkeveld microcosms, a process referred to as habitat filtering (Keddy, 1992; Pontarp *et al.,* 2013). Habitat filtering is a deterministic process where the environment tends to select for organisms with tolerance for that environment, and thus can lead to organisms with similar abilities and traits, and a phylogenetically ''clustered'' tree structure (*i.e.,* more closely related species). Some habitat filtering was present in the pond communities. However, habitat filtering was present at a much larger extent in the meteorite samples. Meteorite samples had an average taxonomic distinctness (avgTD) that was lower than expected, and two of the three samples had a high variation of taxonomic distinctness (varTD). This represents a phylogenetic community structure that is clustered, with OTUs that are more phylogenetically related occurring within the meteorite samples than if the community were structured by random from the original pond samples. The broad variation of this filtered community was more pronounced in CB1 and CB3 than in CB2, demonstrating that the variation between relatedness of the taxa in CB1 and CB3 was higher than in CB2. This high variation indicates that CB1 and CB3 contain closely related species in lower taxonomic levels but have greater divergence at higher taxonomic levels.

The habitat filtering in the meteorite communities may suggest that nutrients were not a limiting factor in the meteorite microcosms but rather that early establishment of microbial communities on new meteoritic material is driven by which species can survive and grow on the nutrients that were available on the meteorite and under anaerobic conditions (habitat filtering) from the larger pool of possible microbial community members (*i.e.,* Blackford Pond communities). Competitive exclusion (*i.e.,* overdispersion of taxonomic relatedness and thus higher than expected avgTD) can occur in oligotrophic environments where species that are closely related are competing intensely for the same resources and one or few species outcompete other closely related species for those resources (Begon *et al.,* 1996). In such cases, the community assemblage is constructed of more distantly related species, with less overlap in habitat requirements. On meteorites, with time however, as available carbon and nutrients decline, competitive exclusion might increase avgTD or help push species to adapt, creating novel niche space, as has been suggested to explain high diversity in oligotrophic lava caves (Barton, 2015).

Community composition analysis showed how some OTUs representative of families that were present in small abundance in the pond samples became highly abundant in the presence of the meteorite. Additionally, five families and 67 OTUs were found in the presence of the meteorite, which

were not found in the source community from the pond sediment. This includes OTU JQ086796.1.1478, which was highly abundant in CB1 but not in any other sample. This may be explained by two possibilities. First, these OTUs may have been present in very low abundance in the pond and were not detected by 16S rRNA sequencing. Second, these families could have been contaminants from the laboratory or meteorite material. We did not, however, observe any growth on the nonbiological controls of the meteorite only (control C), which were handled the same and exposed to the same laboratory conditions, minimizing the chances of this second possibility to have played a large contribution.

Prevalent in the microbial community were the anaerobic respiring Deltaproteobacteria Geobacteraceae and Desulfuromonadaceae. Many species in these families are known to use elemental sulfur or ferric iron as electron acceptor, while organic compounds are used as electron donors and carbon sources. Important metabolisms of Geobacteraceae and Desulfuromonadaceae are anaerobic dissimilatory iron and sulfur reduction. Further research investigating the presence and activity of these metabolisms could include functional gene sequencing and chemical analyses on growth substrates. These metabolisms are thought to have evolved early in the history of life, and potentially have been present in the universal ancestor, as microorganisms closely related to the last universal common ancestor can reduce ferric iron and sulfur compounds (Vargas *et al.,* 1998; Canfield and Raiswell, 1999). Therefore, oxidized iron, sulfur, and organic material from meteorites could have been a source of substrates for iron and sulfur reduction in early life around the Late Heavy Bombardment.

Microorganisms associated with the sulfur and iron cycle have also been reported on L type ordinary chondrites found in the Nullarbor plain in Australia, albeit in low abundance (Tait *et al.,* 2017). The low abundance of these types of organisms can be explained by the different chemical composition of L type ordinary chondrites compared to carbonaceous chondrites (Rubin, 1997; Tait *et al.,* 2017). The fact that sulfur- and iron-reducing microorganisms have been found on meteorites both in enrichment experiments and in natural habitats indicates that meteoritic sulfur and iron could provide partial redox couples and thus serve as a microbial energy source.

Our data show that, consistent with microbial diversity observed in natural meteorites, carbonaceous chondrites supported a highly selective and specific anaerobic community. In particular, sulfur and iron phases seem to select for taxa that are capable of using these compounds in redox couples to conserve energy for growth. Although iron and sulfur compounds, including their oxidized redox states, would have existed in indigenous early Earth materials, this work shows that in the localized presence of carbonaceous chondrite, specific communities are favored and that meteorites on early Earth might have created islands of filtered microbial diversity, provided with their own extraterrestrial nutrient and redox supplies.

Our results are applicable to other young planets. As planetary formation leaves remnant material, we would expect meteoritic infall on any young anoxic planet hosting life. The influence of meteoritic material would especially be important on planets with low surface nutrient availability, where meteoritic material could provide localized nutrients on the planet in locations where it landed. Thus, this work is applicable to understanding the emergence and nature of putative microbial ecosystems on any young planet.

Finally, we note that our work has application to asteroid processing to support a long-term human presence in space. We have shown that specific types of organisms, in particular sulfur- and iron-reducing taxa, can be sustained on carbonaceous meteoritic material alone and can mobilize nutrients from these materials. These taxa, perhaps isolated by using similar experiments to those described here, could be genetically modified, or their metabolic processes could be used in synthetic biology to create new types of organisms that can be sustained on carbonaceous chondrites with no external applied nutrients, and without oxygen. Supplemented with particular metabolic pathways, they might be used to biomine asteroids or transform asteroidal material into useful gases, fuels, and other products to support human settlement.

5. Conclusion

In conclusion, this study shows that the carbonaceous chondrite Cold Bokkeveld can host an anaerobic microbial community dominated by the Deltaproteobacteria Geobacteraceae and Desulfuromonadaceae that can access nutrients and energy sources from the material. The meteoritic material favored a microbial community that contained sulfur- and iron-cycling organisms, showing that it can support microbial growth and select for specific taxa by habitat filtering. The native chondrite supported microbial growth, while the pyrolyzed chondrite was inhibitory and resulted in a decline in microbial cell concentration. This indicates that pristine meteoritic material could have supported microbial communities on early Earth, but the environment on Earth where the material landed would have been crucial for the effects on life, as geothermal activity may have transformed meteoritic resources into inhibitory compounds. These results show how the accumulation of carbonaceous chondrites on early Earth could not only have supported early anaerobic microorganisms but would have selected for specific taxa capable of accessing the nutrients and partial redox couples in these extraterrestrial materials. These results are applicable to other young, bombarded planets that could potentially harbor life, indicating that meteoritic material could locally influence the habitability of young, bombarded planets. Our work also shows the potential to use anaerobic microorganisms to process carbonaceous chondrites for future human settlement using the meteoritic material as the nutrient and energy source in space.

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Authorship Confirmation Statement

ACW and CSC contributed to conception and design of the study. ACW carried out the experimental work. ACW and RP performed data analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

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Abbreviations Used

avgTD = average taxonomic distinctness
CFU = colony-forming unit
nMDS = non-metric multidimensional scaling
OTUs = operational taxonomic units
PD = phylogenetic diversity
SP-SDS = single plate-serial dilution spotting
varTD = variation around the mean taxonomic distinctness