Comparative Microbiology of Typical and Elevated Temperature Landfills

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Executive Summary

While the basic microbial processes of anaerobic decomposition in landfills have been well-known for decades [Halvadakis et al., 1983; Barlaz et al., 1989], studies of microbial diversity using modern sequencing technologies, which make it possible to cheaply and efficiently catalog microbial diversity, are rare [Stamps et al., 2016; Lu et al., 2012]. Due to the current interest in 'hot' landfills, the opportunity exists to study microbial ecology in tandem with environmental data to address deviations from standard microbial composition. Properly functioning landfills are largely unaffected by the heterogeneity of the waste [Spokas et al., 2011; 2015], however, environmental heterogeneity provides a host of potential ecological functions that may be directly or indirectly contributing to the formation of "hot landfill" conditions and the profile of gas compositions observed. Investigations of microbial pathways in "exotic" locations such as hydrothermal and "deep dark" subsurface systems suggest a variety of microbial pathways that may also be functioning in 'hot' landfills, perhaps affecting gas compositions [Meyer-Dombard et al., 2015]. We proposed a complementary field and laboratory study with the goal of aiding in the determination of the causes and indications of hot landfill development, towards distinguishing between biotic and abiotic processes responsible. Our original project objectives had to necessarily be modified due to Covid-19 restrictions on laboratory and field work. Our modified objectives were to:

1. generate baseline microbiological data that can inform ongoing work,
2. investigate whether waste stream modifications have the ability to impact 'normal' microbial community structure,
3. and identify microbial processes that may directly/indirectly enhance the reconfiguration of microbial community composition that could lead to the suppression or prevent flourishing of methanogenic activity.

This pilot project was conceived to coordinate with the previously funded EREF project "Understanding and Predicting Temperatures in Municipal Solid Waste Landfills." Our underlying argument was that landfill operators may currently monitor chemical and physical properties of landfills, but not the biological component. Arguably, the modern management of landfills is based on the fundamental knowledge of the microbiology of typically operating landfills. We should therefore expect that much could be gained by understanding the subsurface microbiology of atypical landfills as well. This was not economically viable in the past. However technology is now advancing rapidly, and microbiological analysis is increasingly cheaper, making the timing of this proposal fortuitous. We argue that, in addition to the newly-achievable economic feasibility of microbiological testing at typical landfills, it will be ultimately beneficial to monitor the microbiology of both typical and atypical landfills for improved understanding of fundamental processes at both types of sites. Improved process understanding can also assist with developing improved site monitoring for critical parameters as well as improved engineering designs and controls guided by site-specific microbiological information when and where atypical fluid and gas chemistries develop.

We addressed the structure and composition of landfill communities that have been exposed to higher than normal temperatures, and whether it is possible that there are ties between modern waste streams and the environmental conditions that allow high

temperatures to form at depth in a landfill. Our original plan included both laboratory experimentation and field-based work. The latter was not possible due to restrictions during Covid-19 caused shutdowns of the University, laboratories, and field access. Our completed work includes a survey of the microbial community of a higher than normal temperature landfill in the southeastern United States, and experimental microcosms that explored the impact of the addition of construction and demolition waste and CECs (Chemicals of Emerging Concern) such as antibiotics and microplastics to the landfill ecosystem.

This work showed that community structure of higher temperature landfills are distinctly different than more normal temperature locations. Both Bacteria and Archaea were impacted by the high temperatures. Further, Archaeal communities may be suppressed in lower temperature areas due to lack of production of metabolic substrates by suppressed Bacteria. This work also explored the impact of amendments mimicking C&D waste and CEC to landfill ecosystems. Specifically, C&D waste was approximated using additions of Na_2SO_4 and $Fe(OH)_3$ (mimicking iron-bearing and wall board C&D waste). CECs explored included a suite of the most common antibiotics and three types of microplastics. The addition of $Fe(OH)_3$, antibiotics, and microplastics changed the composition of microbial communities in simulated landfill environments the most significantly. Iron metabolizing organisms were stimulated, and interactions between iron and sulfur biogeochemistry may have produced an excess of sulfide, suppressing some groups of organisms. Addition of antibiotics and microplastics promoted changes in the microbial communities. Our results overall indicate that the increase of antibiotics in the waste stream with municipal solid waste landfills as the terminus will dramatically change the bacterial community structure, for at least three weeks following application. The addition of microplastics had a delayed but notable impact on community diversity.

In total, eight undergraduates participated in this research. Three graduate student theses have emerged from the work. My students and I have reported our results in eight conference presentations. Jean Bogner, my Ph.D. student Judy Malas, and I have published a review paper on the use of high throughput sequencing on MSW samples. Four additional manuscripts are in preparation from the results of the graduate students' work, and will be submitted as part of the completion of their theses.

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1 Introduction

1.1 Landfills as Ecosystems

Anaerobic decomposition of landfilled waste, similar to anaerobic decomposition of buried organic carbon in terrestrial ecosystems (e.g. wetlands), occurs via a series of hydrolytic, fermentative, acidogenic, acetogenic, and methanogenic microbial reactions (Halvadakis et al., 1983; Barlaz et al., 1989). Major intermediates include carbon dioxide (CO_2,) hydrogen (H_2,), acetate (CH_3COOH) and other carboxylic acids. The end product, landfill gas, is a biogas consisting of methane (CH_4), CO_2, and a wide range of minor and trace components. Biogenic methanogenesis occurs via either (1) reduction of CO_2 with H_2 or (2) cleavage of acetate into CH_4 and CO_2, both of which occur in landfill settings (Bogner et al., 1996). Landfill CH_4 can be aerobically oxidized in cover soils by indigenous methanotrophic microorganisms (Scheutz et al., 2009). Importantly, in anaerobic landfill settings, new work is exploring the possibility that CH_4 can also be anaerobically oxidized via several potential microbial pathways including reactions coupled with sulfate reduction or metal oxide reduction, by nitrite dismutation, or disulfide disproportionation (Joye, 2012; Caldwell et al., 2008; Parsaeifard et al., 2020; Jiang et al., 2022; Xu and Zhang, 2022).

Depending on waste composition, cover soils, hydrology, thermal regime, and other factors, numerous diverse microbial and abiotic reactions can occur in landfill settings, including nitrate, sulfate and iron reduction. Many of these reactions also occur in 'exotic' deep subsurface settings. For example, in deep marine environments, secondary carbonate deposits can be formed as a biproduct of anaerobic CH_4 oxidation coupled with sulfate reduction (e.g., Drake et al., 2015). In landfills, the carbonate crusts sometimes observed in leachate collection systems may also result by this mechanism (Rowe et al., 2000; Mulla Saleh, 2006). The similarity in microbial function and environmental conditions between landfills ('hot' or typical) and the deep biosphere is unexplored.

1.2 How Are Landfills Relevant to the Study of the "Deep Biosphere?"

We argue that landfills, whether operating typically or at high temperature, are analogous to many natural ecosystems in the deep, dark biosphere, and as such have intrinsic value to the wider Geobiology community. The 'deep biosphere" is a concept that has excited the Geobiology community in the last decade - the discovery that microorganisms can occupy the subsurface to astonishing depths, and thrive there, has had a huge impact. The Earth's subsurface has been shown to be not only habitable, but a thriving microbial ecosystem. Recent estimates place the extent of Earth's subsurface biosphere as deep as 5–10 km, with a capacity to accommodate up to 2×10^{14} tons of biomass (Jorgensen, 2012; Kallmeyer et al., 2012). The high end of these estimates equates to more biomass and biodiversity than in Earth's surface environments combined. The role that subsurface habitats play in global biogeochemical cycling has not been described, but is certain to be substantial. We know little about carbon cycling, nitrogen cycling, or any other biogeochemical cycling that takes place in this subsurface biosphere or how these may impact surface processes.

The deep biosphere can be simply defined by any ecosystem that is kept physically separate, if not 100% isolated from, the surface biosphere. We study deep biosphere ecosystems by drilling into the subsurface, visiting the subsurface via caves and mines, and by

sampling deeply-sourced fluids (such as hot springs) at the surface. We also argue that the deep biosphere can include man-made ecosystems such as landfills.

Deep biosphere ecosystems typically lay at the extremes of temperature or pH, may be depleted in resources such as carbon or nitrogen, may have additional environmental stressors such as localized contamination (e.g., elevated trace element concentrations), and operate without the supplement of photosynthesis-derived surface metabolites. This later condition also means the deep biosphere is a 'dark biosphere,' and is often able to function completely divorced from energy derived from sunlight. In other words, carbon sources in the deep, dark biosphere are not being supplied directly by photosynthetic organisms 'fixing' CO_2 into biomass, but rather from other autotrophic or even heterotrophic functions. Methanogenesis is a good example - in the deep, dark biosphere, methanogenesis may function from diagenesis of carbon-bearing sedimentary rock combined with abiotically produced hydrogen gas. Therefore, the connection to carbon produced in surface ecosystems may be removed in time by millions of years. In addition, the deep, dark biosphere is often saturated and anaerobic, thrives on energy provided by 'lithotrophic reactions' - examples are the production and consumption of methane or sulfate reduction - and requires interaction with mineral surfaces.

Diversity of microorganisms is typically determined using an examination of genetic diversity, by means of comparing the genetic sequence of the '16S rRNA gene' between organisms in a given sample. A wealth of information was obtained by this method, but a modern version of sequencing technology has increased this knowledge base by orders of magnitude. Traditional sequencing methods may yield a few thousand DNA sequence 'reads' but new 'high throughput' sequencing techniques have enabled yields of gigabases of reads per sample. Because of this, examining genetic diversity in deep, dark biosphere samples can now also include more than just the 16S rRNA gene. We now also sequence 'metagenomes' of a given sample - essentially partial genomic sequencing from all but the least numerous microorganisms in the sample. This method yields not only a phonebook of 'who's home' but also a profile of metabolic capabilities found in the community. It is a powerful and game changing tool. Examples of these sequencing methods being applied to solid waste problems include Stamps et al., (2016), who found that the microbial composition of 'typical' municipal waste sites varies, and depends on several factors, but the variation could be correlated most strongly with the concentrations of chloride and barium, rate of evapotranspiration, age of waste, and the number of detected household chemicals. Lu et al., (2012) examined the effect of leachate contamination in neighboring aquifers and found various geochemical parameters had a significant impact on the subsurface microbial community structure. These new generation, high throughput methods have much potential in the field of solid waste management, but are seldom applied to 'hot' landfill settings.

1.3 Conditions in Elevated Temperature ('Hot') Landfills

This project focused on the microbial ecology and geochemistry of landfill systems over a thermal gradient. In general, thermal regimes in landfills are typically "mesophilic", e.g., ranging from about 25-45 ^{o}C. However, internal landfill temperatures can infrequently range up to very high values approaching 100 ^{o}C. From field observations at 'hot' sites and laboratory scale studies (e.g., Chae et al., 2010), it is well known that rates for the terminal methanogenesis step can be suppressed at both high temperatures and low pH, resulting in buildup of intermediate

gaseous products such as H_2 and CO_2. At some 'hot' sites, carbon monoxide (CO) is also observed; this may result from subsurface combustion under limited O_2 conditions, but CO can also be produced by diverse microbial pathways without any subsurface combustion (e.g., Ragsdale, 2004).

1.4 Potential Microbial Interactions in 'Hot' Landfills

In general, methanogenesis in most landfills is largely unaffected by the heterogeneity of the waste [Spokas et al., 2011; 2015]. However, environmental heterogeneity provides a host of potential ecological functions that may be directly or indirectly contributing to the formation of 'hot' landfill conditions and the profile of gas compositions observed. Selected waste materials at specific sites (e.g., aluminum dross-mediated exothermic abiotic reactions) are capable of raising internal landfill temperatures, suppressing methanogenesis, and resulting in a biogas product enriched in H_2 and CO_2. Investigations of microbial pathways in "exotic" locations such as hydrothermal and deep, dark subsurface systems suggest a variety of microbial pathways that may also be functioning in 'hot' landfills, perhaps affecting gas compositions [Meyer-Dombard et al., 2011; 2015]. These processes may include: anaerobic oxidation of CH_4, drawing down CH_4 (via sulfate reduction, metal oxide (Fe, Mn) reduction, disulfide disproportionation) [Joye, 2012]; aerobic/anaerobic oxidation of CO, producing CO_2; development of uncharacteristic pH conditions (e.g., via metabolizing S- and Fe-bearing compounds); and aerobic break-down of organic compounds in groundwater-saturated waste, providing a gas mixture rich in CO_2, H_2, and CO. It is thus important to expand the 'hot' landfill discussion to consider identified microbial pathways in similar extreme earth environments such as hydrothermal and deep, dark subsurface systems [Shock et al., 2010; Meyer-Dombard et al., 2015].

While the basic microbial processes of anaerobic decomposition in landfills have been well-known for decades [Halvadakis et al., 1983; Barlaz et al., 1989], studies of microbial diversity using modern sequencing technologies, which make it possible to cheaply and efficiently catalog microbial diversity, are rare [Stamps et al., 2016; Lu et al., 2012]. Due to the current interest in 'elevated temperature' landfills, the opportunity exists to study microbial ecology in tandem with multiphase geochemical indicators (gaseous, liquid, solid) to address deviations from standard microbial pathways. Properly functioning landfills are largely unaffected by the heterogeneity of the waste [Spokas et al., 2011; 2015], however, environmental heterogeneity provides a host of potential ecological functions that may be directly or indirectly contributing to the formation of 'hot landfill' conditions and the profile of gas compositions observed. Investigations of microbial pathways in 'exotic' locations such as hydrothermal and "deep dark" subsurface systems suggest a variety of microbial pathways that may also be functioning in elevated temperature landfills, perhaps affecting gas compositions [Meyer-Dombard et al., 2015]. Our work implemented a complementary laboratory study to the "Understanding and Predicting Temperatures in Municipal Solid Waste Landfills" project previously funded by EREF. To this end, our goal was to aid in the determination of the causes and indications of hot landfill development, towards distinguishing between biotic and abiotic processes responsible.

Our research utilized laboratory 'microcosms' to investigate the response of synthetic landfill communities to additives that mimicked realistic modern waste streams. We also

investigated the microbial composition of soils from a landfill with higher than normal temperatures using high throughput sequencing technologies. A full review of the current application of these technologies to landfill ecosystems can be found in Meyer-Dombard et al., 2020.

2 RESULTS AND DISCUSSION

2.1 Microbial diversity and composition of select elevated temperature locations in a Southeastern US landfill

The taxonomy of discrete samples within three separate bore holes of a landfill in the SE United States was determined by 16S rRNA sequencing (Fig. 1). Data shown are for depths at or below 10m. Bore hole 'A' represents a less elevated temperature location, with temperatures at depth ranging from 54-71°C. In comparison, bores 'B' and 'C' reach temperatures up to 87°C at some depths. Figure 1 reveals that the dominant taxa for 'normal' temperature and elevated temperature landfill soils are similar – most depths are dominated by Bacilli and Clostridia. However, there are typically more Bacilli and significantly more Actinobacteria and Proteobacteria in depth profiles for the higher temperature bore holes. Further, Archaea (methanogens), are absent from the high temperature profiles below 10m depth. A non metric multidimensional scaling analyses of these data (Fig. 2) reveal that the samples >75°C cluster together, and that most of the samples <75°C cluster together. This indicates that microbial communities are at least in part dependent on temperature regimes in landfill soils, and that temperature is likely suppressing groups such as Clostridia and Archaea and encouraging growth of Bacilli, Actinobacteria, and Proteobacteria that are more resistant to increases in temperature in these ecosystems. These results are somewhat unsurprising given general observations that elevated temperature landfills do not produce the expected volume of methane. However, these data also indicate that other ecological functions are likely impaired as groups of Bacteria are suppressed by higher temperatures.

Relationships between the diminishing archaeal population and shifting bacterial populations are still to be determined. For example, the peak archaeal population in bore A (at 21m) was at ~63°C. Several samples in bore B were well within the range of optimum/ maximum temperatures for methane production of 65°C/77°C [Barlaz et al., 2016; Schupp et al., 2020] yet these soils did not support Archaea in significant numbers. Clearly, the overall biogeochemistry of core B did not encourage methanogenic growth, even at lower temperature zones of the bore hole. We suggest that bacterial production of acetate, CO_2 or H_2 was slowed significantly enough in higher temperature zones to also suppress methanogenesis in lower temperature zones. This indicates that temperature alone is not determining whether Archaea are able to thrive in elevated temperature landfills, and that biogeochemical variations due to non-typical bacterial populations should be considered as well. Alternatively, these depths may have experienced higher temperatures than those measured at the time of sampling, at some point in the past.

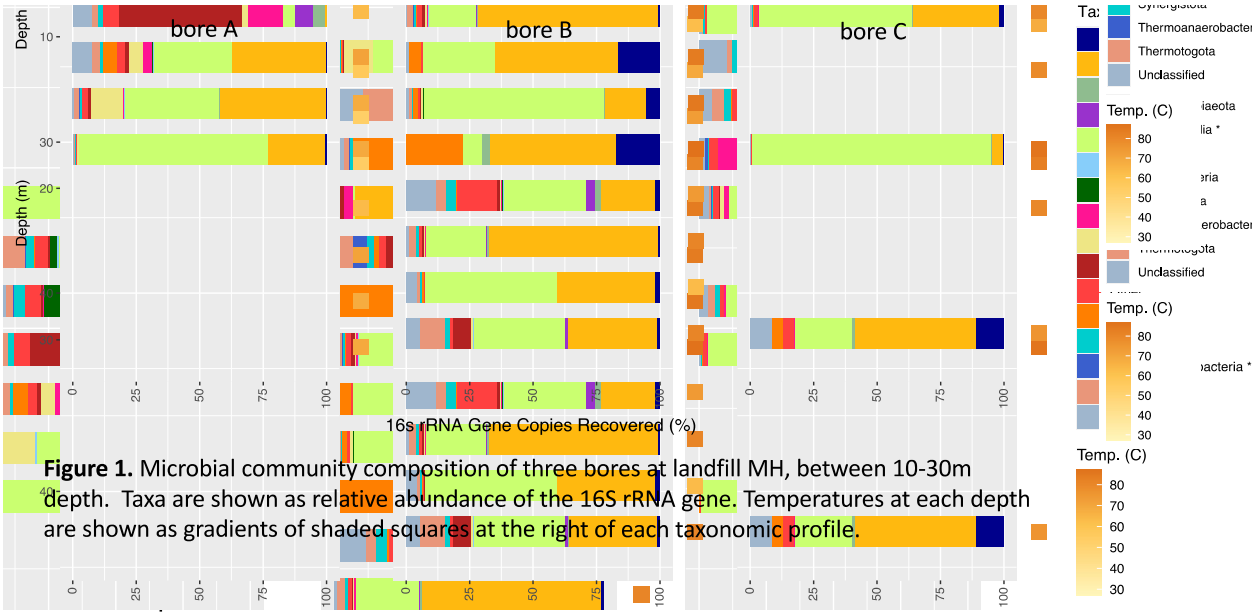


Figure 1. Microbial community composition of three bores at landfill MH, between 10-30m depth. Taxa are shown as relative abundance of the 16S rRNA gene. Temperatures at each depth are shown as gradients of shaded squares at the right of each taxonomic profile.

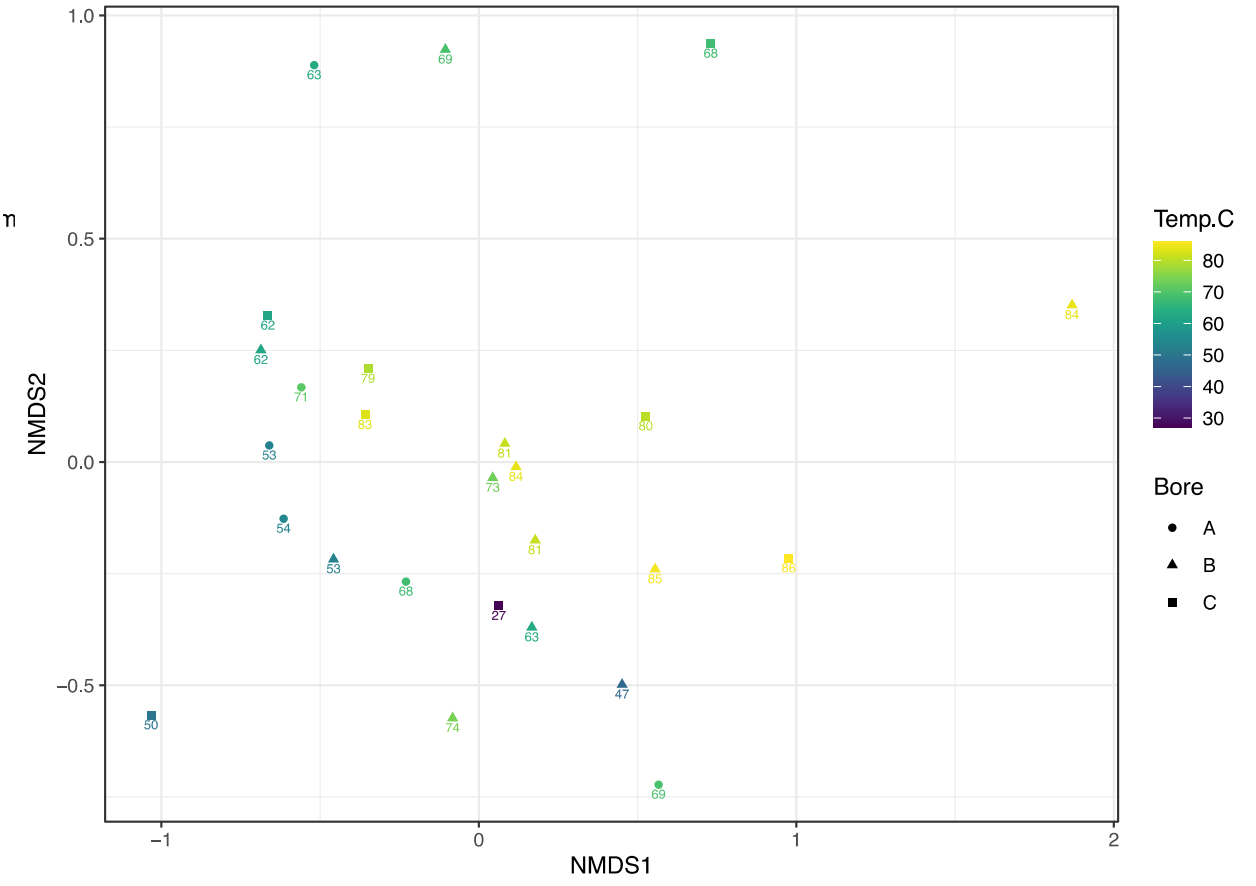


Figure 2. Nonmetric multidimensional scaling analysis of data from bores A, B, and C. Temperature of each sample is color coded, and temperatures are printed below data.

- Depth as proxy for waste age

2.2 Considerations of the impact of construction and demolition waste on microbial community composition and biogeochemistry of landfill ecosystems

A part of our proposed project was to determine if concentrations of substrates added to the waste stream could potentially alter the ecosystem biogeochemistry to the point that methanogenic activity is suppressed. To this end, we set up laboratory microcosms to investigate microbial community structure as a function of added oxidized iron and sulfate, expected components of added construction and demolition waste. The microcosms were run for 65 days, which should have been sufficient for establishment of methanogenic members of the community (as per Barlaz et al., 1989). Triplicate bottles were amended with either Na_2SO_4 or $Fe(OH)_3$ (see methods) at 44 days. Figure 3 shows the microbial community composition as a function of time in the microcosms, as determined by 16S rRNA sequencing. Shown from top to bottom are three each of control bottles (no amendments), bottles amended with $Fe(OH)_3$ (at t4), and bottles amended with Na_2SO_4 (at t4). Effectively, all 3 control bottles and all six amended bottles are identical in set up and treatment from t1-t4.

Despite this homogeneity in experimental set up, there is broad scale heterogeneity in taxa found in the 9 bottles from t1-t4. Most bottles experienced major shift in taxa between t1 and t2, and then began to somewhat stabilize as time passed, typically settling on Ruminococcaceae, Bacillaceae, and Planococcaceae or Oxobacteraceae as the dominant taxa. Control bottles 1 and 2 remained stable over time, with few changes in dominant taxa throughout the experiment – control bottle 3 however was highly variable, especially after t3. Importantly, despite the length of the experiment and anaerobic nature of the bottles, methanogens do not appear as an abundant taxon in any of the bottles. However, these experiments have provided results on the variation in the bacterial communities over time, and given that Bacteria are the primary microbial components of landfill ecosystems these data are very relevant.

Amending bottles with Na_2SO_4 was intended to mimic the addition of gypsum-bearing wall board to the waste stream. The mineral gypsum provides a ready source of alternative oxidant (SO_4^{-2}) to a landfill ecosystem. Sulfate as an available oxidant in anaerobic environments such as saturated landfills can support a variety of metabolic schemes. Among these are sulfate reduction coupled to anaerobic CH_4 oxidation, organic carbon oxidation), and oxidation of dissolved or mineral iron. Further, the product of sulfate reduction, H_2S gas, can participate in other biogeochemical reactions and microbial metabolisms further changing the local chemical profile. Sulfate levels >500mg/L have been shown to experimentally suppress methanogenesis in landfill settings [Moreau-Le Golvan et al., 2003]. In our experiments, the addition of Na_2SO_4 did not appear to overly stimulate sulfate reducers (Fig. 3). The bacterial communities did experience shifts in t5-t6 after the amendment was added, however these shifts can not be shown to be the result of the addition of the amendments as they are no more remarkable than the shifts in taxonomic profiles that occurred prior to the amendments. However, our chemical data indicate that sulfate reducers were active at the end of the experiments (figure 6). Oxidized and reduced iron concentrations in the Na_2SO_4 amended experiments were variable, but higher than the control bottles in some replicates. However, the sulfide concentration was consistently higher than in the control experiments. The simplest

explanation for this sulfide increase is increased activity of sulfate reducing taxa in the Na_2SO_4 amended experiments relative to the controls.

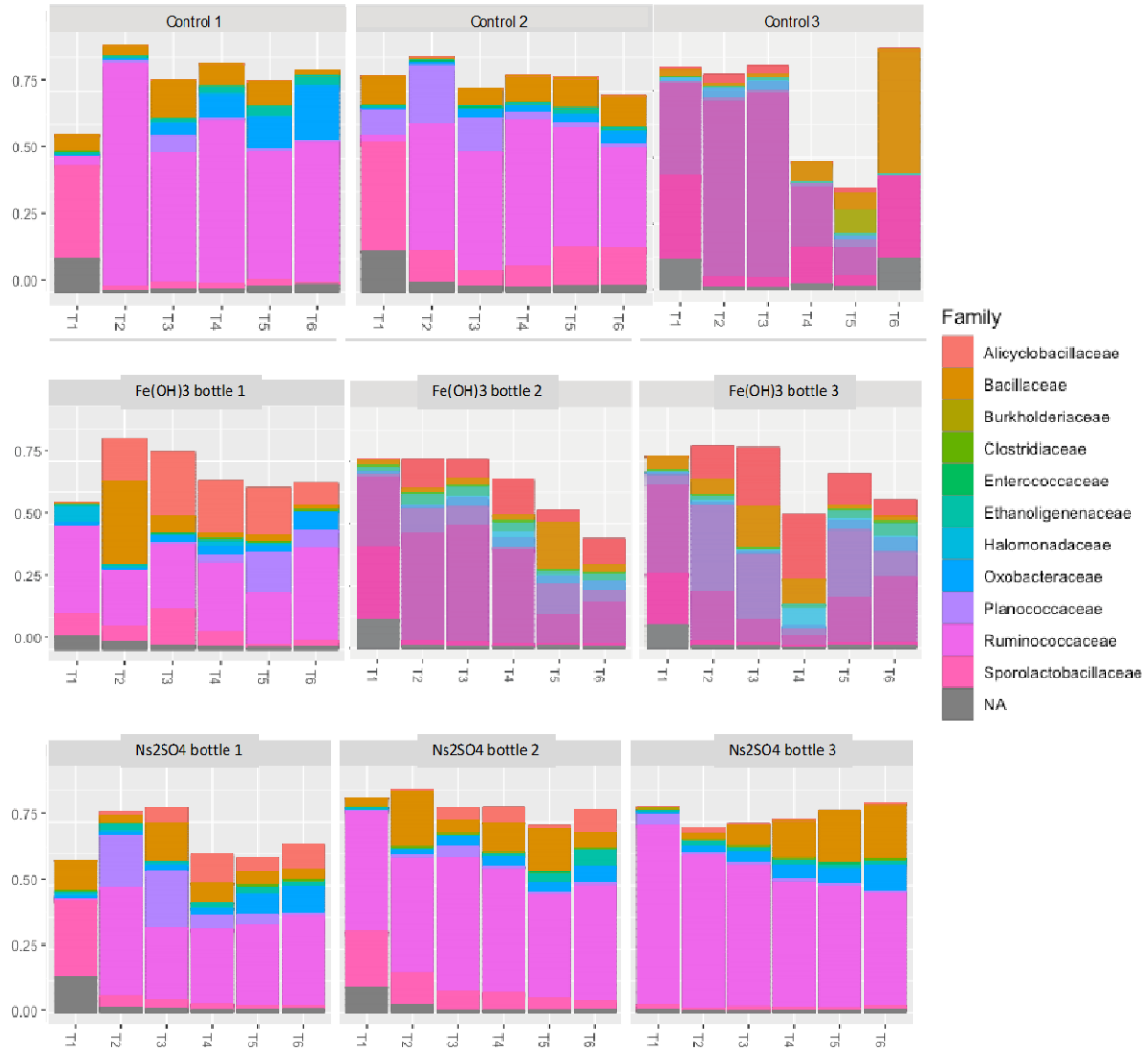


Figure 3. Relative abundance of top 200 microbial taxa in microcosms, as determined by amplicon sequencing of the 16S rRNA gene. Shown are six time points for each replicate bottle. Top row is unamended controls, middle row is experiments amended with $Fe(OH)_3$, bottom row is experiments amended with Na_2SO_4. Triplicate bottles were amended with either Na_2SO_4 or $Fe(OH)_3$ (see methods) at 44 days. T1 = 6d, T2 = 20d, T3 = 29d, T4 = 40d, T5 = 50d, T6 = 65.

The second set of experiments were amended with $Fe(OH)_3$ (oxidized particulate mineral iron), effectively analogous to rust supplied to a landfill environment in construction and demolition waste. In an anaerobic environment, oxidized particulate iron can be microbially reduced by a variety of compounds but in particular is very active with sulfur bearing compounds. The $Fe(OH)_3$ amended experiments are the most heterogenous of all three sets. Bottles 1 and 2 converged on a similar community structure before the amendment was added

at t4 (bottle 3 experienced a large shift both before and after amendment). Bottles 1 and 2 show shifts in specific taxa following the addition of $Fe(OH)_3$ (t5 and t6), namely Planococcaceae and Bacillaceae populations.

Analysis of the fluid in the microcosms at the end of the experiments was analyzed and results are shown in figure 6. The fluid in the $Fe(OH)_3$ amended experiments was significantly depleted in Fe^{+2} and Fe^{+3} relative to the control experiments. Sulfide concentrations were also lower than the controls, in some of the replicates. These results indicate that amending the experiments with $Fe(OH)_3$ activated increased metabolic activity of Fe^{+3} reducing microorganisms, releasing Fe^{+2} into the fluid. The reduced iron was then able to react with sulfide in the fluid, either abiotically or biologically, removing both from solution in some replicates.

A nonmetric multidimensional scaling analysis of the $Fe(OH)_3$ and Na_2SO_4 amended experiments is shown in Figure 5. This figure shows many microcosms converged on a similar group of dominant taxa over time (with t1 representing a large number of outliers). It further illustrates that the bottles amended with Na_2SO_4 had communities that were very similar to each other, and to the control experiments. In contrast, the $Fe(OH)_3$ experiments had highly variable communities especially in the later time points.

2.3 Considerations of the impact of the changing, modern waste streams on microbial community composition and biogeochemistry of landfill ecosystems

Our study was also interested in the effect of changing waste streams, primarily modern abundances of plastics (specifically microplastics) and antibiotics being added to landfill ecosystems. Similar to the experiments in the section above, we set up laboratory microcosms to investigate microbial community structure as a function of added antibiotics and microplastics (see methods for specific compositions). The microcosms with antibiotic amendments were run for 65 days with amendments occurring at 44 days, while the microplastic amended experiments were run for 73 days with the amendments occurring at 41 days. Figure 4 shows the microbial community composition as a function of time in the antibiotic amended microcosms, as determined by 16S rRNA sequencing, this time at the genus level of taxonomic differentiation. Again, effectively, all 3 control bottles and all three amended bottles are identical in treatment between t1-t4.

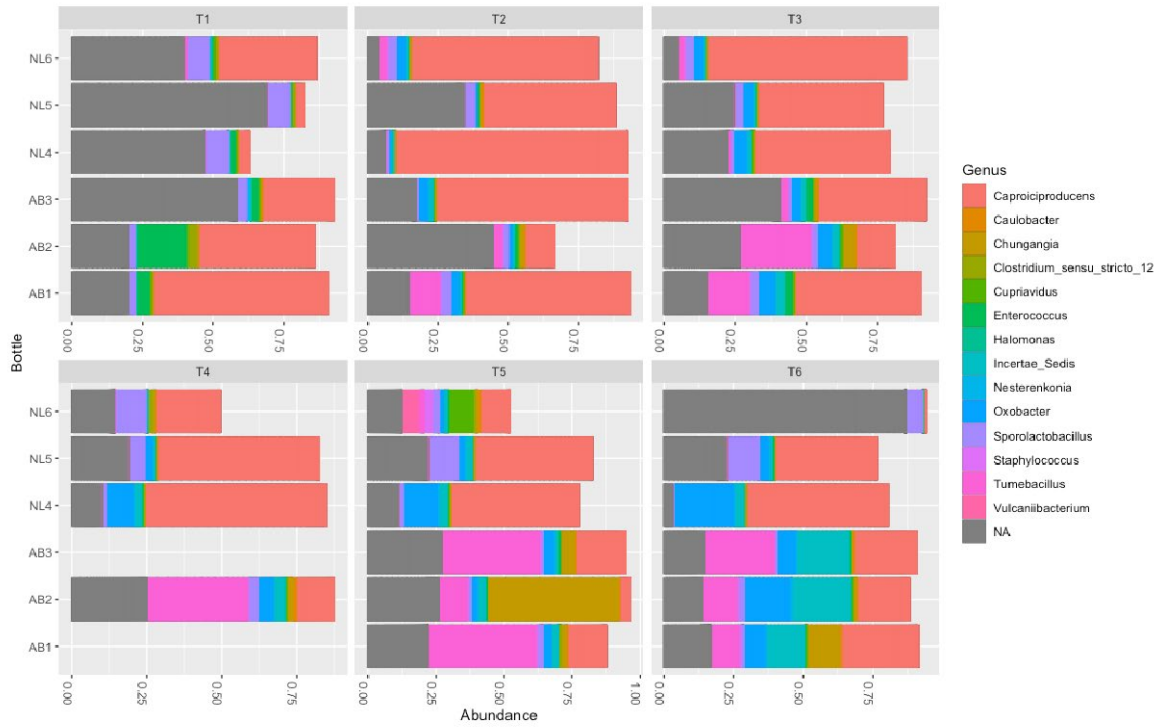


Figure 4. Relative abundance of top 200 microbial taxa in microcosms amended with an antibiotic mixture, as determined by amplicon sequencing of the 16S rRNA gene. Shown are six time points for each replicate bottle. The top three bars are the three replicates for the unamended control bottles ("NL"). The bottom three bars in each plot are antibiotic amended bottles ("AB"). Triplicate bottles were amended with antibiotics (see methods) at 44 days. T1 = 6d, T2 = 20d, T3 = 29d, T4 = 40d, T5 = 50d, T6 = 65.

Figure 4 shows that large proportions of the communities in the antibiotic experiments could not be resolved at the genus level due to non-specificity in the 16S rRNA gene sequences. However, of the resolvable genera, *Caproiciproducens*, a genus of Clostridiales, are the dominant taxa in all three control bottles from t1-t6 and all three experimental bottles between t1-4. After the antibiotic amendment was added, the microbial communities in the experimental bottle set change dramatically. The *Caproiciproducens* community was suppressed after t4, and the populations of *Tumebacillus, Chungangia, Oxobacter,* and a strain of uncertain taxonomic affiliation increased over the control populations. The only cultured example of *Caproiciproducens* was isolated from a wastewater treatment plant and produces caproic, acetic, and butyric acids [Kim et al., 2015]. The growth of this strain is enhanced when cocultured with other anaerobes that produce ethanol, acetic acid, or butyric acid. No data are available concerning the antibiotic susceptibility of *Caproiciproducens*, therefore, its suppression in t5-t6 post-antibiotic addition may either be due to antibiotic susceptibility or because the enhancing co-culture organisms were suppressed. These surviving dominant strains are largely anaerobic respirators, fermenters, and acetogens and include antibiotic resistant Bacilli. *Tumebacillus* strains have been isolated from a wide variety of natural and artificial environments (fresh and waste water, agricultural and arctic soils, animal guts), respire anaerobically, and have been shown to be resistant to norfloxacin and novobiocin but

susceptible to other antibiotics (e.g. Wu et al., 2015) including the tetracycline used here (no data available on the other antibiotics used in this

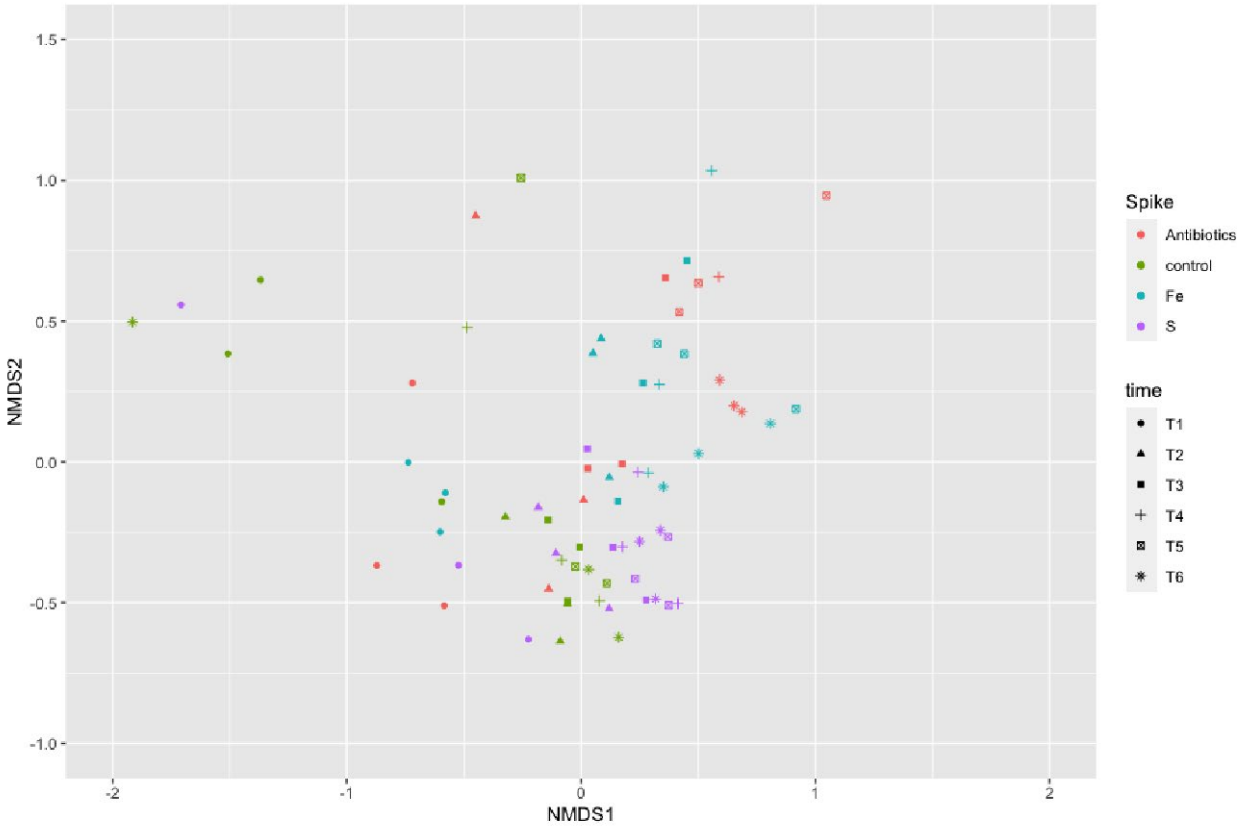


Figure 5. Nonmetric multidimentional scaling of data from the antibiotic, $Fe(OH)_3$, and Na_2SO_4 amended microcosms as well as the unamended control bottles. Each experiment is color coded, and the six sampling time points are shown in different symbols. Triplicate bottles were amended with antibiotics (see methods) at 44 days. T1 = 6d, T2 = 20d, T3 = 29d, T4 = 40d, T5 = 50d, T6 = 65.

experiment). The genus *Chungangia* only has one isolated representative in culture, *Chungangia koreensis* [Kim et al., 2012], which is a strict anaerobe. The genus *Oxobacter* also only contains one isolated strain in culture, which produces acetate among other organic acids [Krumholz and Bryant, 1985]. No data are available on the antibiotic susceptibility of either *Chungangia* or *Oxobacter*.

The NMDS analysis shown in Figure 5 also contains the data from the antibiotic amended experiments. Here, it is especially evident that the microbial communities were clearly affected after the amendment was added, clustering the data for t5 and t6 all in one area of the NMDS plot.

Figure 6 shows results of fluid analysis of redox concentrations in the antibiotic amended bottles. Sulfide concentrations are significantly higher in the experimental bottle compared to the controls. As noted above, populations of *Tumebacillus* increased after the addition of antibiotics. *Tumebacillus* is a known sulfide producer, so may be directly responsible for this change in fluid chemistry in the experimental bottles.

These results overall indicate that the increase of antibiotics in the waste stream with municipal solid waste landfills as the terminus will dramatically change the bacterial community structure, for at least three weeks following application. While we were unable to document the impact on methanogenic communities in these experiments, the interruption of normal organic degradation may lead to the suppression of methanogenesis as substrates such as acetate, CO_2, and H_2 may be produced in less abundance as the bacterial community adjusts to the shift in ecosystem roles. The impact of CECs on the microbial communities in landfills is largely unknown with few exceptions [Threedeach et al., 2012; Wang et al., 2015]. Antibiotics of

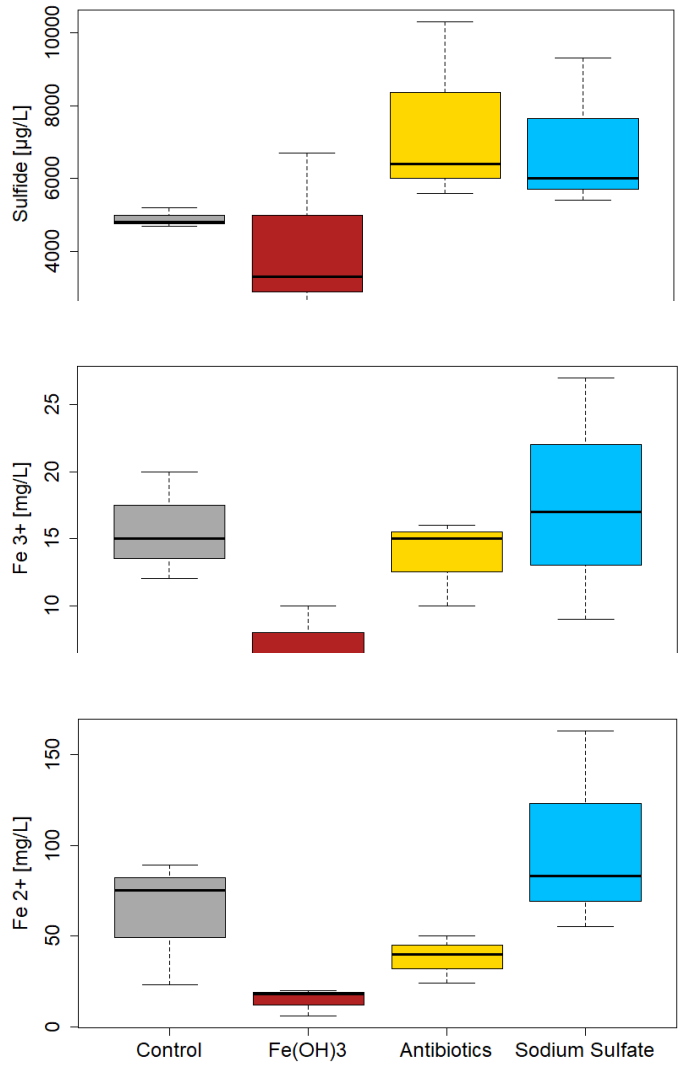


Figure 6. Results of analysis of redox chemistry of microcosm fluid at the end of the experiments. Shown are results of the $Fe(OH)_3$, Na_2SO_4 and antibiotics microcosms.

different classes have been shown to potentially increase or decrease the concentration of landfill gas emissions [Wu et al., 2017]. Greater ecological implications emerge as well - landfills may serve as a reservoir of antibiotic resistant genes, potentially generating antibiotic

resistance in proximal ecosystems where dust from landfill soils could transfer these genetic properties [Song et al., 2016].

Other modern waste stream compounds of concern include microplastics. Several recent studies have focused on the emergence of microplastics as a concern in the environment [e.g., Kettner et al., 2017; Danso et al., 2019; Jacquin et al., 2019], and one study finds that landfills may be a potential source of microplastics [He et al., 2019]. To this end, we performed microcosm experiments similar to the above described, where a mixture of size fractions and classes of plastics were added after 41 days and sampled over 73 days.

Figure 7 summarizes the relative abundance data for all replicates over seven time steps. This representation is useful for watching broad changes over time, and it can be seen that, like the other microcosms discussed above, much heterogeneity is present. Some taxa are so highly variable over the course of the experiment, that no trends can be observed for post-amendment time points. Some taxa appear to be completely unaffected by the addition of microplastics. However, several taxa do appear to change abundance following the amendment of the experiments, accounting for the trend in abundance leading up to the addition of microplastics at t4. These include most notably the Betaproteobacteria and Pseudomondales, both of which were negatively impacted and lost abundance after amendments were added, and two taxa that may have benefitted by the loss of these other community members, increasing over t6/t7 (Rhizobiales and Azospirillaires). In contrast to experiments amended with antibiotics, the response of the communities is delayed and isn't apparent until t6/t7.

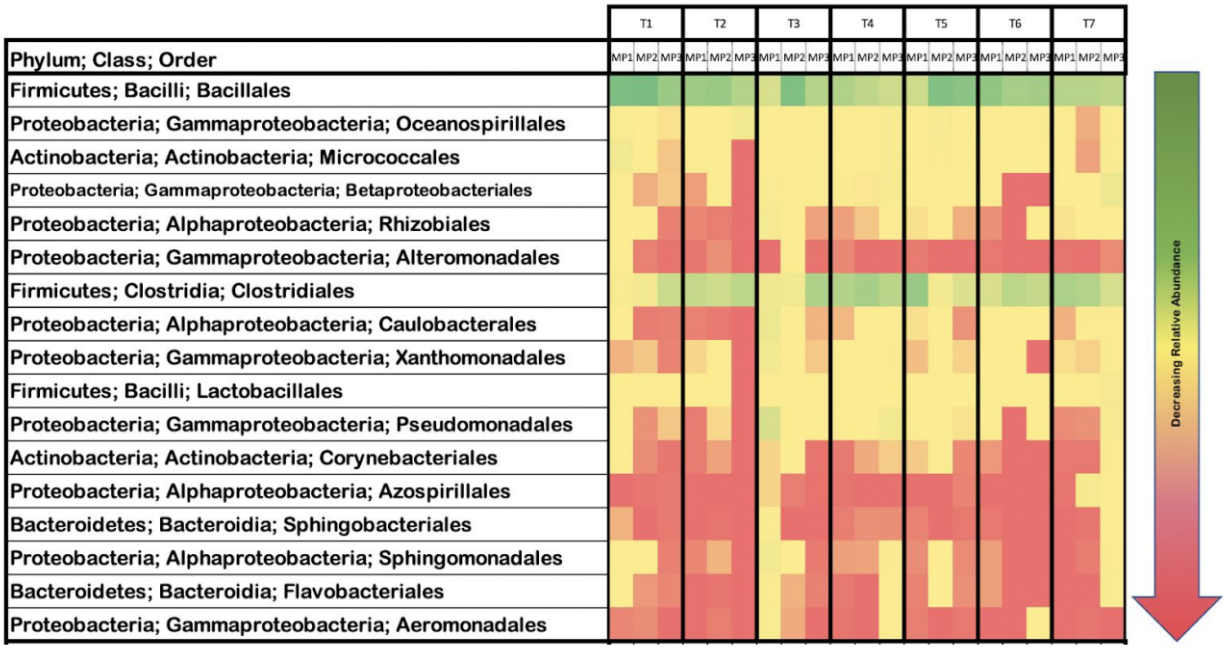


Figure 7. Heat map showing microbial relative abundances at seven time steps. Triplicate bottles are shown in each column. Microplastic amendments were added at T4. T1 = 7d, T2 = 28d, T3 = 37d, T4 = 41d, T5 = 52d, T6 = 66d, T7 = 73d.

This trend is further apparent in figure 8, which is an NMDS plot for the microplastic amended experiments. The communities began extremely heterogeneous at t1, much like the other

microcosm experiments discussed above, and then begin to converge on higher similarity through t2-t4. Post amendment t5 shows little dissimilarity from t2-t4, but t6 and t7 are again widely heterogeneous and distinct from t4-t5 communities. Overall, these results indicate that the addition of microplastics had a delayed but notable impact on community diversity.

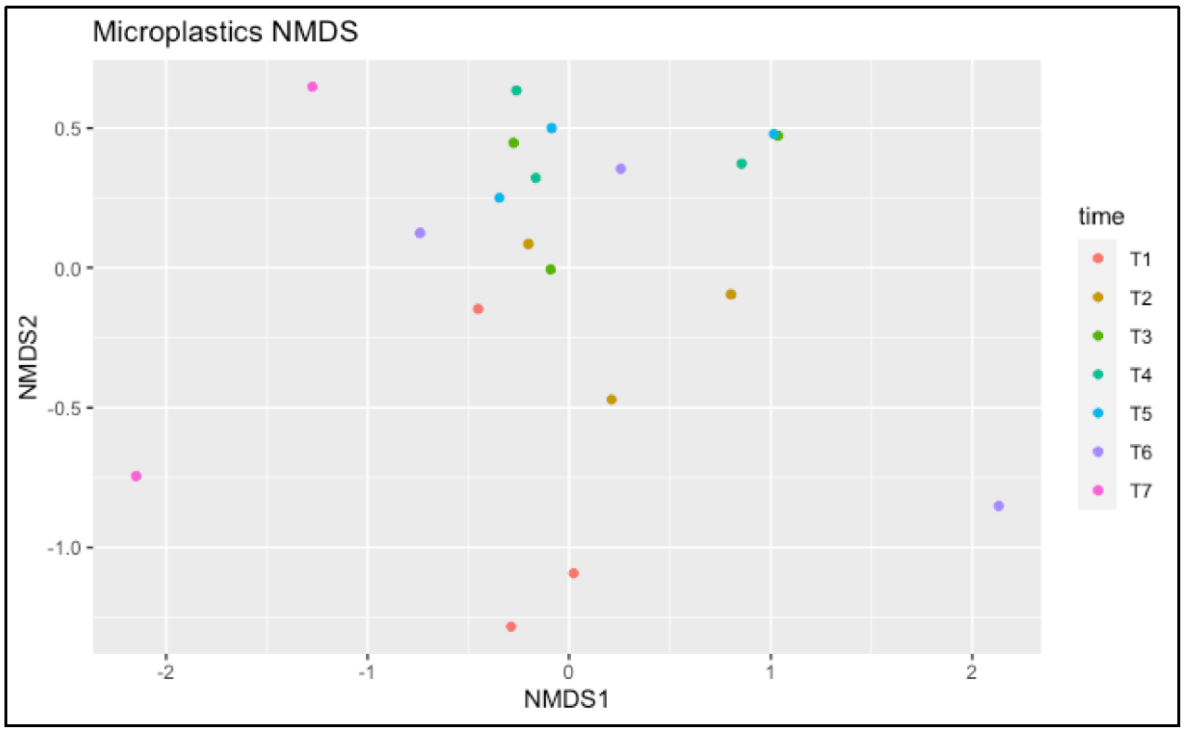
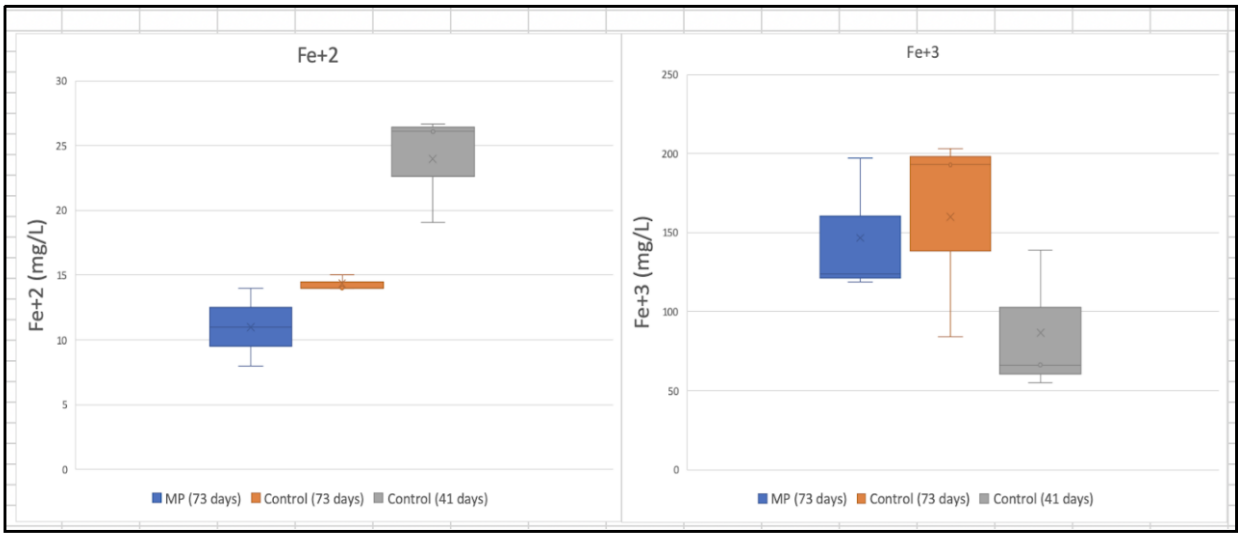


Figure 8. NMDS analysis for the microplastic amended experiments, showing seven time steps. Microplastic amendments were added at T4. T1 = 7d, T2 = 28d, T3 = 37d, T4 = 41d, T5 = 52d, T6 = 66d, T7 = 73d.



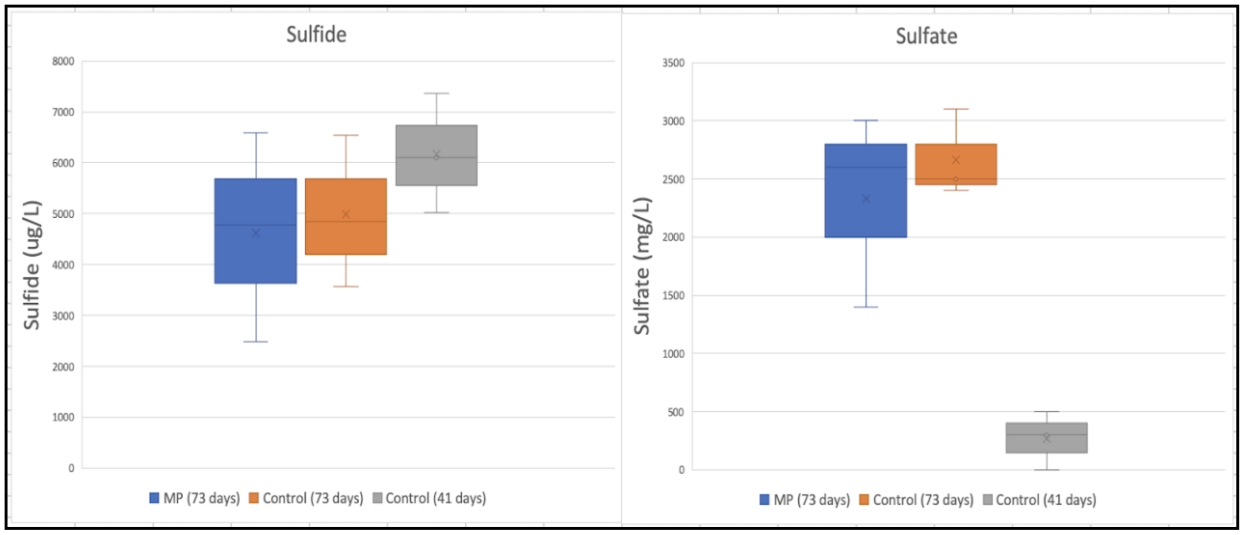


Figure 9. Results of analysis of redox chemistry of fluids in microplastic amended experiments.

The addition of microplastics also influenced the fluid chemistry in the experimental bottles (figure 9), but most notably in the concentrations of Fe^{+2}. Our assumption is that the addition of microplastics encouraged the growth of iron reducing Bacteria, which may be among the taxa that experienced increased abundance post-amendment.

3 Conclusions

Higher than normal temperatures in landfill environments are broadly of concern to landfill owners and the MSW research community. To date, the cause of elevated temperatures in landfills is debated, but our work has shown that both high temperature and changing, modern waste streams impact the Bacterial (and by necessity Archaeal) communities in native and experimental landfill ecosystems. This work showed that community structure of higher temperature landfills are distinctly different than more normal temperature locations. Both Bacteria and Archaea were impacted by the high temperatures. Further, Archaeal communities may be suppressed in lower temperature areas due to lack of production of metabolic substrates. This work also explored the impact of amendments mimicking C&D waste and CEC to landfill ecosystems. The addition of $Fe(OH)_3$, antibiotics, and microplastics changed the composition of microbial communities in simulated landfill environments. Iron metabolizing organisms were stimulated, and interactions between iron and sulfur biogeochemistry may have produced an excess of sulfide, suppressing some groups of organisms.

Future work will further explore the impact of individual antibiotic classes (as opposed to a generic mix, as was used here). Our original experimental plan included the exploration of the impact of changing temperatures in a laboratory-based landfill environment - however De la Cruz et al. (2021) have already published in this area so we will not repeat their work. Further surveys of high and normal temperature landfills at depth, using high throughput sequencing technologies, will be needed to parse the specific impacts on bacterial and archaeal communities.

4 Materials and Methods

4.1 Acquisition of samples

Natural landfill samples from a higher temperature site in Florida, (United States), were obtained via bucket auger. Soils were homogenized and then stored frozen until analysis. Soils were sampled by F. De la Cruz and M. Barlaz and shipped on dry ice to UIC.

4.1 Microcosm experimental design

Each set of microcosms was set up identically, with the exception of the additives intended to mimic the addition of specific types of waste in a modern waste stream. In general, microcosms were performed in triplicate, and included a 'live' control with no additives. 'Dead' controls were attempted as well, by three cycles of autoclaving and freezing the synthetic landfill substrate, but spore forming Bacteria were recalcitrant in the matrix. Synthetic landfill substrate included portions of glass, metal, plastic, cellulose (sawdust or wood shavings, as well as paper), sand, and food waste. Each 120ml serum bottle was filled to ~30% with the substrate, and then to ~60% with water. Each bottle also received nucleation materials for the use of DNA extraction. Providing solid surfaces that were uniform across all experiment for the colonization by microorganisms negated any variation in materials retrieved from each bottle. These surfaces were 10mm glass beads (a non-reactive material) that were placed inside 0.5ml microcentrifuge tubes in which ~10 holes had been drilled to allow free movement of leachate through the tube and across the surfaces of the glass beads. Serum bottles were capped in an anaerobic chamber to provide a non-oxygenated head space for the experiments.

Additives:

Sulfate: To mimic the addition of sulfate bearing C&D waste, Na_2SO_4 was added to three of the microcosms after 44 days, in the concentration of 3mg/L.

Iron: To mimic the addition of iron-bearing metals in C&D waste, an oxidized iron substrate in the form of $Fe(OH)_3$ was added to three of the microcosms after 44 days, in the amount of 7.1mg/L.

Antibiotics: Seven of the most commonly used antibiotics were added to three of the microcosms after 44 days. These were: bacitracin, amoxicillin, cephalexin hydrate, amoxicillin trihydrate (also called augmentin), tetracycline, azithromycin, and sulfamethoxazole. These were added as 1000 ng/L of each individual antibiotic. This concentration is within the ranges of various antibiotics found in modern waste streams of MSW landfills (e.g., Wu et al., 2015; Song et al., 2016).

Microplastics: Microplastics in a mixture of polypropylene (PP), polystyrene (PS), and polyethylene (PE) were added to three of the microcosms after 41 days in size fractions of $\leq$ 4 mm, < 2 mm, and < 1 mm.

4.2 DNA extraction/sequencing/processing

Microcosms were opened in an anaerobic chamber at several time points, and 4-6 of the glass beads were removed, leaving the rest in the bottles which were recapped in the chamber. DNA was then extracted from the glass beads using the PowerSoil DNA extraction kit

(QIAGEN), following the manufacturers instructions. For the natural landfill samples, landfill material was added directly to the bead beating tube in the kit, following manufacturers recommendations. DNA quality was checked using the PCR, and products of the 515F/806R primer set were sequencing using the Illumina Miniseq platform at the DNA Sequencing facility at UIC. Forward and reverse reads were merged using the software PEAR. Full length data were quality checked, trimmed, filtered, and chimeric sequences removed using the software DADA2 and Phyloseq. High quality reads were then compared to the Silva nr v.132 database and taxonomic similarities determined by BLAST. Statistical analyses were performed with Phyloseq.

4.3 Chemistry of microcosm leachate

Limited chemical analysis of the microcosm leachate at the end of each experiment was performed using a portable spectrophotometer, as in Cardace et al, 2015. The leachate was handled as minimally as possible prior to analysis to prevent abiotic oxidation of sulfide and Fe^{+2}.

5 Acknowledgements

The authors would like to acknowledge M.A. Barlaz and F. De la Cruz for several useful discussions in the preparation of this project and for sharing samples from the high temperature landfill site in FL. In addition, six undergraduates were involved in the laboratory work for this project, and their help was fundamental in the work. They are listed by name below in Appendix A (students 1-6). Three graduate students are completing thesis work pertaining to this project. They are Judy Malas, Michael Tanzillo, and Sarah Khoury (see Appendix A).

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7 Appendix A

Students involved in the project:

1] Ms. Perla Orozco, undergraduate (graduated May, 2018)
2] Ms. Blanca Escutia, undergraduate (graduated May, 2018)
3] Ms. Ester Yim, undergraduate (graduated May, 2018)
4] Ms. Alexandra Guzman, undergraduate (graduated May, 2019)
5] Mr. Ian Patete, undergraduate (graduated May, 2019)
6] Ms. Gracie Fisher, undergraduate (projected graduation, May, 2022)
*7] Mr. Michael Tanzillo, undergraduate (graduated May, 2019)**
*8] Ms. Sarah Khoury, undergraduate (graduated May, 2020)**
9] Ms. Judy Malas, PhD program (projected graduation, May 2023)
10] Mr. Michael Tanzillo, Masters program (projected graduation, May 2021)
11] Ms. Sarah Khoury, Masters program (projected graduation, May 2022)
** joined graduate program after completion of undergraduate program*

Papers and Conference Presentations (**student authors in bold**):

- **Khoury, S., Morales, M., Nguyen, N.**, Meyer-Dombard, D. (2022). Microplastic - soil separation methods. 10th Annual Midwest Geobiology Symposium, Chicago.
- **Khoury, S.**, Meyer-Dombard, D.R., Bogner, J., **Malas, J, Fischer, G.A.** (2021). Microplastics: Abundance and Effect on Microbial Life in Landfills, Wetlands, and Grassland. Midwest Geobiology Symposium, Indianapolis, Indiana.
- **Tanzillo, M.**, Meyer-Dombard, D.R., Bogner, J.E. (2020) Influence of Elevated Temperatures on the Microbiome of a Municipal Solid Waste Landfill. American Geophysical Union, Fall Meeting, 2020.
- **Malas, J., Khoury, S., Tanzillo, M., Fischer, G.A.**, Bogner, J., Meyer-Dombard, D.R. (2020) Impact of changing waste streams on microbial ecology and biogeochemical cycling in deep landfill ecosystems. American Geophysical Union, Fall Meeting, 2020.
- **Tanzillo, M.**, Meyer-Dombard, D.R., Bogner, J.E. (2020). Influence of elevated temperature on the microbiome of a municipal solid waste landfill. Geological Society of America annual meeting, 2020. Abstract#356851.

- **Malas, J., Khoury, S., Tanzillo, M., Fischer, G.A.**, Bogner, J., Meyer-Dombard, D.R. (2020). Impact of changing waste streams on microbial ecology and biogeochemical cycling in landfill ecosystems. Geological Society of America annual meeting, 2020. Abstract#358928.
- **Khoury, S., Malas, J., Tanzillo, M., Fischer, G.A**., Bogner, J., Meyer-Dombard, D.R. (2020). Abundance of microplastics in landfills and their effects on microbial processes. Geological Society of America annual meeting, 2020. Abstract#358929.
- **Khoury, S., Malas, J., Tanzillo, M., Fischer, G.A.**, Bogner, J., Meyer-Dombard, D.R. (2020). Abundance of Microplastics in Landfills and their Effects on Microbial Processes. Undergraduate Student Research Forum, University of Illinois at Chicago.
- **Malas, J., Khoury, S., Tanzillo, M., Fischer, G.A., Patete, I.D.**, Bogner, J.E., Meyer-Dombard, D.R. (2019). Trash or treasure? Biogeochemical cycling in landfill ecosystems. Abstract B76-546328 (poster presentation). American Geophysical Union, Fall Meeting, 2019.
- **Malas, J., Khoury, S., Tanzillo, M., Fischer, G.A., Patete, I.D.**, Bogner, J.E., Meyer-Dombard, D.R. (2019). Trash or treasure? Biogeochemical cycling in landfill ecosystems. 8th Annual Midwest Geobiology Symposium, St. Louis, MO.

Student theses:

- Michael Tanzillo, MS thesis. "Characterizing the Microbiome of a Municipal Solid Waste Landfill Experiencing Atypical Temperatures." Defended, 8 March, 2023. University of Illinois Chicago.
- Sarah Khoury, MS thesis. Putative title, "Microbial responses to Chemicals of Emerging Concern in Natural and Manmade Soil Ecosystems." Defense expected, 31 May, 2023.
- Judy Malas, PhD thesis. "Microbial ecology and evolution in extreme environments." At least one chapter will contain work from the publication listed below. Defense expected May, 2024.

Manuscripts in preparation**:** Three graduate students are also working on writing and submitting first-authored manuscripts based on work from this project, as part of their thesis projects:

- **Malas, J., Tanzillo, M., Khoury, S., Fischer, G.**, Bogner, J.E., Meyer-Dombard, D.R. Microbial responses to specific pharmaceutical and construction and demolition waste in lab-buit landfill microcosms. Submitted to FEMS Microbial Ecology, March 2023.
- **Tanzillo, M., G.**, Malas, J., Khoury, S., Bogner, J.E., Meyer-Dombard, D.R. Community structure and abundance of microorganisms in a temperature impacted municipal solid waste landfill. To be submitted to Geobiology Journal.
- **Khoury, S., G.**, Bogner, J.E., Meyer-Dombard, D.R. Microplastics in the solid waste stream: effects on microbial community structure. To be submitted to Environmental Microbiology.

Published materials:

- **Meyer-Dombard, D.R.**, Bogner, J.E., Malas, J. (2020) A review of landfill microbiology and ecology: A call for modernization with 'next generation' technology. *Frontiers in Terrestrial Microbiology*, 11: Article 1127.