Understanding the Anthropocene through the lens of landfill microbiomes

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Rapid increases in human population and per-capita waste production have resulted in accelerated growth in the size and number of landfills. We propose that microbiota within landfills can contribute to a better understanding of the Anthropocene thus far. Because landfills are characterized by conditions that are uncommon in natural, non-anthropogenic environments, they present a novel, rapidly growing, and essentially unstudied ecological system. Anthropogenic impacts may lead to unique adaptations in resident microbial communities, resulting in novel assemblages, activities, and features that differ from those in natural environments. Modern tools like molecular and bioinformatic techniques allow scientists to characterize these communities in unprecedented detail. A nuanced understanding of the adaptations, such as novel degradation abilities, that arise from such unusual selection pressures could facilitate improvements in bioremediation. Furthermore, similarities between landfill formation and geological deposition processes suggest that landfills will be preserved in future geological records and, consequently, may be suitable for dating anthropogenic influences at the local scale. Studying landfill microbiomes could therefore help to define the Anthropocene and reveal its consequences.

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The Anthropocene epoch is generally defined as a new geological period created by the impact of rapidly intensifying human activities on the environment (Waters *et al.* 2016), although the definition and acceptance of this term are still debated in the geological community (SQS 2017). One of the main challenges pertaining to the study of the Anthropocene is that it is still in its infancy, and its duration so far covers only a brief moment in geological time. However, this provides an opportunity to observe phenomena that define the early stages

In a nutshell:

- The microbial communities that underlie human-created environments can inform our understanding of when the Anthropocene epoch began and how it is progressing
- Landfills may represent a non-standard environment where we can study rapid, human-induced microbial evolutionary shifts
- Modern landfills generate novel microbial communities that could be used for environmental remediation

¹Department of Food Science and Technology, University of California-Davis, Davis, CA *(yachmon@ucdavis.edu); ²Department of Biological and Agricultural Engineering, University of California-Davis, Davis, CA; [‡]current address: ExxonMobil Research and Engineering, Annandale, NJ; ³Department of Geophysics and Planetary Sciences, Tel-Aviv University, Tel-Aviv, Israel; [†]current address: Kibbutz Mishmar Haemek, Israel; ⁴School of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; ⁵Department of Biotechnology and Food Engineering, Guangdong Technion Israel Institute of Technology, Shantou, China of the Anthropocene as they occur. We highlight the opportunities that come with studying the beginning of the Anthropocene from a microbial ecology perspective. Specifically, we argue the following: (1) the unique anthropogenic environment of landfills (localized sites for burying anthropogenic waste that contain complex mixtures of materials synthesized or modified by humans). These waste materials harbor their own microbiota and interface with microbiota in the surrounding natural environment that can mix and generate unique microbial communities; these communities (2) may serve as an additional local anthropogenic dating tool and (3) may offer opportunities to investigate rapid, human-induced evolution in novel environments, and to capitalize on new traits and functions emerging in these environments.

We propose that environments created and dominated by anthropogenic activity may serve as effective starting points for investigating the progression of the Anthropocene. Hereafter, we refer to these environments as "xeno-ecological niches" (XENs), a term we derived from the Greek prefix "xeno" (meaning "foreigner") and "oikos" (meaning "home", and which gave rise to the English prefix "eco"). Although such XENs are often referred to as novel, heavily disturbed, managed, or engineered, we argue that from a microbial perspective they represent a novel set of conditions that differ substantially from most natural environments, and should therefore more appropriately be called XENs. Examples include mines, urban areas, industrial areas, and to a lesser extent agricultural environments (eg farmland) and landfills, all of which have expanded greatly over the past century (Figure 1). In landfills especially, conditions are characterized by the presence of diverse materials, including exceptionally high concentrations of naturally occurring materials (eg cellulose in the form of paper) and varying amounts of synthetic substances (eg plastics). Microorganisms inhabiting landfills are subjected to selection pressures based on their ability to use these resources. Understanding the resulting changes in the microbiota could aid in the development of tools to deal with the consequences of the Anthropocene; for instance, new bacterial strains from landfills could be used to remediate petroleum-contaminated soil (Dashti *et al.* 2009) or help to mitigate other environmental problems.

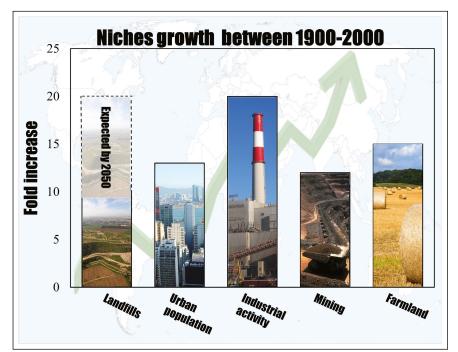
In our view the most promising XENs for the purpose of research – that is, those that provide a relatively comprehensive and detailed picture of anthropogenic changes – are modern urban landfills. In particular, we explain why inactive urban landfills, specifically those that are sealed (ie covered with soil) to prevent further environmental contamination while buried waste is undergoing stabilization processes, are good places to start (Figure 2). We also outline possible research strategies as well as obstacles that might be encountered.

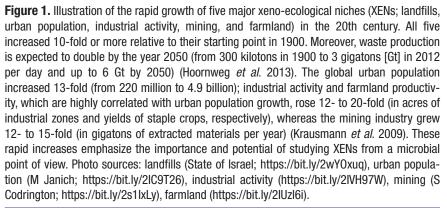
Developing markers of the Anthropocene from landfill microbiomes

Examining the microbiology of landfills may be especially fruitful because landfill microbiota are often exposed to a wide range of chemical compounds that are uncommon in natural environ-

ments. As shown in Figure 3, landfills receive waste from a wide variety of other XEN environments, ranging from urban and industrial areas to mines, which means that they may accumulate a broad range of harmful compounds, including heavy metals, antibiotics, pesticides, radioactive elements, and pharmaceutical byproducts. For this reason, they may act as "sink" habitats (see Figure 3 for a description; Table 1). Together with other physical conditions, which may include high temperatures, lack of oxygen, and increased physical pressure (especially in sealed landfills), the presence of contaminants at these sites exposes microbiota to unique selection pressures that may eventually lead to new assemblages, strains, and functions. Unlike megaflora and megafauna, the populations of which are often substantially reduced or driven to extinction by anthropogenic effects (Dirzo et al. 2014), some microbes thrive in mines (Méndez-García et al. 2015) and landfills (Stamps et al. 2016). Intensive research in relatively "old" landfills is needed to assess the chemical and physical compositions of these sites, as well as for microbial sampling and analysis.

Not all sealed landfills are suitable for study; therefore, we propose that actual investigations of sealed landfills should





begin with a careful consideration of their qualities and history as related to the specific research goals. To facilitate this research, we recommend a combination of geological, molecular, and bioinformatic approaches. Traditional trenching, core drilling, and soil sampling techniques can be used (Figure 4) in combination with next-generation DNA sequencing and network analysis.

By using tools such as metagenomics (characterizing the genetic content of a microbial community), metatranscriptomics (characterizing the gene transcripts in a microbial community as a measure of gene expression), metaproteomics (characterization of the proteins within a microbial community), and metabolomics (characterization of metabolites within a microbial community as a measure of metabolic activity, including engagement of specific biochemical pathways) (Jansson and Baker 2016), researchers can obtain highresolution depictions of the phylogenetic, transcriptional, and biochemical activities of landfill microbial communities at specific landfills must be carried out in parallel with the application of geological dating tools, and with a review of the land-



Figure 2. An example of an old, sealed urban landfill in the coastal area of Israel. This site (called Hiriya) was active from 1952 until 1998, when it was closed and converted to a recreational park. This "trash mountain" contains ~25 million tons of waste, and could potentially be used for landfill—microbiome research. (a) A side view of the site and (b) an on-site recycling facility that creates electricity from methane produced by microbial activity inside the landfill.

fill operating records. Together, these approaches can be used to identify the specific microbial fingerprint of each layer or each distinct parcel within a given site. Advanced bioinformatics approaches, such as relying on microbial network analysis from 16S ribosomal RNA (rRNA) gene sequencing data (Barberán et al. 2012) or conducting and using metagenomic data (Fierer et al. 2012), can further enhance our understanding of landfill microbiomes and the complex interactions that occur between species inhabiting such environments. For instance, recent work focusing on the microbiomes of leachates from 19 non-hazardous landfills highlighted the potential that microbiomes have as anthropogenic markers (Stamps et al. 2016). Profiling may initially focus on 16S rRNA gene sequencing along with metagenomic and metatranscriptomic data before advanced mechanistic questions are addressed. Measuring changes in relative gene frequencies can provide insight into the pathways that landfill microbial communities use to tolerate or eliminate the anthropogenic factors described in Table 1. For example, this method can be applied to look for genes related to antibiotic resistance, such as multidrug resistant genes, efflux pump genes, or quinolone resistance genes (Martínez 2008). Another option would be to search for genes associated with the degradation of pesticides such as DDT and/or glyphosate-based herbicides (Matsumura and Krishna Murti 1982).

As suggested earlier, that initial research should focus on landfills with features suitable for attaining the objectives of the planned research. These include landfills for which there are detailed records of waste disposal, where coring and sampling can be carried out, that have been sealed for a long period, and where disposal occurred for a substantial period of time. Microbial data can then be integrated with geological and chemical data to establish interactions and correlations across different landfill layers; examining these interactions across different landfills might facilitate the identification of common trends and improve our understanding of the temporal changes that transpire as landfills age. These broader questions can proceed simultaneously with narrower research questions focused on specific sites.

Research to characterize landfill microbiota and their genomes at both phylogenetic and functional levels will help to identify novel assemblages and functional adaptations resulting from anthropogenic effects. However, in addition to elucidating these effects on microbial ecology, the research may also deliver innovative tools to improve environmental health. Discovery of microbial tolerance mechanisms that help these organisms to overcome the harsh landfill environment may be facilitated by collating ecological data across many landfill sites with appropriate metadata; this work could

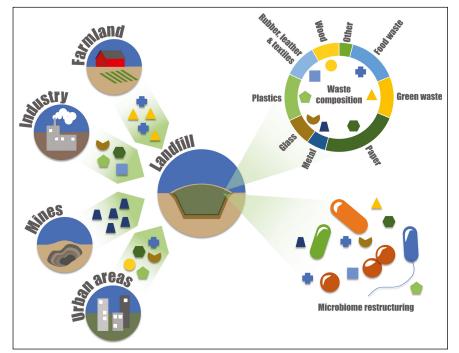
potentially lead to numerous major discoveries. For example, the discovery of a new microbial tolerance mechanism, such as the ability to degrade petroleum hydrocarbons, siloxanes, and other toxins, could be exploited for environmental remediation (Table 1).

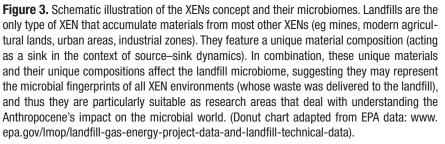
Accounting for landfill variability when developing anthropogenic markers from microbiomes

Both the Anthropocene time line (Braje and Erlandson 2013; Monastersky 2015) and its impact (Crutzen 2006; Bennett et al. 2016) are continuing subjects of debate. The most plausible starting date for the Anthropocene is the period known as the Great Acceleration (1945-1955; Waters et al. 2016); growing consensus on this date is fueled by evidence of the rapid increases in pollution (eg atmospheric carbon dioxide, pesticides, antibiotics, radioactive fallout from nuclear tests) that occurred over this time period. From an ecological point of view, defining the Anthropocene has implications for environmental health studies and policies. However, additional tools are needed to allow researchers to accurately date and better understand the changes that have occurred within this extremely short geological period. At present, several efforts to describe and define the Anthropocene have been completed (Zalasiewicz et al. 2011; Lewis and Maslin 2015; Waters et al. 2016), and landfill studies can complement this work. These studies primarily focused on examining physical and chemical characteristics, such as temperature, and concentrations of carbon, carbon dioxide, methane, and nitrate, whereas other research has focused on the detrimental impacts of human activity on biodiversity (the "sixth extinction wave"; Dirzo *et al.* 2014). However, the effectiveness of using microbes as defining tools has yet to be tested.

Considering the anthropogenic impact on the microbial world represents a new research direction (Gillings and Paulsen 2014). The concept of the Earth's microbiome has attracted a great deal of scientific attention recently (Blaser et al. 2016; Peay et al. 2016; Pointing et al. 2016), but to the best of our knowledge the potential use of microbiomes as tools for elucidating the time line of the Anthropocene remains largely unexplored (Gillings and Paulsen 2014; Gillings et al. 2015). The challenge in defining the onset of the Anthropocene is its geological recency. Whereas most geological phenomena are measured in thousands to millions of years, life cycles of most macroscopic organisms scale from days to years, and those of microbes can be on the order of minutes to hours (Weller and Wu 2015). The ability to resolve microbial adaptions over a variety of timescales will be enabled by advances in DNA sequencing and bioinformatics, which will likely continue to improve the sensitivity, speed, and cost of profiling microbiota. Although it

would be misguided to treat the separated ecological "islands" that landfills comprise as if they were universal dating tools, it may still be possible to establish distinct microbiological "clocks" or chronologies for each landfill and then examine similarities among their evolutionary pathways. As these tools become more accessible, there is also the possibility of monitoring the environment within landfills for these biomarkers in real time (Gillings and Paulsen 2014; Pointing *et al.* 2016). Landfill microbiomes are suited to such activities because of their spatial and temporal heterogeneity.





Framing landfills as geological entities within the Anthropocene

Modern urban landfills are a recent phenomenon, first appearing in the early 1900s (www.epa.gov/lmop/landfill-gas-energy-project-data-and-landfill-technical-data). After the Second World War, landfill construction proliferated and spread worldwide (Hoornweg *et al.* 2013), creating large artificial deposits and making these sites one of the fastest growing anthropogenic niches (Figure 1). Landfills lend

Table 1. Anthropogenic factors in modern landfill microbiomes		
Factors	Possible targets	What to look for
Antibiotics	Targeting specific genes such as <i>tet</i> or <i>aphA3</i> (Zhu <i>et al.</i> 2013) and many others (Li <i>et al.</i> 2015)	Changes in the amounts and types of antibiotic resistance genes with correlation to chemical data and geological deposition
Pesticides	Effects of different pesticide types on the microbial community or on specific genes (Jacobsen and Hjelmsø 2014)	Correlations between pesticide usage as reflected in the deposits and the microbiomes
Detergents	An increase in the abundance of lipases (Andualema and Gessesse 2012)	Correlations between detergent usage as reflected in the deposits and the microbiomes
Plastics	Relative changes in the abundance of fungal species related to plastic degradation (Ghosh <i>et al.</i> 2013)	An increase in plastic-degrading microbes/genes correlated with increases in the deposition of plastics
Temperature	Changes in microbiomes due to temperature variations (Karhu et al. 2014)	Correlations between global climate change and changes in microbial communities
Water	Changes in microbiomes due to drought (Alster et al. 2013)	Correlations between long drought periods and changes in microbial communities

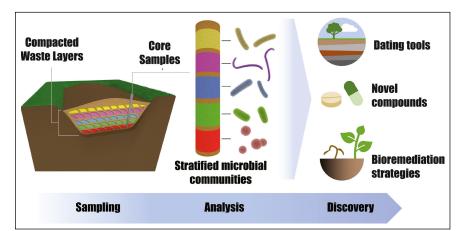


Figure 4. Schematic illustration of core sampling in a sealed landfill (ie an inactive landfill that has been covered by soil) to study shifts in the microbiomes across different layers. Combining classical geological techniques of core extraction with modern biological methods and molecular tools (eg next-generation sequencing) may allow us to distinguish and profile the stratified microbiomes. Such research could lead to the discovery of populations or strains with unique characteristics (eg novel antibiotic resistance, material degradation abilities, etc).

themselves to analysis by geological methods due to their stratification; they are composed of substances that in some cases are already somewhat layered, are not overly "engineered", and accumulate further materials in a way that roughly mimics natural deposition. In the context of human activity, this happens over a relatively long time span (tens of years) and in many cases follows the principle of superposition (ie that the deepest layers are the oldest), like many archaeological sites (Figure 4). Strata in landfills can often be very accurately dated if reliable records have been maintained or if dated artifacts are found within the layers. Furthermore, the chemical composition of each layer is reflective of human activity at the time of deposition. They are also more likely to be preserved or "fossilized" in a

Table 2. Factors that can affect microbial communities in landfills and further increase heterogeneity in time and space, both within and between sites

Challenges	Considerations
Seasons	Parameters such as seasonal temperature and rainfall should be taken into account
Geographic location/ climate zone/culture	Average temperature, humidity, and type of deposits should be adjusted according to the specific location, as both environ- mental conditions and the nature of landfill deposition may vary
Leachates	The effect of leachates, such as material drainage, into deeper layers
Soil types	Different soils harbor different core microbial communities that can create a founder effect or differential assemblies that need to be taken into account
Lifetime/duration of activity	Each landfill has a different time line that needs to be considered when different landfills are being compared
Size	The size of the landfill area is another parameter that must be standardized

similar state and form to their initial appearance following stabilization processes than are other types of XENs, and they are in situ (Ritzkowski *et al.* 2006).

Although the appearance of large urban landfills around the world took some time, as is the case with almost all real stratigraphic units, this is negligible on a geological timescale. The appearance of these landfills coincides almost exactly with the period of the Great Acceleration, and they are representative of the rapid changes in the physical attributes of the Earth system that occurred after 1950 (Steffen et al. 2015). As such, landfills are one of the Great Acceleration's defining characteristics, and are in many ways similar to the time-honored geological concept of "facies". One can argue that millions of years in the future, when differences of 10,000 years or so become immaterial, there will be no better remaining evidence for the beginning of the Anthropocene than the first appearance of this facies, whatever date is finally decided on for its

beginning. Research is needed to characterize microbial communities in human-perturbed environments and to identify phylogenetic shifts that mark human influences.

Challenges associated with using landfill microbiomes as anthropogenic clocks and markers

As mentioned above, there are numerous challenges associated with researching landfills as potential models for determining the timing, and understanding, of anthropogenic impacts. Several obstacles must be overcome before landfill microbiomes can be used either as reliable local dating tools or as a reliable spatial and temporal marker of anthropogenic activity (Table 2). For instance, landfills undergo several "aging" processes (eg as a result of acid production or methanogenesis), which can be reflected in microbial community composition, and earlier microbial assemblages may be altered or obliterated altogether by later processes within the landfill. Eventually, landfills may reach what is referred to as "functional stability", which is generally defined by lower rates of landfill gas emission and acid production. As a result, sealed and stabilized landfills should be the primary choices for such research. The fact that layers change as a function of time and location within the landfill must be acknowledged when sampling their microbiomes, and therefore preliminary research is needed to identify and minimize "background noise". Research on "setting" landfill anthropogenic clocks - by studying the ways in which microbiomes respond to various landfill waste deposition and sealing practices - can also provide a wealth of ecological data. To best manage the large amount of information that may be generated, an online database for storing and sharing landfill sequencing data and associated metadata should be

established. A rigorous sampling program run by a large agency (private or governmental, as in the case of human genome sequencing; Venter *et al.* 2001) should also be created, and should include as many landfills as possible worldwide. Alternatively, early research can intensively study a small number of carefully selected landfill sites and in this way reduce many of the variables that are mentioned in Table 2, while acknowledging the limitations of examining only a specific location or a limited number of sites.

Conclusions

We propose here that landfills, and XENs in general, be investigated as potential sources of unique microbiomes and biological functionality. Understanding the new assemblages and novel toxicant resistance generated by natural selection within landfill communities during the Anthropocene can aid in the development of innovative tools to deal with its consequences. Of course, any method(s) eventually chosen to study microbiomes in landfills will have to be conducted alongside conventional geophysical and geochemical dating and analysis. Despite the short geological time span that the Anthropocene represents, modern dating techniques (eg cesium-137, with a half-life of 30 years) can be used to address such challenges of short-term accurate dating. This field of inquiry will require cross-disciplinary collaboration among geologists, microbiologists, ecologists, archaeologists, and others. Such research may help to unravel critical aspects of human-environment interactions that can both define our past environmental effects and yield new microbial remediation pathways to mitigate our future impacts.

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