

EDITORIAL

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Microbiome multi-omics can accelerate human excrement composting research

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Abstract

In this editorial, we discuss the need for a new, long-term strategy for managing human excrement (feces and urine) to facilitate health equity and promote environmental sustainability. Human excrement composting (HEC), a human-directed process driven by highly variable and diverse microbiomes, provides a means to advance this need and we discuss how microbiome science can help to advance HEC research. We argue that the technological advancements that have driven the growth of microbiome science, including microbiome and untargeted metabolome profiling, can be leveraged to enhance our understanding of safe and efficient HEC. We conclude by presenting our perspective on how we can begin applying these technologies to develop accessible procedures for safe HEC.

“In a given time, progress aiding, with mechanisms being perfected, and knowledge increasing, [we will return human] manure to the fields. From this simple act will result, for the whole social community, a reduction of misery and an improvement of health.”

~*Les Misérables*, Volume 5, Book 2, Victor Hugo (1862) [1]

Modern sanitation needs a good flushing

Worldwide, over two billion people lack access to toilets or latrines, and more than 450 million people engage in open defecation, often in exposed or environmentally sensitive areas [2, 3]. Environmental and human health impacts from poor or non-existent sanitation systems persist, in both underdeveloped and developed areas [4].

Historically, human excrement (feces and urine) served as potent fertilizers contributing to soil health,

agricultural productivity, and local economies [5, 6]. However, the Industrial Revolution brought rapid urbanization and denser populations, which led to excrement accumulation in streets, drinking water contamination, and the spread of human disease [7]. In response, sanitation management systems were developed that markedly improved public health but inadvertently disconnected people from their environmental impacts and fostered a myopic view of human excrement (HE). Unintended consequences of this transition were foreseen by astute observers [1], and now manifest as natural resource depletion (e.g., drinking water for flushing toilets, fossil fuels to synthesize and transport fertilizer rather than cycling nutrients locally) and infrastructure that can be prohibitively expensive to build, operate, and maintain [4]. Consequently, many populations have little choice but open defecation, exacerbating environmental disruption (e.g., eutrophication, water and soil contamination) and health disparities (e.g., diarrheal diseases). Although HE continues to be used in agriculture in parts of the world [8–12] it remains a largely untapped resource that is nutrient-rich, readily available, and underappreciated.

The 2015 United Nations “Transforming our World” initiative defined seventeen Sustainable Development Goals. Goal six aims to “ensure availability and sustainable management of water and sanitation for all.”

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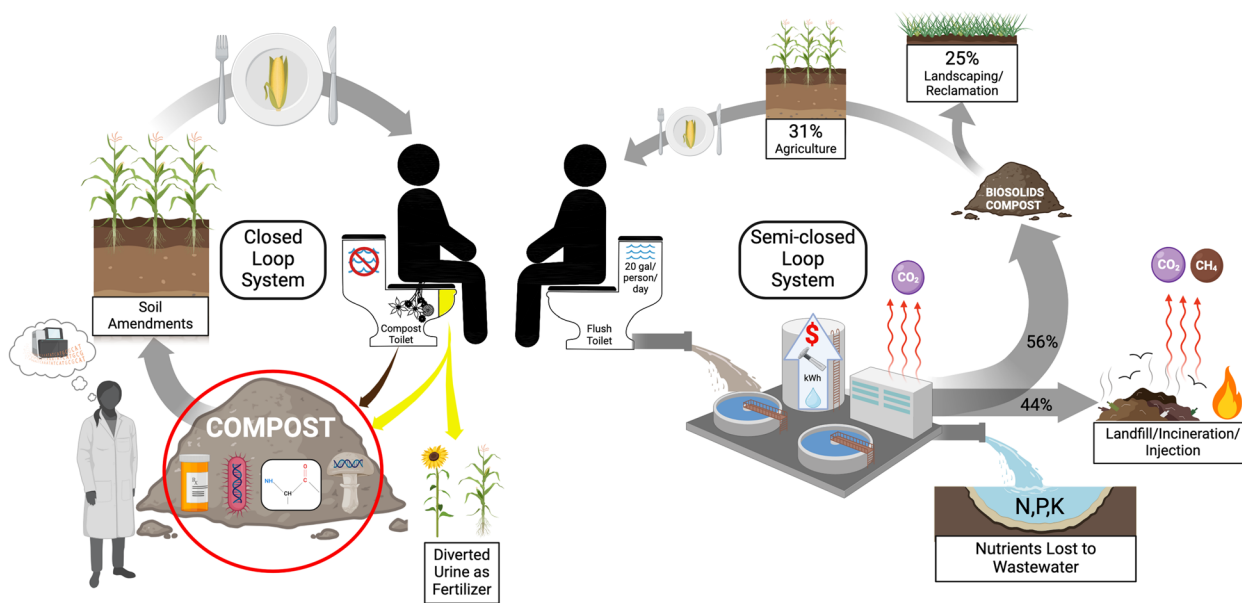


Fig. 1 Environmental benefits of composting toilets. Composting toilets (left) facilitate closed-loop systems as the nutrients yielded by HEC can replace those obtained from synthetic fertilizers in agriculture. Additionally, HEC requires infrastructure that is significantly less complex and more affordable, relative to traditional wastewater treatment systems (right). Wastewater treatment plants in the United States consume 30 terawatt-hours (TWh) of energy annually, resulting in the emission of 41.7 million metric tons of carbon equivalents in 2020, while returning a relatively small portion of the nutrients present in human excreta to agricultural systems [13, 14]. Microbiome multi-omics can further advance HEC research (red circle), for example, enabling untargeted tracking of pharmaceutical residues during composting or bioprospecting for enzymes that efficiently degrade recalcitrant compounds. Figure created with BioRender.com. Values presented in the figure were obtained from [15]

Expanding the use of composting toilets (CTs) for HE management can contribute to attaining this goal. CTs are designed to collect HE for treatment in systems that promote aerobic decomposition, relying on bacteria, fungi, and other organisms to break down organic matter into a safe, stable, humus-like substance. The resulting compost can be used as a soil amendment, and regulatory standards or guidelines often govern its application to ensure safety and environmental protection. Unlike traditional flush toilets, CTs do not require water for flushing. They are cost-effective, transformative alternatives for HE management that provide versatile solutions across diverse socio-economic settings at personal, municipal, national, and global scales. Their broad adoption has the potential to reduce environmental stresses, human health disparities, water consumption, and pressures on aging wastewater treatment infrastructure. Additionally, these systems can generate safe, nutrient-rich soil amendments, contributing to a circular economy and promoting environmental stewardship (Fig. 1). The organisms and systems involved in the excrement-to-compost transition, and the technologies that can be applied to better understand them, are familiar to human and environmental microbiome scientists. We are therefore well-positioned as a field to

advance this Sustainable Development Goal for humanitarian and environmental gains.

Illuminating microbial dark matter

Composting is a human-directed biological reaction driven by highly variable and incredibly diverse microbiomes. Extensive research has described microbial succession in composting [16–21], identified optimal ranges of variables driving the composting processes (e.g., carbon to nitrogen ratio, moisture content, pH) [22, 23], and evaluated safety considerations in finished compost, such as pathogen load [24, 25]. Notably, researchers have observed drastic reductions in the diversity of antibacterial-resistant genes during composting of dairy manure [26, 27] and nearly 100% degradation of the antibiotics metronidazole and ciprofloxacin [28] through thermophilic composting.

Meanwhile, “-omics” technological innovations of the past two decades [29–31] have expanded our capabilities for studying microbial systems and have much to offer our understanding and practice of human excrement composting (HEC). DNA sequencing and mass spectrometry, which drive -omics data generation, can serve as next-generation data sensors, allowing us to probe tens of thousands of species (the majority of which we currently do not know how to grow in culture), hundreds of

thousands of genes, and tens of thousands of small molecules. When two or more of these technologies, such as metagenomics, metabolomics, or metatranscriptomics, are applied together to investigate complex communities of microorganisms, we refer to this as “microbiome multi-omics.” We argue that microbiome multi-omics when applied in replicated HEC time series experiments, can enable us to develop a systems-biology-based understanding of the HEC reaction.

Microbiome science has already yielded insight into composting. For example, the accessibility of marker gene sequencing, which targets specific conserved regions of genomes (e.g., 16S ribosomal RNA) for taxonomic profiling [29], has enabled compost researchers to identify the impact of feedstocks, bulking materials, and compost preparation methods on microbial diversity [8, 17] and to track reductions in antibacterial resistance gene diversity [26]. Studying microbial succession with marker gene sequencing alongside typical measures of compost maturity (e.g., low microbial activity, between 10:1 and 20:1 C:N ratio, pathogen levels within regulatory standards) can expand our knowledge of taxa indicative of compost safety [10], demonstrate when human gut-associated taxa are depleted [32], suggest microbial taxa that could be used in compost inoculants [33], and contribute to waste-based epidemiology [34]. However, marker-gene sequencing approaches, by their nature, do not provide information on *all* organisms present in a sample and may be limited in resolution (e.g., only providing identification at the bacterial genus level, rather than at the species level).

Alternatively, metagenomic sequencing, which does not target specific genomic regions but rather aims to sequence *all* DNA present in a sample, enables broader detection and higher-resolution annotation. The considerably higher cost of metagenome sequencing often means that it makes sense to start with marker gene sequencing of many samples, for example along a dense time series, and then consider metagenome sequencing on a resulting subset of samples of interest. Metagenomic sequencing has been employed in composting systems, for example, to assess the regulation of β -glucosidase genes responsible for cellulose degradation by microbial communities across various composting phases [35]. Additionally, it has been utilized to investigate the functional profiles of microbiomes across composting phases [36] and to monitor the abundance of a wide range of antimicrobial and heavy metal resistance genes in compost and manure [37].

Microbial activity in composting systems can be studied by applying DNA sequencing technologies to profile microbial mRNA abundance using metatranscriptomics

[36, 38] and/or mass spectrometry to track small molecule production (e.g., microbial metabolites), for example, to support the development of standardized detection assays for pollutants commonly present in HE (e.g., pharmaceutical residues, personal care products). In addition to human, animal, and plant health considerations, such as endocrine disruption and ecotoxicology arising from pharmaceuticals in soil amendments, ensuring that antibiotics and antifungals are degraded is important for reducing the potential for the evolution of new drug resistance mechanisms.

Combining these individual -omics approaches can enable efforts such as bioprospecting for enzymes that degrade recalcitrant compounds, such as lignocellulose [39], or ecotoxicological molecules, such as pharmaceuticals. For example, in a replicated HEC time series experiment, the decreasing abundance of targeted molecules could be linked with active microbial enzymes. Organisms that encode those enzymes in their genome could subsequently be identified, validated for the desired activity, and included in compost starter communities that increase the efficacy of target degradation. Components of this workflow have been applied already in composting systems [33, 40–42], and we hypothesize that integrating multi-omics technologies in this way could yield a standard protocol to bioprospect for organisms and/or enzymes that could serve as useful HEC inoculants.

Integrating multi-omics with other related approaches, including qPCR targeting relevant pathogens [43] or antibiotic resistance genes [26], and/or targeted amplicon sequencing such as pan-enterovirus assays [44], can accelerate our understanding of HEC and support research to advance current small-scale [45] and large-scale [46] best practices. It is important to note, however, that the multi-omics approaches described here have varying potential to reveal information about the individuals or populations using the systems being studied, for example linking drug use from metabolomics assays with personally identifying or ancestry-informative genetic markers obtained incidentally during metagenome sequencing. This creates ethical challenges to their application in wastewater treatment or HEC systems that must always be carefully managed [47, 48].

Toward sustainable excrement management

CTs are most commonly known for their diverse sustainability implications, including reducing water consumption and promoting nutrient cycling [49–51]. For instance, an average American uses 310 L of drinking water per day, 24% of which is used for flushing toilets [52]. We estimate that if residential toilets in the USA

were hypothetically replaced with CTs, 9.1 trillion liters of water per year¹ would be saved by not flushing alone, equivalent to the entire annual allotment of Colorado River water to the arid lower basin states of Nevada, Arizona, and California [53].

Certain models of CTs divert urine into the ground or designated collection chambers [54], thereby reducing odors associated with ammonia volatilization and enhancing the desiccation of HE. This process facilitates safer disposal and enables energy recovery [55]. Building on the previous example, if urine-diverting composting toilets were used, the nutrients collected from diverted urine could be repurposed as fertilizers to grow 48 million metric tons of wheat per year [56–58].

The broad implementation of both small and large-scale HEC systems faces massive cultural [8, 59, 60], political [61], and regulatory barriers [62–64]. The convenience of modern sanitation allows us to flush and forget rather than viewing HE as a resource, and misconceptions abound that HE-derived products are inferior and unsafe. A lack of awareness of alternative waste treatment systems, legal liability concerns, international plumbing codes, and local building regulations (if they exist) can be cost-prohibitive or restrictive regarding the installation of in-home CTs. A deeper understanding of the microbiological processes that drive the excrement-to-soil transition, aided by the technological innovations outlined here, can accelerate advances in the perspectives, policies, economies, and infrastructure necessary to support broader deployment and adoption of HEC. In spite of the challenges, we firmly believe HEC represents an essential component of achieving health equity and sustaining life on Earth (or on other planets, for that matter).

Growing awareness of global sustainability problems has prompted interest in revitalizing traditional agricultural practices, and the emergence of innovative, successful HEC enterprises to address the logistical challenges is underway. Organizations such as SOIL (<https://www.oursoil.org>), Sanergy (<https://www.sanergy.com>), Sanivation (<https://sanivation.com>), and Rich Earth Institute (<https://richearthinstitute.org/>) utilize varied approaches to upcycle HE into compost, animal feed through harvesting of black soldier fly larvae [65, 66], biomass fuels, and fertilizers. These innovations not only reduce resource consumption, but can provide low to no-cost fertilizers, food for livestock, and a source of energy for cooking or heating homes. We hypothesize that a better understanding of HEC microbiology would expand

economic opportunities, for example through the development of inoculants or substrates that accelerate or standardize the process.

Small-scale initiatives are critical steps toward the urgently needed paradigm shift in HE management. By collaborating with community organizations and leaders, researchers can pinpoint communities that could benefit from CT installations. Through education and public outreach (e.g., demonstration sites on university campuses or at community centers) these collaborations can cultivate economic opportunities and drive technological innovations while raising awareness and fostering acceptance of circular excrement management. These efforts can provide models for scaling up in areas where new approaches are most needed, or where there is the greatest appetite for transformation.

Collectively, the knowledge gained by applying microbiome multi-omics to study HEC systems will help reduce the spread of human disease through contaminated soil and water, substantially decrease water and energy consumption, and retain nutrients in closed-loop cycles. We strongly encourage researchers, policymakers, community organizers, and funding agencies to invest resources in HEC microbiome research to harness the transformative power these microbiomes offer to create a more equitable and sustainable planet. In short, Hugo's "given time" is now.

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Authors' contributions

J.M. and J.G.C. wrote the main manuscript. M.J. assisted with writing and editing the manuscript. All authors reviewed the manuscript.

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No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

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Competing interests

The authors declare no competing interests.

¹ 310 L per person per day × 365 days per year × 24% × 333,300,000 people = 9.1 trillion L/year

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