Advancing Urine Separation for Sustainable Food, Energy, and Water systems

by

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Abstract

Inefficient nutrient management is an increasingly urgent challenge at the heart of the food-energy-water nexus, posing a threat to public and environmental health. Nitrogen (N) and phosphorus (P) emissions from agriculture and waste systems lead to detrimental disruptions in natural environments, such as harmful algal blooms. These blooms can significantly reduce water quality and potentially expose users to harmful toxins. Additionally, the resource-intensive processes involved in manufacturing N and P fertilizers and removing nutrients from mixed waste contribute to greenhouse gas emissions and land degradation. Efficient nutrient management requires a circular approach that redirects N and P flows in waste to beneficial uses, such as in agriculture. Urine separation promotes circular nutrient management by separating urine from other components of wastewater at the toilet and processing it into a fertilizer. However, a number of questions remain about the public and environmental health benefits of urine separation. The objective of this dissertation is to compare the public and environmental health impacts of urine separation to conventional toilets, fertilizer use, and urban waste management.

From a public health perspective, the general population interacts with urine separation during collection at the toilet. Previous research has connected conventional toilet usage with virus exposure. To determine how urine-diverting toilets (UDTs) affect user exposure to viruses, virus emission levels from flushing were compared between a UDT, which has a diverter in the toilet, and a standard mix-flush toilet (MFT) that only has one compartment. The results demonstrated that the MFT had high emissions of viruses excreted in urine, potentially exceeding the minimum number of viruses that cause infection. In contrast, the UDT removes urine-associated viruses from the toilet before a flush, reducing their emission levels. The lower levels of urine-associated virus emissions from the UDT and high frequency of flushing associated with urination suggests that UDTs provide a significant reduction in potential exposure to urine-associated viruses. The emission values determined in this study can be used directly in future risk assessments for both MFTs and UDTs.

Environmental health impacts of urine separation were assessed at two different scales, namely at the field scale in terms of impacts on soil health, and at the system scale in terms of sustainable nutrient cycling in communities. At the field scale, a greenhouse experiment was conducted to compare the impacts of urine-derived fertilizers (UDFs) to other fertilizers on soil health and N cycling. The short-term experiment revealed that the UDF increased plant yield by a comparable amount to the inorganic fertilizer with no compromise to soil health. Due to their similarities in N availability, the effects on soil N cycling were more similar between UDF and inorganic fertilizer than to the organic fertilizer. When compost was applied with UDF, there were higher N_2O emissions per gram of N applied, but the ratio of N loss (as leachate and N_2O emissions) to harvested by the plant significantly decreased. The similar behavior of the UDF and inorganic fertilizer suggests that UDFs can substitute inorganic fertilizers, significantly reducing resource consumption in agriculture. Additionally, there is an opportunity for UDFs to contribute to ecological nutrient management goals by combining their application with organic fertilizers.

At the system scale, a mass balance of N and P flows was used to quantify the potential benefits of nutrient recovery from food waste and wastewater (source-separated urine and sewage sludge) for nutrient circularity, sustainable waste management, and fertilizer offset in New York City (NYC). The analysis found that urine has the largest proportion of recoverable N whereas food waste has the most recoverable P. Almost half of the nutrient inputs used to produce food for NYC can be replaced by recovered nutrients. Additionally, a suitability analysis revealed specific sewersheds in the city where NYC Department of Environmental Protection (DEP) can implement urine separation to meet stringent nutrient discharge permits amid projected population increase. This dissertation advances a circular economy for nutrient management by quantifying the potential public and environmental health benefits of urine separation. Stakeholders such as toilet users, farmers, agricultural policy makers, conservation organizations, and city agencies can use these results to make informed decisions about implementing urine separation.

Chapter 1 Introduction

Anthropogenic use of nitrogen (N) and phosphorus (P) in the agricultural and food systems have allowed major advancements in food security and economic development at the cost of disrupting natural biogeochemical cycles. Environmental N and P are primarily inert, but industrial processes such as the energy-intensive Haber-Bosch process and phosphate rock mining, are used to convert them into reactive N (Nr) and P (Pr) (Woods et al., 2010; Mallin & Cahoon, 2020) for anthropogenic use, especially in industrial agriculture. Convenient access to Nr and Pr have fostered a culture of imprudent production, usage, and wasting of N and P. This has created a steady increase in environmental Nr and Pr (Galloway & Cowling, 2002; Carpenter et al., 1998) and thus a high risk of irreversible damage to the natural environment (Steffen et al., 2015). Increased environmental Nr and Pr negatively affects both public and environmental health through harmful algal blooms, reduced water quality, and increased greenhouse gas (GHG) emissions among other detrimental effects (Wolfe & Patz, 2002; Mallin & Cahoon, 2020; Carpenter et al., 1998; Galloway & Cowling, 2002). Due to the importance of N and P in maintaining public and environmental health, there is a responsibility for engineered processes to mitigate Nr and Pr flows for more sustainable food, energy, and water (FEW) systems.

Engineering pathways for nutrient recovery from waste can reduce environmental Nr and Pr and also create a source of N and P that requires less energy-intensive production. Nr and Pr are lost to the environment due to management inefficiencies in agriculture, food consumption, and waste management. Current N and P use in these systems result in significant losses as they are converted from fertilizers to wastewater. Only 4-15% of N that is applied as fertilizer for food

production is consumed, most of which is lost during crop production. The remaining N that is not consumed is lost as direct emissions from agriculture or embedded in organic waste (Leach et al., 2012; Galloway & Cowling, 2002). Of the N that is consumed, most is excreted in wastewater. When wastewater resource recovery facilities (WRRFs) do not have the capacity to convert it back into inert forms, they are directly discharged into the environment. Even with sufficient processing, some N is emitted as N_2O (Law et al., 2012). As a result, critical strategies for mitigating environmental Nr and Pr include reducing the use of manufactured, inorganic N and P fertilizers and improving N and P use efficiency (NUE, PUE) during crop production, in addition to minimizing direct emissions of nutrients as waste and indirect emissions from waste management processes. For example, converting food waste and wastewater into agricultural soil amendments is a synergistic approach that reduces the release of Nr and Pr while offsetting the negative effects of traditional fertilizer production. In this case, the resources used for traditional waste management and inorganic fertilizer production can be redirected to nutrient recovery from the waste.

Among waste flows, the recovery of N and P from municipal wastewater has the potential to provide multiple public and environmental health benefits. N and P in wastewater effluent remains a major source of environmental Nr and Pr, especially for water bodies surrounding dense metropolitan areas (Liang et al., 2019; Forkes, 2007; Vaudrey, 2017; Carey & Migliaccio, 2009). N and P enters municipal wastewater via human excrement flushed down the toilet. The nutrients are diluted with water and combined with a complex mixture of organic and inorganic constituents in the sewage, greywater, urban runoff, and industrial waste waters. Most wastewater resource recovery facilities (WRRFs) in the US were designed over 80 years ago primarily to protect public health and remove carbonaceous or organic content. Advanced wastewater treatment processes to

remove N and P were only developed and implemented in the 1960-1970s (Lofrano & Brown, 2010). With increasing concern about eutrophication and other detrimental impacts of nutrient pollution, environmental agencies have enforced increasingly stringent N and P discharge regulations that require WRRFs to adopt more advanced N and P treatment processes (Son & Carlson, 2012; Carey & Migliaccio, 2009). Removal of diluted N and P at WRRFs requires resource-intensive processes that contribute up to 40% of a utility's energy usage, which in turn can account for 15-30% of a municipality's energy bill (DOE). Many WRRFs are approaching their design age (Water Infrastructure Network, 2000) and this provides an opportunity to design innovative wastewater management that takes advantage of the nutrients in wastewater.

Source separating and processing urine into a fertilizer provides a more efficient approach to wastewater nutrient management. Urine contributes approximately 80% of N and 50% of P in municipal wastewater despite making up less than 1% of the volume (Höglund, 2001). Urine separation can therefore separate the majority of N and P in wastewater prior to dilution. Furthermore, urine can be processed into fertilizers (Martin et al., 2020) to 1) reduce the demand for commercially produced inorganic fertilizers by generating approximately 30, 8, and 33% of the global per capita N, P and potassium demand, respectively (Wilsenach & Loosdrecht, 2003), and 2) reduce N and P loading at WRRFs to help them achieve increasingly stringent treatment standards and improve resource use efficiency of their treatment processes. For example, circularizing N and P flows by urine separation can reduce GHG emissions, energy demand, water consumption, and eutrophication potential as compared to conventional wastewater treatment and fertilizer production (Hilton et al., 2021) with implications for public and environmental health and sustainable waste management.

Despite the potential benefits of urine separation, there are significant challenges and uncertainties preventing large-scale implementation. Over the last 30 years, research has focused primarily on technologies to process urine into a safe fertilizer (Maurer et al., 2006) and on assessing the feasibility and efficiency of urine separation systems at building (Boyer & Saetta, 2019) and regional scales (Noe-Hays et al., 2015). While European municipalities have started to adopt urine separation at the building scale (Wald, 2022; Johansson et al., 2009), implementation in the US are primarily limited to household and community scales (Noe-Hays et al., 2015). Critical questions remain about the effects of urine separation and urine-derived fertilizer (UDF) use on public and environmental health, as well as the infrastructural and sociopolitical barriers to wide-scale adoption.

1.1 Overview of Dissertation

The objective of this dissertation is to understand how urine separation can improve public and environmental health in the sanitation, agriculture, and waste management sectors. Using urine as a fertilizer is not an uncommon practice at the household level in low/middle income countries (Bracken et al., 2007), and this dissertation builds on existing practices and research to expand this technology to building and institutional scales in high-income countries. Within this context, this dissertation addresses the impacts on public health for toilet users, environmental health during application, and urban sustainability at the system level (Figure 1-1). A review of these three types of impacts is provided in Chapter 2.

One aspect of public health is user exposure to virus emissions from toilet flushing, which can put users at risk of virus infection in confined spaces. In Chapter 3, flush experiments were conducted with urine-diverting (UDTs) and mix-flush toilets (MFTs) to compare their virus emission levels, especially for viruses excreted in urine. Virus emissions from the UDT were lower

than those from the MFT. Additionally, urine-associated viruses were emitted at high levels from the MFT, highlighting the importance of studying them in this context. Overall, this research demonstrates a potential benefit of urine separation on public health and provides critical information for future microbial risk assessments.

Beyond the toilet, separated urine can be processed into a urine-derived fertilizer (UDF) for field application. In Chapter 4, the impacts of a UDF on agricultural soil health and soil N cycling were compared with inorganic, organic, and combined use of organic fertilizers with a UDF in a greenhouse setting. UDF was just as effective in increasing plant yield as the inorganic fertilizer, but not as well as the organic fertilizer. Differences in the amount of carbon and the form of N in the organic fertilizer resulted in different N loss pathways as compared to the UDF and inorganic fertilizer. Additionally, combined use of UDF with compost reduced the N loss to harvested ratio as compared to sole UDF application, but it also increased N_2O emissions per gram of N applied and did not improve soil health. This chapter is one of the first studies to test the effects of UDF and UDF with compost on multiple indicators of soil health and N losses. Results from this chapter advances our understanding of UDF impacts on soil health and identifies a strategy for improving the ecological impacts of UDF application.

At the system level, urine separation can impact sustainability goals for urban municipalities. In Chapter 5, a material flow analysis (MFA) was used to quantify nutrient recovery benefits and impacts on urban nutrient efficient in New York City (NYC) for source-separated urine and other waste streams. I then applied a suitability analysis to identify sewersheds that are most suitable for urine separation in the city. A majority of the recoverable N and P were in source-separated urine and food waste, respectively. Consequently, urine separation has an important role for sustainable nutrient management, particularly for N, and provides benefits

beyond the WRRF and water utility. Additionally, we evaluated key criteria to consider for implementing urine separation in an urban setting using a suitability analysis.

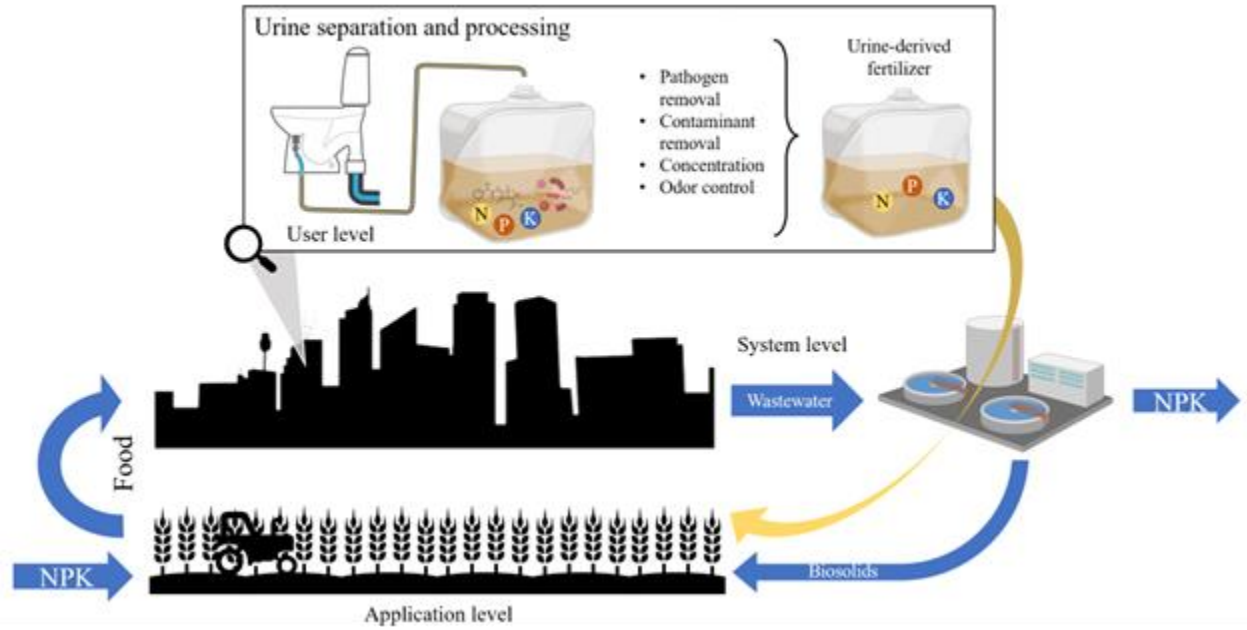

Figure 1-1. Nutrient (N, P, and potassium (K)) flows entering, exiting from, and exchanging between urban and agricultural communities. Food waste flows are not included but are a significant contribution to NPK emissions into the environment. Blue arrows are the current nutrient management practices, and the yellow arrow is the potential for urine separation to return nutrients from urban communities to agriculture.

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Chapter 2 Background and Motivation

As described in Chapter 1, urine separation has the potential to reduce environmental Nr and Pr, and subsequently alleviate the public and environmental health impacts associated with nutrient use. Impacts can occur at different levels of the technology, each of which involve different stakeholders. A number of questions remain that are critical for farmers and waste management agencies to make informed decisions about implementing urine separation. This chapter provides background on previous research on urine-associated viruses and emissions from toilet flushing, fertilizer impacts on soil health, and urban nutrient management, and identifies important research gaps that are addressed in the dissertation research.

2.1 User Level: Urine-Associated Virus Emissions From Flushing Toilets

Urine separation occurs by physically separating urine from toilet water and other excrements. Individuals would primarily interact with urine separation at the toilet. The standard toilet in the US and other high-income countries is a mix-flush toilet (MFT), which combines flush water, all excrements, and toilet paper prior to a flush. Urine-diverting toilets (UDTs) have a unique design that physically removes urine from the rest of the waste stream, often by having urine drained from the front of the bowl, and the remaining toilet contents conveyed to sewage pipes from the back of the bowl. For widespread adoption of urine separation, existing MFTs will need to be replaced by UDTs or UDTs will need to be installed in new institutional and commercial buildings. These areas are relevant for urine separation because they typically have high human activity and subsequently, high density of urine generation and greater likelihood of

implementation. There are a variety of commercially available UDTs, but flush UDTs, as opposed to dry UDTs, are most similar to a MFT. Even with a flushing UDT, users are required to adjust some of their toilet use behaviors (e.g., to prevent blockage of the urine diverter). Since toilets have a critical role in maintaining public health, changes made to toilet design and user interactions with the toilet may inadvertently impact public health.

Although toilets are one of the most important tools for proper sanitation, virus-laden bioaerosols generated from toilet flushing can expose users to infectious pathogens (Barker & Jones, 2005, Sassi et al., 2018, Gerba et al., 1975). Viruses are excreted at high levels in human excreta, a portion of which can be infectious (Atmar et al., 2008, Abney et al., 2021). When flushed, a toilet containing high levels of viruses can emit virus-containing particles into the restroom (Barker & Jones, 2005, Sassi et al., 2018, Gerba et al., 1975). Understanding the drivers of emission levels is important for assessing the risk of viral infections from toilet flushing. Risk assessments of disease from toilet flushing are limited and these studies have only focused on viruses excreted in feces from MFTs (Carducci et al., 2016, Overbey et al., 2021). They found that toilet flushing resulted in a higher risk of virus infection than working at a wastewater treatment plants or landfill (Carducci et al., 2016) and similar risk to being exposed to aerosols from a faulty drain in a sewer pipe (Shi et al., 2021), suggesting that toilet flushing can create a significant risk of virus infection. The risk of infection from toilet flushing is particularly relevant for health care settings, cruise ships, and airplanes, where virus-laden droplets and aerosols are emitted into confined spaces (Johnson et al., 2013).

Each person flushes a toilet, on average, 7 times a day. Of those flushes 6 are following urination-only toilet uses (Rose et al., 2015). Human viruses are present at high levels in urine. Specifically, up to 10^9 gene copies (gc) mL^{-1} of adenoviruses (HAdV, Hanaoka et al., 2019), $3x10^4$

gc mL^{-1} of West Nile virus (WNV, Barzon et al., 2013), and 10^{10} gc mL^{-1} of human polyomaviruses (HuPyV, Randhawa et al., 2004) as well as infectious WNV particles have been measured in urine. These viruses are associated with a range of illnesses including gastroenteritis, cold-like symptoms, and kidney deterioration. The urino-oral route is suspected to be an important source of infections for HuPyV (Berger et al., 2006), and for other viruses such as Cytomegalovirus (Cannon et al., 2011). Despite the high frequency of flushes with urine and the high levels of certain viruses in urine, there has been relatively limited research on urine-associated viruses in toilet settings. A better understanding of the emissions of urine-associated viruses from toilet flushing would inform the risks of infection associated with them.

UDTs have the potential to release fewer viruses, especially those excreted in urine, based on their design. Bioaerosol and droplet emissions from toilet flushing are dependent on its flush energy (Lai et al., 2018), which could differ between MFTs and UDTs. In institutional and commercial settings where restrooms are shared by the public and often with high traffic, MFTs typically have a flush-o-meter mechanism, which utilizes the building's water pressure for higher flush energies. Most flush UDTs are cistern toilets, which utilize a water tank for flushing and typically have a lower flush energy (Johnson et al., 2013). By replacing MFTs with UDTs that have lower flush energies, there may be lower virus emissions. Additionally, UDTs physically separate urine from the toilet bowl reservoir and send the urine directly down the drain. This prevents urine and its contents from being emitted during flushing. Of the viruses that are excreted into the toilet bowl, urine-associated viruses can be emitted at different levels from the UDT due to the different physical and chemical characteristics of urine and feces that affect mixing in the toilet bowl, adsorption of viruses to surfaces (Jeyachandran et al., 2010), and the formation of droplets (Okubo & Kobayashi, 1998). Understanding the impacts of toilet bowl contents after

excretion of waste and toilet type on emission levels can inform risk assessments of viral infections from toilet flushing.

2.2 Application Level: Comparative Soil Health Impacts of Using UDFs

Collected urine can be processed into a UDF to circularize N and P flows and offset the increasing demand for inorganic fertilizers. In 2019, it was estimated that the global demand for inorganic fertilizers was 106 Mt N, 46.3 Mt of phosphorus pentoxide, and 36.3 Mt potassium oxide (FAO, 2021). The demand for NPK was forecasted to increase by 6, 10, and 13%, respectively, between 2016 and 2022 (FAO, 2019). Inorganic fertilizers provide soluble nutrients for plants in high concentrations, but they can have negative impacts on soil health with long term use, including soil acidification, reduction of soil organic matter, and lower biodiversity (Singh, 2018). These effects have been contributing to global soil health degradation, risking the ability of soils to sustain their functions and ecosystem services (Lal et al., 1990). Alternative nutrient sources, such as legume nitrogen fixation and waste-derived fertilizers including UDFs, could help meet the increasing demand for fertilizers and rising interest in sustainable agricultural practice. The impact of these practices on soil health needs to be assessed for their wide-scale adoption.

Soil health is defined as the capacity of the soil to continue performing its ecological functions. It is measured by assessing soil health indicators that encompasses its physical, chemical, and microbiological characteristics: soil organic matter, bulk density, cation exchange capacity, pH, and microbial biomass (NRCS, n.d.). A particularly important component of soil health that has consequences for nutrient management is the nutrient use efficiency (NUE) and N cycling potential of the soil microbial community. These indicators help farmers make informed decisions on the type of fertilizers to use and understand fertilizer impacts on N losses as gaseous emissions and leachate (Stuart et al., 2014). N is primarily mediated by soil microbes, and thus

understanding soil microbiology is critical to understanding the impacts of fertilizer on soil N cycling (Nelson et al., 2016; Kuypers et al., 2018). Additionally, biological indicators are increasingly important for viewing soil as a living ecosystem, observing how soil organisms are affected by treatments added to soil, and understanding how soil biology impacts other health properties (Cardoso et al., 2013).

Fertilizer type, nutrient availability, and composition determine their impacts on soil health and N cycling. UDFs contain similar forms of N (e.g., urea and ammonium) as inorganic fertilizers, which suggests the two will have similar effects on N cycling. A review of studies comparing UDFs with inorganic fertilizers reported similar NUE rates for both, but UDFs had slightly lower values, at 75-100% of the NUE for inorganic fertilizers (Martin et al., 2020). These types of soluble fertilizers can increase N losses as leachate and N_2O emissions (Galloway & Cowling, 2002), but they provide an immediate pulse of plant-available N. In contrast, many waste-derived soil amendments such as manure, compost, and biosolids are rich in organic matter, which can improve water retention, nutrient mineralization, and soil physical properties (Johnston, 1986). However, these fertilizers are relatively low in nutrient content and availability as they are bound in recalcitrant organic matter and can reduce crop yield (De Ponti et al., 2012). Many waste-derived fertilizers also contain xenobiotic compounds such as heavy metals, trace contaminants, and pathogens that may negatively affect soil, plant, and human health (Mortvedt, 1995; Venglovsky et al., 2006). Urine also contains pathogens and trace contaminants, but their levels are lower than in biosolids and animal manure and they can be reduced relatively easily through processing (Martin et al., 2020). There are remaining questions about the comparative impacts of UDFs and organic fertilizers on soil N cycling and broader soil health to determine their respective roles in sustainable agriculture.

Like any fertilizer, UDF use should incorporate ecological nutrient management (ENM). ENM utilizes the ecological understanding of soil nutrient cycling to achieve optimal plant growth while maintaining long-term soil functionality and mitigating nutrient losses (Drinkwater & Snapp, 2022, Blesh et al., 2022). Inorganic fertilizers provide accessible N for crop growth, but this also results in high rates of N losses. UDFs have similar NUE to inorganic fertilizers, and thus may also cause high N losses. One strategy for reducing N loss and incorporating ENM into UDF use is combined application with organic fertilizers. In previous studies, substituting a portion of inorganic fertilizer with organic fertilizers increased crop yield by as much as 150% while improving soil enzymatic activity and nutrient availability (Jat et al., 2015). This suggests that combining UDFs with organic waste-derived fertilizers can also improve crop yields and soil qualities. Demonstrating that UDFs can offset inorganic fertilizers in an ENM context is critical for mitigating Nr emissions with urine separation.

A better understanding of the impacts that UDFs have on soil health can help farmers, consumers, policy makers, and utilities determine if UDFs are appropriate for their sustainability goals. When farmers in Switzerland and Germany were surveyed about UDFs, 24% of the 231 responses indicated an ecological concern with UDFs (Lienert & Larsen, 2010). The perception of the risks and benefits of UDFs was the strongest predictor of UDF acceptance. When information about the risks and benefits of UDFs was available, UDFs were perceived similarly to biosolids, both of which were perceived better than inorganic fertilizers (Cohen et al., 2020). To inform consumers and farmers about the environmental health impacts of UDFs, this chapter compares the soil health effects, particularly on N cycling, between UDFs, inorganic, and organic fertilizers and evaluates how UDFs can be used in an ENM context.

2.3 System Level: The Role of Urine Separation in Urban Nutrient Efficiency and Sustainable Waste Management

At the system level, urine separation can be implemented as a part of broader waste management efforts and improve urban nutrient management efficiency. Analyses of nutrient efficiency in large cities found that most nutrients enter cities from rural areas in the form of food and exit in reactive forms in waste that can contribute to nutrient pollution (Liang et al., 2019; Forkes, 2007, Vaudrey, 2017). Current waste management typically require high consumption of resources and have negative environmental consequences. For example, 50% of food waste is landfilled in the US (EGLE, n.d.), making it the largest contributor to the 122.6 million metric tons of carbon dioxide equivalent emitted from landfills per year (EPA, n.d.) in addition to the environmental impacts of food waste hauling. A significant source of urban nutrient pollution is wastewater (Liang et al., 2019, Forkes, 2007, Vaudrey, 2017), but 40 % of nutrients in wastewater were not removed in 2008 (NACWA, 2011), and an even smaller fraction is recovered. N and P removal from wastewater, particularly N, is an energy intensive process that contributes up to 40% of a utility's energy bill (DOE, n.d.).

WRRFs have a critical role in minimizing nutrient pollution by removing nutrients from wastewater and urine separation can improve WRRF treatment performance. Conventional biological nitrogen removal (BNR) is achieved by facilitating heterotrophic nitrifier and denitrifier growth which requires high sludge age, high oxygen requirements, and chemical inputs for supplemental carbon and alkalinity. Process modeling of BNR demonstrated improved efficiency of BNR processes and reduced resource needs at WRRFs with urine separation (Jimenez et al., 2015), at a reduction of 25-64% eutrophication potential, 29-47% GHG emissions, 50% of freshwater consumption, and 26-41% of energy demand as compared to conventional wastewater

treatment and fertilizer production (Hilton et al., 2020). For some WRRFs, urine separation may be one of the only options to meet increasingly stringent N discharge permits and increasing urban density. N inputs into urban WRRFs are expected to increase as more of the global population moves into cities, requiring WRRFs to expand or upgrade the existing infrastructure (Huh et al., 2020; Vörösmarty et al., 2010). In some cases, WRRFs are landlocked and cannot expand, creating a challenge for cities that are already limited in space. Urine separation can alleviate the burden on WRRFs to remove nutrients and allows them to redirect their focus to carbon/energy capture and trace contaminant removal but quantifying the scale and assessing the feasibility of implementing is necessary for adoption.

With growing interest in a circular nutrient economy, municipal sustainability goals often incorporate some form of nutrient recovery (DEP) to improve resource use efficiency of waste management and mitigate the environmental health impacts of insufficient nutrient management. Redirecting the focus of waste management from nutrient removal to recovery reduces Nr and Pr emissions into the environment and resource consumption by offsetting inorganic fertilizer production. A framework to quantify the recoverable nutrients of a city, compare it to the nutrient needs for local and regional agriculture, and quantify the environmental benefits of different levels and types of recovery is needed to characterize the relative importance of urine separation for sustainable waste management goals.

New York City (NYC), the largest US city by population, is an important model city for evaluating the benefits and feasibility of urine separation. NYC is rapidly developing, and more sustainable and cost-effective approaches are needed to accommodate the expected influx of wastewater at the city's WRRFs. In NYC alone, diversion of a small percentage of N from centralized sewage can lower costs for N removal by up to $9 million annually, reflecting the

reduced costs of carbon supplementation, and biosolids processing and disposition associated with the supplemental carbon addition. Urine separation operates at the intersection of the nutrient (food), energy, and water (NEW) cycles. Therefore, a key first step in understanding the relative role that urine separation could play in NYC is to use a Material Flow Analysis (MFA) to quantify and evaluate the N and P flows into and out of the city. Similar analyses of this scale in Detroit (Liang et al., 2019) and Toronto (Forkes, 2007) found that wastewater was a significant contribution to environmental N emissions and the recovery of nutrients from wastewater was limited by stringent restrictions on land application of biosolids. In NYC, a MFA of N and P can quantify the impacts of diverting nutrient flows from WRRFs on costs and energy demands and determine where nutrient inefficiencies are within the city. Specifically, the MFA can determine the level of urine separation that new developments must achieve to allow for complete elimination of the supplemental carbon addition at BNR WRRF facilities and minimize N emissions from WRRFs. If the findings of this analysis are similar to those of Detroit and other large cities, they could provide a strong case for urine separation in NYC and provide a template for other cities to determine how urine separation can fit into their broader sustainability strategies.

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Chapter 3 Virus Emissions From Toilet Flushing: Comparing Urine-Diverting to Mix-Flush Toilets

3.1 Introduction

Human viruses can be excreted at high levels in the vomit, feces, and urine of infected individuals. In feces, up to 10^{12} gene copies (gc) of viruses per wet gram can be excreted during norovirus (HuNoV), adenovirus (HAdV), and rotavirus infections (Abney et al., 2021; Atmar et al., 2008; Hanaoka et al., 2019). Many viruses are excreted in urine including West Nile, Nipah, Rabies, Rubella and Smallpox viruses (Abney et al., 2021; Barzon et al., 2013). HAdV and human polyomavirus (HPyV) JCPyV and BKPyV can be excreted at particularly high levels in urine, namely 10^{10} genome copy (gc) mL^{-1} (Hanaoka et al., 2019; Randhawa et al., 2004; Urbano et al., 2016). Whereas virus genome copies in human excreta can be high, the fraction of these genomes that are part of infectious virions are often not known.

Flushing a toilet generates droplets and aerosols. When the toilet contains excreta with viruses, flushing creates a possible route of exposure for individuals in the restroom. This is particularly relevant for HuNoV, HAdV, and HPyV as they can be excreted at high levels and are linked to a range of illnesses including gastroenteritis, cold-like symptoms, and kidney deterioration, respectively (CDC, n.d.; Ahsan & Shah, 2006; Minnesota Department of Health, n.d.). Viral infection risks from environmental exposure are commonly characterized using the

quantitative microbial risk assessment (QMRA) framework. A limited number of QMRAs have been conducted on virus emissions from toilet flushing. They suggest that this exposure route can create a significant risk of virus infection (Carducci et al., 2016; Overbey et al., 2021; Shi et al., 2021). For context, a study on the risks of HAdV infection from inhaling contaminated bioaerosols in different settings estimated that the exposure to aerosols from toilet flushing is higher than that of a faulty drain in a sewer pipe and from working at a wastewater treatment plant and municipal solid waste landfills (Carducci et al., 2016).

Understanding and mitigating pathogen exposure risks from toilet flushes requires an understanding of the amounts and drivers of virus emissions from toilets (D. L. Johnson et al., 2013). Viruses emitted from toilets are present in large droplets that settle onto surfaces or in small droplets that evaporate to become droplet nuclei (WHO, 2014). The latter remain in the air for hours and travel with the air plume. Most studies have recovered infectious viruses from the air (Barker & Jones, 2005) and on surrounding surfaces (Sassi et al., 2018) after flushing. Viruses on surfaces and in air may remain infective for hours to months (Abad et al., 1994; Gerba et al., 1975). For example, dried hepatitis A virus and human rotavirus on surfaces were infective for more than two months (Abad et al., 1994). Gerba et al. detected infectious viruses in bioaerosols for up to 6 hours after toilet flushing, some of which settled over a 4-hour period and contaminated nearby surfaces (Gerba et al., 1975). While these studies have focused on the detection of viruses in either bioaerosols or droplets, Gerba et al. captured total droplets that reach the toilet seat (Gerba et al., 1975). This approach can provide a more comprehensive understanding of the total virus emissions from flushing.

Toilet types can affect the amounts of viruses emitted. Previous studies have focused primarily on the common mix flush toilet (MFT), which flushes excrement from one compartment

(Barker & Jones, 2005; Gerba et al., 1975; Lai et al., 2018; Sassi et al., 2018). In these toilets, higher flush pressures are associated with higher virus emissions (D. Johnson et al., 2013; Lai et al., 2018). Alternative toilet technologies such as low and dual flush, composting, dry, and incinerating toilets can address the United Nations Sustainable Development Goal related to clean water and sanitation. Urine diverting toilets (UDTs), for example, separate urine from the rest of the waste stream at the toilet so that nutrients in urine can be recovered and processed into fertilizer (Udert & Wächter, 2012). In UDTs, urine can be collected with or without a flush, the latter likely leads to an overall decrease in virus emissions.

Studies on virus emissions from toilets have primarily focused on fecal-borne viruses (Barker & Jones, 2005; Gerba et al., 1975; Lai et al., 2018; Sassi et al., 2018) even though urine can contain high levels of human viruses. Urination accounts for an estimated 6 of the 7 daily toilet flushes per person; (Rose et al., 2015; Silverman et al., 2008) the higher frequency of urine flushes could result in higher exposures to urine-associated viruses. Virus release may be impacted by the toilet contents, such as the higher amount of protein in toilet water containing urine and feces, which can affect adsorption of viruses to surfaces (Jeyachandran et al., 2010) and the formation of droplets (Okubo & Kobayashi, 1998). Likewise, virus properties, such as size or isoelectric point (IEP), may also affect the release of viruses because these characteristics impact sorption (Gerba, 1984; Lai et al., 2018). Characterizing the influence of toilet water contents and virus characteristics on virus emissions is necessary to conduct informed risk assessments of toilet flushing.

This study aims to compare the total amount of virus emissions from flushing a UDT and an MFT. We used a method to capture viruses emitted from the toilet water during flushing and compared the emission levels from a commercially available UDT and MFT installed in an

institutional restroom. Experiments were conducted with two surrogate viruses; bacteriophages MS2 and T3 were selected as surrogates for human viruses common in urine and feces based on their similar physicochemical properties such as size, IEP, and presence of envelope (Table 3-1). We also tested the effect of protein in the toilet water on virus emissions, using a protein concentration that can be found in urine. The measured emission levels of the surrogate viruses were extrapolated to estimate the emissions of human viruses HuNoV, HAdV, and HPyV when the maximum reported viral loads are present in feces and urine and flushed from the MFT, flushed from the UDT, and diverted from the UDT.

3.2 Methods

Toilet information

Two toilets on a university campus were used in this study to represent a MFT and a UDT (Figure 3-1). The MFT was a Kohler model 4330-0 (Kohler, Wisconsin, USA) installed in 2016 with a small flush volume of 4.8 L and a large flush volume of 6.1 L. The UDT was a Wostman Ecoflush (Wostman, Saltsjö-boo, Sweden) installed in 2016 with a small flush volume of 0.3 L and a large flush volume of 2.5 L. Only the large flushes were used in this study.

Figure 3-1. Photos of the MFT (A) and UDT (B).

Virus surrogates

Bacteriophages MS2 and T3 were used as surrogates for human ssRNA viruses (e.g., HuNoV) and human dsDNA viruses (e.g., HPyV and HAdV), respectively (Table 3-1). MS2 is a non-enveloped, ssRNA bacteriophage that is commonly used to represent enteric viruses of similar size and genome type such as HuNoV. T3 is a non-enveloped, dsDNA bacteriophage that we used to represent human dsDNA viruses such as HPyV and HAdV. MS2 (ATCC 15597 - B1) and T3 (ATCC 11303 - B3) were propagated in their *E. coli* hosts (ATCC 15597 and 11303). Following chloroform extraction and PEG precipitation,(EPA, 2001) the viruses were further concentrated by 100kDa ultrafiltration (MilliporeSigma UFC901024) and filter sterilized with polyethersulfone (PES) 0.22 μm filters (Celltreat 229747). The concentrated virus stocks (10^{11} pfu mL^{-1}) were stored at 4^{o}C until use.

Table 3-1. Surrogate virus properties as well as properties of a human ssRNA virus found in feces and human dsDNA viruses found in urine.

Virus	Genome type	Genome size (kbp)	Size (nm)	Isoelectric point (IEP)	Max fecal concentration (gc g^{-1})	Max urine concentration (gc mL^{-1})	References
T3	dsDNA	38	50	2.0-5.0	N/A	N/A	28, 33
Human polyomavirus (HPyV)	dsDNA	5	44	N/A	N/A	10^{10}	29, 6
Adenovirus (HAdV)	dsDNA	26-45	90	4.5	10^{11}	10^{10}	30, 33, 34, 3
MS2	ssRNA	3.6	27	2.2-3.9	N/A	N/A	31, 33
Norovirus (HuNoV)	ssRNA	7.7	27-38	5.5-6.0	10^{12}	N/A	32, 33, 1

Virus solution

Based on reported mean 24-hour urine and feces shedding rates and median daily urination and defecation events, (Rose et al., 2015) we estimated that 130-710 mL of urine and 100-300 grams of feces are excreted into a toilet per event. Up to 10^{11} gc of HAdV (Lion et al., 2010) and 10^{12} gc of HuNoV (Atmar et al., 2008) have been measured in 1 g of feces and 10^{10} gc of HPyV (Urbano et al., 2016) and HAdV (Hanaoka et al., 2019) in 1 mL of urine. Based on typical virus concentrations and excrement amounts, we simulated a virus loading event by adding 10^{10} pfu of the surrogate viruses into the toilet. Specifically, 10 mL of stock containing 10^{9} pfu mL^{-1} of MS2 and T3 in phosphate buffered saline (PBS, Gibco 10010023) or PBS with 1% (83.3 mg L^{-1}) bovine serum albumin (BSA, Dot Scientific DSA30075-25), was added to the toilet. To test the effects of

protein content on virus emissions, experiments were conducted with and without BSA in the toilet bowl water. Based on previous reports of average urination and defecation frequency, urine and feces protein content, and urine and feces volume, (Rose et al., 2015) we estimate that up to 20.8 mg L^{-1} and 11.25 g L^{-1} of protein is present in the toilet following urination and defecation events, respectively.

Toilet experiments

Toilet bowl surfaces were sanitized with a 70% ethanol solution and flushed before experiments. A 10 mg L^{-1} sodium thiosulfate solution (Fisher Scientific S474-500) was added to quench residual chlorine in the water. Total and free chlorine were measured using a Hach meter DR 900 and DPD pillows (Hach 2105669) to ensure that both were below detection limits before experiments. Experiments were done in ambient conditions in the restroom.

For the flushing experiments, virus stock solution (10 mL) was added to the toilet water. In the UDT, most of the urine is diverted to the front of the bowl, but we performed our experiments to quantify virus emissions for urine and feces that are deposited into the toilet water. The toilet bowl water was mixed for 1 minute with a sterile serological pipette. Control experiments were done with ten mL of water added to a second MFT that was not used in the virus experiments. Prior to flushing, a 1-mL aliquot of toilet water was collected to quantify the initial virus concentration in the toilet bowl water. Polyethylene film (Office Depot 32007-OD) was placed over the toilet bowl area and the toilet was flushed (Figure 3-2). After one minute, the film was removed and a sterile cotton gauze pad (Dukal 2283) that had been soaked in a PBS solution containing 1% BSA was wiped over the film to recover viruses. The gauze pad was then placed in ten mL of 1% BSA in PBS and the solution was vortexed at maximum speed for one minute. Recovery experiments suggested that 88.8% of T3 and 130.1% of MS2 was recovered with this

approach (Appendix A). Infectious viruses were quantified using a double overlay agar plaque assay with a limit of quantification (LOQ) of 20-250 pfu mL^{-1} and a limit of detection (LOD) of 10 pfu mL^{-1}. Plaque assay negative and positive controls were conducted with each experiment to rule out contamination and problems with the assays. The fraction of viruses emitted was calculated as the total number of viruses (in PFU) recovered divided by the total number of viruses (in PFU) added to the toilet. When the amount recovered from the toilet was below the LOQ, the fraction of viruses emitted was calculated using the LOD of 10 pfu mL^{-1} in the numerator.

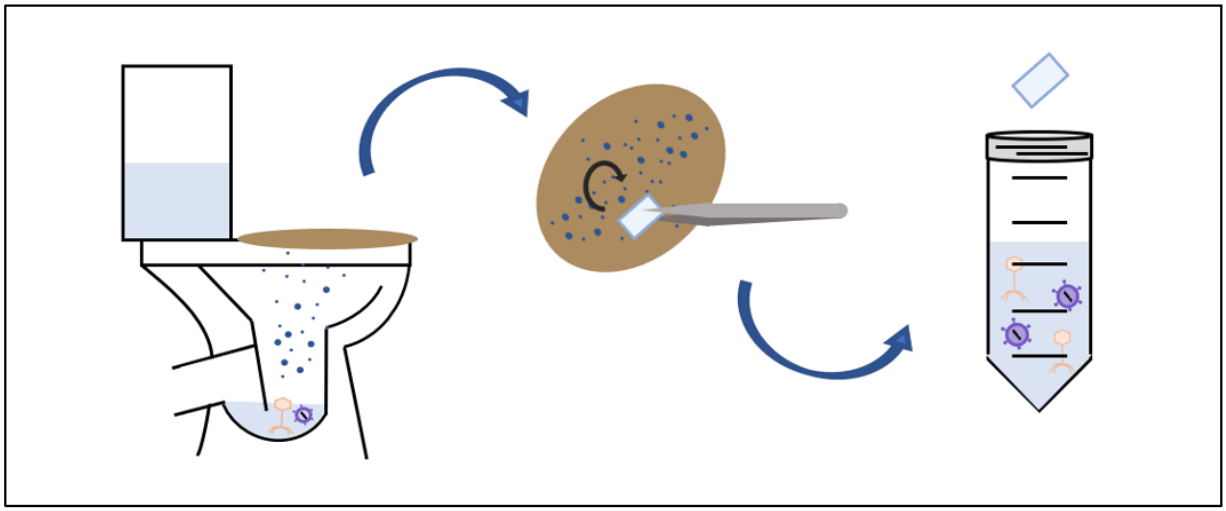

Figure 3-2. Graphical representation of the experiment procedure: a recovery surface was placed over the toilet bowl area and the toilet was flushed. Droplets and aerosols emitted on the recovery surface were captured with a soaked cotton gauze pad and suspended into solution by vortexing.

Particle size distribution of the virus emissions

We used toilet emission particle size distributions reported in a previous study (Knowlton et al., 2018) and our measured fraction of viruses emitted to estimate the virus emissions in different particle size ranges. The study by Knowlton et al. conducted ten flushes with fecal waste in a hospital toilet and measured droplets near the toilet before and one minute after the flush. They categorized droplets into six bin sizes, including 0.3, 0.5, 1, 3, 5, and 10 μm (Knowlton et al., 2018). We used WebPlotDigitizer to extract the average particle concentration in each bin

presented in their publication and used the resulting data to calculate the percentage of the total emission volume emitted in each size bin. Using the assumption that the number of viruses in a particle is directly proportional to the volume of the particle, we calculated the fraction of viruses emitted for each bin size.

Data analysis

Plaque assay data were log-transformed and analyzed using GraphPad Prism. A Shapiro-Wilk test was used to validate the lognormality of the data and multiple unpaired, parametric student-t tests with Welch correction were performed to assess statistical significance ($p < 0.05$).

3.3 Results and Discussion

Emissions from the UDT were significantly lower than from the MFT.

Of the 10^{10} pfu MS2 and T3 added to the MFT water, an average of 25×10^2 pfu MS2 and 4.3×10^2 for T3 were emitted when the toilet water was not supplemented with protein. In terms of the fractions of total viruses added to the toilet, these values are equivalent to 9.6×10^{-7} for MS2 and 13×10^{-7} for T3. Compared to previous studies in which viruses were flushed with an MFT and then measured either in air or on surfaces, our results are slightly higher. For example, 2.4×10^3 MS2 pfu m^{-3} was measured in the air after a mixed flush water tank toilet was flushed containing 10^{10} pfu (Barker & Jones, 2005). This is equivalent to 2.4×10^{-7} fraction of added viruses. Sassi et al. quantified 1.9×10^4 pfu of MS2 on the surrounding floor and 3.4×10^5 pfu on the surface of the toilet seat after flushing a toilet containing equivalent to 0.19×10^{-7} and 3.4×10^{-7} fractions of the total added viruses (10^{12} pfu of MS2), respectively (Sassi et al., 2018). Compared to a study in which all droplets at the toilet seat level were measured, our results were similar – Gerba et al. measured an average of 8.6×10^2 pfu of poliovirus, which is equivalent to 30×10^{-7} fraction of added viruses (Gerba et al., 1975). The higher fractions measured in these

studies may be reflective of the total emissions capture method as compared to separate air and surface sampling in previous studies.

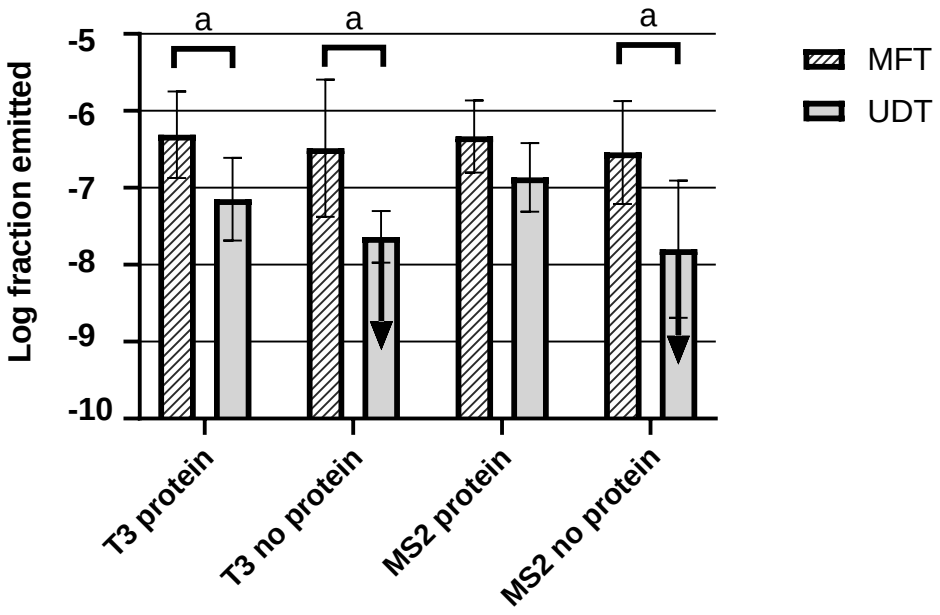

Figure 3-3. Virus emissions from toilet flushing. The log fraction emitted is the average fraction of viruses (pfu) that was captured on the recovery surface, normalized by the number of viruses (pfu) added into the toilet water, then $\log_{10}$ transformed. Error bars indicate the standard deviation for each set of experiments (N=6). The letter a above the bars indicates statistically significant differences at $p < 0.05$ using unpaired, parametric student-t tests with Welch correction. A down arrow is used to represent mean bars that include data below the LOD.

Fewer viruses were emitted when the same number of viruses added to the toilet water were flushed with the UDT compared to the MFT. Specifically, the mean fractions of viruses emitted from the UDT were 1.6×10^{-8} for MS2 and 2.3×10^{-8} for T3 (Figure 3-3). On average, the fraction of viruses emitted from the MFT was greater than that of the UDT by 1.2-$\log_{10}$ ($p = 0.02$) and 1.3-$\log_{10}$ ($p = 0.02$) for T3 and MS2, respectively, when not amended with protein. The MFT emitted more virus than the UFT by as much as 2.0-$\log_{10}$ for T3 and 2.3-$\log_{10}$ for MS2 (Figure 3-4). The same trends between the toilets were observed when protein was added to the toilet bowl (Figures 3-3 and 3-4). It is worth noting that in several of the UDT experiments, T3 or MS2 levels recovered following the flush were below the detection limit, whereas T3 and MS2 were always

recovered following flushes with the MFT (Figure 3-3). These results demonstrate that the toilet type affects the number of viruses emitted from the toilets.

The differences in the virus emissions between the two toilet types can be driven by the different flush energies as well as the different toilet water volumes. The flush volume of the MFT is larger than that of the UDT by 1.3 L, which may generate more water droplets. Flush energy is a toilet characteristic that has been studied more extensively in previous research.[19,20,36] Like many residential toilets, the UDT has a water tank attached to the toilet bowl that provides pressure to flush the toilet. The MFT is a commercial toilet that utilizes a flushometer to draw water pressure from the water supply line. Typically, toilets with flushometers have a higher flush pressure than water tank toilets (D. Johnson et al., 2013). In a previous study, Lai et al. found that flushometer toilets resulted in higher bacterial emission levels than from a water tank toilet. Additionally, they found statistically significant greater emission levels in a flushometer toilet with 400 kPa flush than with a 200 kPa flush (Lai et al., 2018). A similar observation was made in water tank toilets (Newsom, 1972). Combined, our current results with viruses and previous studies with bacteria suggest that the type of toilet and specifically flush pressure are important for the emissions of viruses from the toilet water.

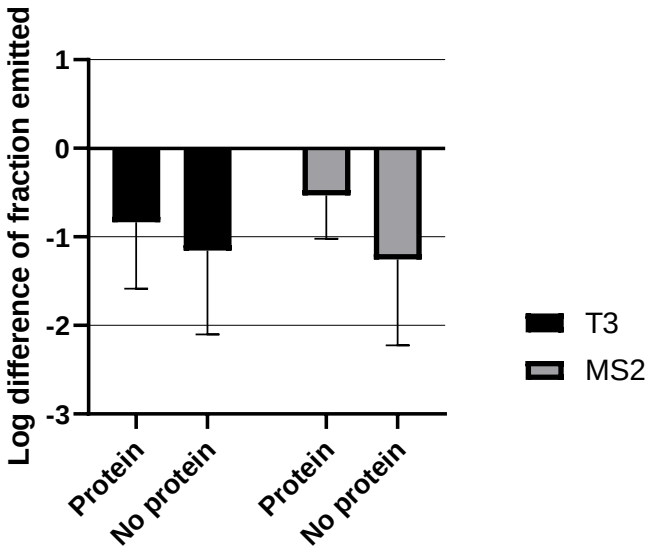

Figure 3-4. Comparing the log difference of fraction emitted for all conditions. Log difference above zero indicates that the fraction emitted was greater for the UDT. Log difference below zero indicates that the fraction emitted was greater for the MFT. For all experimental conditions, the MFT emitted more viruses. Error bars indicate the standard deviation for each set of experiments (N=6).

Protein in the toilet resulted in slightly greater emissions.

We tested the effect of protein added to the toilet bowl along with the viruses, as urine and feces result in elevated toilet water protein levels. The experiments with protein added to the toilet bowl water consistently yielded greater virus emissions than the experiments without added protein (Figure 3-3). In the MFT, T3 and MS2 emissions in the experiments with added protein were greater than in the experiments without added protein by 0.17-$\log_{10}$ ($p = 0.70$) and 0.21-$\log_{10}$ ($p = 0.55$), respectively. These differences were more pronounced with the UDT toilet, with T3 and MS2 emissions in the protein experiments greater by 0.49-$\log_{10}$ ($p = 0.09$) and 0.93-$\log_{10}$ ($p = 0.05$), respectively.

Protein content can affect virus adsorption to surfaces and aerosolization of the toilet contents. For example, viruses adsorb to toilet surfaces, (Gerba et al., 1975) and the extent of sorption can be affected by protein content in urine and feces (Jeyachandran et al., 2010). In

addition to competing for adsorption sites, protein components in the toilet water can affect how droplets are formed during a flush (Okubo & Kobayashi, 1998). Namely, the presence of protein can reduce surface tension of droplets, (Alvarez et al., 2008) subsequently reducing their size (Tolman, 2004). It remains unclear, however if the total volume of all emissions is affected by the presence of protein. The addition of BSA generally resulted in higher emissions from the toilet, but the differences were not statistically significant.

Protein in the toilet resulted in differences between the two toilets.

The difference between viruses emitted from the MFT and the UDT was greater when the toilet water was not supplemented with protein. Specifically, for MS2 the difference in the fraction emitted between the toilets was 0.53-$\log_{10}$ with protein present and 1.3-$\log_{10}$ without protein present. For T3, the difference was 0.83-$\log_{10}$ with protein present and 1.2-$\log_{10}$ without protein present (Figure 3-4). While all experiments in the MFT and all experiments with supplemental protein added were above the LOQ, five and two, out of six replicates in the UDT without added protein were below the LOD for T3 and MS2, respectively (Table A-1). One possible explanation for our observed impacts from protein and toilet type is that there are compounding effects of protein, toilet flush energy, flush volume, and virus types. Lai et al. previously correlated variables such as pathogen size and flush pressure to pathogen emission levels (Lai et al., 2018) although they did not consider protein content or viruses. Future work should explore correlations between protein content and additional matrix properties (e.g., presence of feces) with virus emissions from toilet flushing.

MS2 and T3 were emitted at similar levels.

In all conditions except the UDT flushes amended with protein, T3 was emitted at slightly greater fractions than MS2. The differences in fractions emitted between MS2 and T3 were

between 0.02-$\log_{10}$ and 0.28-$\log_{10}$ across all conditions ($0.34 < p < 0.95$). The similarity between MS2 and T3 emissions suggests that the differences in MS2 and T3 properties, namely their size and isoelectric points, did not affect their emissions in a considerable manner. T3 is approximately 1.9-fold larger than MS2 and the two viruses have similar isoelectric points (~3.5 for MS2 and 2-5 for viruses similar to T3) (Michen & Graule, 2009). Other studies have suggested that with smaller pathogen size, emissions may be greater. For example, Lai et al. observed that the smaller *Staphylococcus epidermidis* had emission levels 21 times greater than that of *E. coli* and found a statistically significant correlation between bacterial size and the amount of bacteria emitted in toilet flushes (Lai et al., 2018). Likewise, MS2 viruses were emitted at higher levels than *E. coli,* and this difference was attributed to the different organism sizes (Gerba et al., 1975). The fact that MS2 and T3 are more similar in size than *S. epidermidis* vs. *E. coli* and *E. coli* vs MS2 may explain why we did not observe more emissions for the smaller virus. MS2 and T3 are similar in size and have similar IEPs to many human viruses that are excreted in urine and feces (e.g., HuNoV: 5.5-6, HAdV: 4.5, HPyV: N/A); our results suggest that the emission behaviors of these viruses may be similar to the surrogate viruses measured here. More research is necessary to understand the role that virus size and IEP play on emissions. Likewise, the impact of lipid envelopes on some viruses should be studied to understand the emission behaviors of viruses like coronaviruses, Influenza virus, and Ebola virus. These types of virus characteristics could affect how viruses partition to the toilet bowl or to fecal matter present in the toilet water.

Estimated human virus emissions can exceed infectious levels.

We applied the fractions of the surrogate viruses emitted in our study, adjusted by the recovery experiments (Appendix A) (calculated with pfu) and the levels of human viruses in feces and urine reported in the literature (as gc) to estimate the range of human viruses that could be

emitted from those deposited in the toilet water (Table 3-2). We first conducted control experiments to confirm that the ratios of gc to pfu in the virus solutions added into the toilet were not significantly different than those of the recovered samples from the toilet flush (Table A-2 and A-3).

In feces, HuNoV and HAdV can be excreted into and emitted from toilets at a range of levels. HuNoV levels in human stool samples, for example, have been reported in the range of 10^4-10^{12} gc per wet gram (Atmar et al., 2008; Chan et al., 2006). An infected individual excretes an average of 128 g of feces per day and defecates, on average, 1.2 times per day, averaging 107 g of feces per event (Rose et al., 2015). The estimated viral loading into a toilet per event for HuNoV is therefore 10^6 – 10^{14} gc. Based on the emitted fractions of MS2, the surrogate virus we used for HuNoV, we estimated that HuNoV can be emitted at up to 39 x 10^7 and 6.7 x 10^7 gc per flushing event from the MFT and UDT, respectively. Similarly, HAdV is excreted in feces at a reported maximum of 10^{11} gc per gram of feces (Lion et al., 2010). Based on our results for T3, the surrogate virus we used for HAdV, we estimate HAdV is emitted at up to 7.5 x 10^7 gc per event from the MFT and 0.27 x 10^7 gc from the UDT (Table 3-2). For HAdV, the emission levels of viruses in the toilet water were similar from the MFT and UDT.

Virus emissions from flushing during urination events can be as high as those from defecation events. We used the maximum concentrations reported for HAdV and HPyV in urine (10^{10} gc mL^{-1}) (Hanaoka et al., 2019; Randhawa et al., 2004; Urbano et al., 2016) and the average volume of urination events (237 mL calculated from the reported daily volume and frequency) (Rose et al., 2015) to estimate the amounts of these viruses that are present in toilet bowl water following urination events (10^{12} gc). Based on the emitted fractions for T3 measured in this study, we estimated that HAdV and HPyV can be emitted at up to 166 x 10^5 gc per urination event in the

MFT and 6 x 10^5 gc in the UDT (Table 3-2). While we report the maximum emissions for HAdV and HPyV from the UDT here, the toilet bowl of the UDT is designed to physically separate urine from the toilet water; consequently, the number of viruses emitted from the UDT from urine events is also affected by the efficiency of the UDT at separating urine from the toilet water. If all urine was separated by the UDT, the emissions for viruses excreted in urine would be zero and up to 7-$\log_{10}$ fewer viruses from urine would be emitted with the UDT compared to the MFT.

Table 3-2. Estimated virus emissions from each toilet for HAdV, HPyV, and HuNoV using the minimum and maximum fractions emitted from experiments with surrogate viruses.

	MFT		**UDT**	
	Minimum	Maximum	Minimum	Maximum
HPyV, HAdV in urine (gc/event)	4.6 x 10^4	1.7 x 10^7	1.8 x 10^4*	5.9 x 10^5
HAdV in feces (gc/event)	2.1 x 10^5	7.5 x 10^7	8.1 x 10^4*	2.7 x 10^6
HuNoV in feces (gc/event)	3.9 x 10^6	3.9 x 10^8	2.8 x 10^5*	6.7 x 10^7

Data with * indicates that the minimum fraction emitted used in the calculation was below the LOD.

We estimated that HuNoV can be emitted at up to 10^8 gc from the MFT and 10^7 gc from the UDT per flush in the worst-case scenario. The worst-case scenario was calculated using the maximum reported viral loads in feces and urine and the highest fraction emitted for the surrogate virus in our study. Given that the probability of infection from human challenge experiments is 0.1 for a dose of 10^3 gc HuNoV to 0.7 for a dose of 10^8 gc, (Teunis et al., 2008) our estimates suggest that the amounts of HuNoV emitted from flushing are within the range of the infectious doses.

Infectious doses of HAdV from ingestion are in the range of 10-500 $TCID_{50}$ (Gutekunst et al., 1967). Aerosol infectious doses are lower, at approximately 0.5 $TCID_{50}$ (Yezli & Otter, 2011). Assuming a gc to infectious virus ratio of 1×10^{-3} for HAdV, (McBride et al., 2013) up to 8×10^{4} and 2×10^{4} infectious HAdV viruses are emitted with a flush of feces or urine in the MFT, respectively; these values are within the range of infectious doses. Infectious doses for HPyV are not available at the time of this study, and so we cannot compare the estimated amount of HPyV emitted and infectious doses.

Our emission results combined with literature on shedding and infectious doses suggest that some viruses may be emitted from toilet flushing at levels that approach or exceed infectious doses. It is highly unlikely, however, that people in a restroom would be exposed to the total number of viruses emitted from a flush. Emissions that are smaller than 5 μm evaporate quickly and travel with the air plume (WHO, 2014). Environmental factors such as humidity, temperature and air exchange rate will impact the density of infectious viruses in the restroom after a flush, as will user-dependent factors such as inhalation rate and time spent in the restroom. Emissions greater than 5 μm settle onto fomites near the toilet. Humidity, temperature, and surface material will affect the rate that these viruses are inactivated, and user behaviors such as contact with fomites, handwashing, and time spent in the restroom can affect exposure to infectious viruses.

Most studies measured either droplets or aerosols generated from flushing, whereas we captured the total number of infectious viruses emitted from the toilet bowl, similar to a method used by Gerba et al. (Gerba et al., 1975). The total emission approach bypasses the challenges of choosing an appropriate sampling location and the assumption that the flush emissions are uniformly distributed in the air and surfaces surrounding the toilet. The emissions we measured estimate the total virus emissions directly from the flush, leading to more comprehensive data for

exposure and risk assessments. A QMRA using our emission levels can be used to quantify the risk of virus infection from toilet flushing and inform toilet use and maintenance behaviors. Because QMRAs are unique to specific viruses and exposure routes, namely inhalation and ingestion, we estimated the fraction of viruses emitted in different particle size ranges using the particle size distribution of flush emissions from a previous study (Knowlton et al., 2018) and our average fraction emitted for the surrogate viruses when protein was added to the toilet (Table 3-3). More viruses are emitted in the larger particle size ranges due to the larger volume of the larger particles. In future work, this data can be coupled with virus loading into the toilet, dose-response data, exposure time, and contact and inhalation frequency to quantify an individual's exposure to infectious virus emissions from toilet flushing and their risk of infection.

Table 3-3. Log_{10} fraction emitted of different particle sizes from toilet flushing.

	dsDNA viruses		**ssRNA viruses**	
Particle size (µm)	**MFT**	**UDT**	**MFT**	**UDT**
0.3	-7.5	-8.3	-7.7	-8.2
0.5	-7.4	-8.3	-7.7	-8.2
1	-7.2	-8.1	-7.5	-7.9
3	-6.6	-7.5	-6.9	-7.4
5	-6.4	-7.3	-6.7	-7.1
10	-6.7	-7.6	-7.0	-7.5

3.4 Conclusions and Future Work

This work expands on toilet flushing as a source of exposure to viruses and compares emission levels of fecal-borne and urine-associated viruses from flushing a MFT and a UDT to inform future QMRAs. Like previous studies, we report that virus emissions were lower from the toilet with lower flush pressure – we found that the UDT emitted fewer viruses than MFTs. For viruses excreted in feces, emissions of viruses excreted into the toilet water were reduced by up to 2.3-$\log_{10}$ per flush in the UDT. Because UDTs can collect urine from the toilet bowl without a flush, up to 7-$\log_{10}$ fewer viruses from urine can be emitted with the UDT compared to the MFT. In MFTs, specific focus on urine-associated viruses is warranted as they are excreted at high levels into toilets, higher protein levels in urine can increase their emission levels, and urine accounts for most daily toilet flushes. In particular, HPyV can be emitted at high levels, but more data including infectious virus loading in urine and dose-response data are necessary to quantify the risk of transmission from urine during toilet flushing. MS2 and T3 were emitted at similar levels, but more work is needed to confirm that virus structure does not affect emissions, including the presence of a lipid envelope. Although we used T3 as a surrogate virus for HAdV because they are both dsDNA viruses that can be excreted in urine, HAdV is a larger virus (Table 3-1), and this difference may result in lower emissions. While our results demonstrate that toilet type had the greatest effect on virus emission levels of the factors we studied, future work should evaluate higher protein levels, incorporation in feces (e.g., sorption to organic material and emissions of fecal particles containing viruses), and a range of flush energies on virus emissions. A systematic approach to evaluating these properties is important for understanding how toilet design and environmental controls can affect human exposure to viruses from toilet flushing. Finally, the

emission data we gathered in this study can be used in future QMRAs to quantify an individual's risk of infection from viruses emitted during toilet flushing.

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Chapter 4 Application of Urine-Derived Fertilizers for Ecological Nutrient Management

4.1 Introduction

Current nitrogen (N) management is resource-intensive and has negative impacts on environmental health. Inert N is extracted from the atmosphere and converted into reactive forms of N (Nr) primarily through the energy-intensive Haber-Bosch process (Galloway et al., 2004). In 2005, 86% of N produced from Haber-Bosch was used as inorganic fertilizers (Galloway et al., 2004), but as much as 96% of the N applied ended up as losses and waste to the environment (Galloway & Cowling, 2002). Of the N that is incorporated into crop biomass and consumed, 80% is excreted in human urine and mixed into wastewater (Bingham, 2003). In 2008, 40% of wastewater N was directly released into the environment (NACWA, 2011) and the remaining fraction was converted back into inert forms using energy-intensive processes. The net increase in and displacement of environmental Nr have increased atmospheric ozone concentrations, soil acidity, and hypoxia in surface water (Wolfe & Patz, 2002; Mallin & Cahoon, 2020; Carpenter et al., 1998; Galloway & Cowling, 2002), resulting in direct and indirect damages to ecological and human health. There is an increasingly urgent need to reduce agricultural and wastewater contributions to environmental Nr.

Processing human urine into a urine-derived fertilizer (UDF) can circularize N in the agrifood system and reduce Nr attributed to wastewater emissions and inorganic fertilizer production. Urine can be separated at the toilet to prevent dilution with conveyance water and contamination with chemicals in greywater and fecal-borne pathogens, allowing for a more

concentrated and cleaner waste-derived fertilizer. In some cases, urine separation can reduce greenhouse gas (GHG) emissions by 47%, energy use by 41%, water consumption by 50%, and eutrophication potential by 64%, as compared to conventional wastewater treatment and fertilizer production (Hilton et al., 2021). Globally, if UDFs were used to replace inorganic fertilizers, the recovered nutrients would offset an estimated 16-21% of current inorganic N fertilizer use (Trimmer et al., 2019), contributing to the Sustainable Development Goals of zero hunger, clean water and sanitation for all, and climate change solutions (Larsen et al., 2021).

UDFs are considered waste-derived fertilizers but are chemically more similar to inorganic fertilizers than other waste-derived fertilizers. When excreted, urine primarily contains N in the form of urea. After processing, urea can be hydrolyzed to form NH_4^+ and nitrified to form NO_3^-. These forms of N are also commonly used in inorganic fertilizers. Prior field experiments revealed that UDFs performed as well as inorganic fertilizers in increasing plant yield and had N use efficiencies (NUE), which is the ratio of N harvested to N applied, similar to inorganic fertilizers (Martin et al., 2020). In contrast, other waste-derived fertilizers such as composted municipal solid waste, animal manure, and biosolids contain organically-bound N, which can result in lower NUE in the short term (Martin et al., 2021). Beyond N, many waste-derived fertilizers are also high in organic matter, which can benefit soil health (e.g., improve water retention, nutrient exchange capacity, and microbial activity) and also influence the biogeochemical pathway for N in soil.

Fertilizers can often contain undesirable constituents that might cause a public health risk or negatively impact soil health. Inorganic fertilizers, particularly those that contain phosphates can be high in heavy metals (Nacke et al., 2013). Depending on the waste source, waste-derived fertilizers can also contain heavy metals, as well as pathogens, pharmaceutical compounds, personal care product compounds, and polyfluoroalkyl substances (PFAS) (Rashmi et al., 2020;

Bloem et al., 2017; O'Connor et al., 2022). Compared to other waste-derived fertilizers, UDFs contain lower levels of heavy metals, pathogens, and contaminants (Martin et al., 2020). There are established processes for removing contaminants (Kopping et al., 2020; Udert & Wächter, 2012) and pathogens (Martin et al., 2020; Höglund, 2001) as urine is converted to a fertilizer. The remaining constituents in UDFs such as salt, micronutrients, and enzymes do not post a direct public health threat but might affect soil health. Whereas previous research has focused primarily on assessing and addressing risks posed by UDFs on public health, there is little data on how UDFs impact soil health. A comprehensive assessment of how UDFs affect soil health in contrast to inorganic and other waste-derived fertilizers can inform stakeholders in the agricultural sector about the use of UDFs.

An ecological nutrient management (ENM) approach to using UDFs is essential to their role in mitigating Nr emissions. ENM utilizes the ecological understanding of soil nutrient cycling to achieve optimal plant growth while maintaining long-term soil functionality and mitigating nutrient losses (Drinkwater & Snapp, 2007; Drinkwater & Snapp, 2022; Blesh et al., 2022). In conventional agriculture, only 50% of N applied is in the harvested crop (Galloway & Cowling, 2002) and the remaining N is lost as crop residue, leachate, and gaseous emissions. Soluble forms of N in UDFs suggest that N losses from UDFs would be similar to those of inorganic fertilizers. One ENM strategy for reducing N losses from soluble fertilizers is to apply them with slow-release, organic fertility amendments such as compost. Applying inorganic fertilizers with organic amendments increased crop yield by as much as 150% with improvements to soil enzymatic activity and nutrient availability (Jat et al., 2015) while reducing N in the leachate (Yang et al., 2021). If applying UDFs with organic fertilizers yields similar results, UDFs could play a critical role in simultaneously mitigating Nr emissions from waste management and agriculture. However,

increased mineralizable C in the soil (Mitchell et al., 2013), as a result of organic fertilizer application, can increase N_2O emissions (Ding et al., 2007). A better understanding of N loss magnitude and pathways from using UDFs alone and with organic amendments can inform improved uses of UDFs.

The high potential for UDFs to mitigate Nr emissions and provide environmental benefits for the food-energy-water nexus suggests the need to improve their use from an ENM perspective for more sustainable agriculture. NUE is one indicator of potential conservation of nutrients at the ecosystem scale, but more information is needed about the magnitude and types of N loss pathways. This is particularly important for comparing UDFs with organic amendments or combined use with organic amendments because they have different forms of N that affect their availability to plants. NUE can be estimated as the ratio of total N harvested to total N applied, however N loss is better understood by directly measuring leachate and gaseous emissions. These processes are affected by microbial N cycling and the potential for N cycling can be informed by the microbial community composition and the quantity of N functional genes (NFGs) involved in the N cycle (Levy-Booth et al., 2014). Commonly measured NFGs target different steps in the nitrification and denitrification processes including ammonia monooxygenase (amoA), nitrite reductase (nirS), and nitrous oxide reductase (nosZ). These microbial indicators, in addition to other soil biological health indicators, are particularly suitable for studying short term changes in N cycling (Gil-Sotres et al., 2005) such as the first season after replacing inorganic fertilizers with UDF or organic amendments. For example, plant growth promoting benefits have been observed with vermicompost in just one growing season and with small quantities (Arancon et al., 2004, 2005; Lazcano et al., 2013).

In this study, we conducted a greenhouse experiment to evaluate soil health indicators, NUE, and N cycling for various treatments including UDF, inorganic, organic, and combined use of organic with UDF and inorganic fertilizer. We hypothesized that the similar forms of N in UDF and inorganic fertilizer would result in similar increases in plant yield, NUE, and N losses via leaching, all of which would be higher for the compost treatment. We also hypothesized that adding compost to UDFs and inorganic fertilizers would increase plant yield, improve soil health, and increase N loss as gaseous emissions by providing a source of organic carbon. Due to the short-term nature of our experiment, the results are applicable in the context of changing fertilizer source for soils that have received long-term applications of inorganic fertilizers for intensive agriculture. In addition to measuring N losses directly as N_2O emissions and N in leachate, we selected soil health and N cycling indicators that can be more sensitive to short-term changes such as particulate organic matter (POM), mineral associated organic matter (MAOM), enzyme activity, and NFG abundances. This study advances our understanding of fertilizer impacts on soil N cycling and soil health and informs ecologically-sound use of UDFs to advance sustainable N management goals.

4.2 Methods

Potted plant greenhouse experiment design

We conducted an experiment using potted plants in a controlled environment greenhouse at the University of Michigan Matthei Botanical Garden from February 21 to April 18, 2023. Green Wave mustard (*Brassica juncea*) was chosen for the experiment because it grows quickly and requires a moderate amount of N. Soil for the experiment was collected from the top 0-15 cm of an agricultural field in Washtenaw County, Michigan in November 2022. The field had a long-term management history of corn production with high inputs of inorganic N fertilizer. We selected

this soil to evaluate the short-term effects of changing the main N source on soil health and N cycling processes. The soil was a slightly acidic sandy loam with approximately 1% organic matter (OM). Macronutrient levels in the soil are summarized in Table 4-1. The soil was sieved to 2 mm and well-mixed with vermiculite (Ferry-Morse, Norton, MA) at one volume of vermiculite to four volumes of soil.

Table 4-1. Soil physical and chemical properties

Property	Value	Units
Organic matter	0.8-1.1	%
POM C	0.2	%
MAOM C	3.3	%
pH	5.7-6.3	N/A
Nitrate	7-8	ppm
Phosphorus (weak bray)	61-88	ppm
Potassium	40-78	ppm

Treatment description

The six treatments included the following: control (CL), inorganic fertilizer (I), UDF only (UDF), compost only (CT), inorganic fertilizer and compost (IC), and UDF and compost (UC). The treatments were 21-0-0 ammonium sulfate (Ferti-lome, Bonham, TX) for inorganic N fertilizer, 3-4-3 Revita Pro Plus (Ohio Earth Food, Hartville, OH) for compost, and UDF that was collected and processed by Rich Earth Institute (Brattleboro, VT). The Revita Pro Plus compost is a blend of composted poultry manure, Leonardite ore, and kelp. We purchased UDF from a community-scale UDF system that collected urine, concentrated it using a freeze-thaw system (Noe-Hays et al., 2022), and pasteurized it at 80°C for 90 seconds (WHO, 2006). After receiving

the UDF, we adjusted the pH to 7 using 5 M HCl and mixed it with 40 g of AquaSorb activated carbon (Jacobi Carbons, Columbus, OH) per L of UDF on an orbital shaker at 180 RPM for 24 hours to remove organic contaminants as demonstrated in previous studies (Kopping et al., 2020). We conducted a bench-scale activated carbon experiment with ammonium chloride to ensure that there were no significant losses of ammonium. The chemical properties of the final amendments applied to the soil and the amount applied to each pot are summarized in Table 4-2.

Table 4-2. Fertilizer properties and amount applied

	Inorganic	Compost	UDF
Product	Ammonium sulfate	Ohio Earth Food RevitaPro Plus	Rich Earth Institute UDF
pH	7.0	6.9	7.7
N (%)	20.8	5.7	28.9
P (%)	0.1	2.2	1.9
C (%)	0.9	30.5	23.6
C:N	0.04	5.4	0.8
S (%)	24.1	1.8	1.4
Na (%)	0.03	0.5	8.1
Amount applied per pot for each treatment	0.5 g – I 0.2 g – IC	3.9 g – CT 1.9 g – IC, UC	10.5 mL – U 5.2 mL – UC

All treatments received equal plant available N (PAN) application rates of 50 mg N/kg soil or approximately 127 kg/ha, which is based on nutrient guidelines from Cornell University (Reiners et al., 2019). PAN was calculated as the sum of the inorganic N and the mineralizable organic N (Equation 4-1, Ryals et al., 2021), where K_{min} is the mineralizable N. For poultry litter, we assumed the K_{min} was 39.6% (Geisseler et al., 2021). Organic N (Org-N) was less than 1% of

the total N for both UDF and inorganic fertilizer and was therefore not included in the calculation of PAN.

Equation 4-1. $PAN = NH_4{-}N + [NO_3{-}N + NO_2{-}N] + K_{min}\ (Org{-}N)$

In the combined compost treatments, IC and UC, half of the target N rate was from compost and half was from inorganic fertilizer or UDF. Compost was applied at the start of the experiment, immediately before seeding. The UDF and IF were applied twice, during an initial application before seeding and side-dressing to simulate realistic N fertilizer application in the field. The initial application consisted of applying 100% of the compost treatment and 75% of the inorganic and UDF treatments. The remaining 25% of the N from the inorganic and UDF treatments were applied on day 29. Six batches of 11.5 kg of our potting soil mixture were each well-mixed with 900 mL of water, then each batch received an initial application of one of the six fertilizer treatments. After mixing the fertilizers into the soil, the mixture was divided into six half gallon pots. Each pot was lined with a 10 µm filter (Eisco Labs, Victor, NY) and a thin layer of HCl-rinse silica sand (Ryals et al., 2021) before soil was added into the pot.

Experiment conditions

Immediately after potting the soil, nine mustard seeds were planted into the soil at an approximate depth of 0.65 cm. During the growing season, plants were watered with 50-100 mL of deionized water daily depending on soil dryness and provided with artificial lighting for 14 hours per day for the first 40 days and 16 hours per day thereafter. We simulated a rain event on days 1, 8, 29, 30, and 39 by watering each plant with 200 mL of water. On day 22, after each plant had more than four leaves, we thinned seedlings to one plant per pot. On day 29, we applied a side-dressing for the remainder of the target N application rate for inorganic fertilizer and UDF around the base of the plant. At the end of the experiment, on day 56, each pot was

deconstructed to collect aboveground biomass, belowground biomass, bulk soil, and sterile soil samples for analysis. Sterile soil samples were collected as 5-6 cores around the base of the plant using a sterile spatula. There was extensive pest damage to one pot for each treatment and they were not sampled for our analyses.

Biomass analysis

After 56 days, above- and below-ground biomass were separated from the soil and dried at 60°C for at least 48 hours, weighed, and ground to 2 mm in a Wiley Mill. Total C and N in the biomass was measured by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI). We calculated N harvested for each plant by multiplying the plant dry mass by its N content.

Soil health analyses

We assessed a suite of biological and chemical indicators of soil health, focused on soil N cycling processes. A subsample of approximately five grams of fresh soil was stored at -20°C until processing for bacterial community composition and N functional gene abundances. To assess soil biological health, we measured enzyme activity for beta glucosidase (BG) as an indicator of soil OM quality and quantity, N-acetylglucosaminidase (NAG) as an indicator of N mineralization, and phosphatase (PHOS) as an indicator of phosphorus cycling. For potentially mineralizable nitrogen (PMN), samples were homogenized and sieved to 2 mm before extraction with 2 M KCl. Soil moisture was analyzed gravimetrically. Extractions were stored at −20°C and later thawed before analysis for NH_4^+.

The remainder of the soil was collected and stored at 4 °C for chemical analyses. A subsample of each sample was subsequently fractionated by size into POM and MAOM (Cotrufo et al., 2019). We measured the C:N ratio and N content (% by weight) in the POM and MAOM

fractions of the soil in addition to the total soil. First, 10 g of each dried, sieved (<2 mm) subsample received 30 mL of 0.5% sodium hexametaphosphate and went onto a reciprocal shaker for 18 hours. Samples were then poured onto a 2 mm sieve over a 53 µm sieve in a large pan. Glass beads were extracted from the 2 mm sieve, and then any soils on the 53 µm sieve were rinsed thoroughly and washed into a pre-weighed aluminum pan labeled for the POM fraction. Subsequently, the large pan beneath the sieve was rinsed into another pre-weighed aluminum pan labeled for the MAOM fraction. Aluminum pans were then completely dried at 60 ℃ for 2-5 days. Dried POM and MAOM fractions were transferred to scintivials, and subsequently, each fraction was analyzed for C% and N% by dry combustion on a Leco TruMac CN Analyzer.

Gas and leachate fluxes

Leachate was collected from four of the six replicates on days 1, 8, 29, and 39 after simulating a rain event with 200 mL of water. Prior to watering, a deep collection tray was placed under each pot. Water that leached out was collected approximately 24 hours later and stored at -20℃ until processing. Leachate samples were thawed overnight and then analyzed for NH_3 and NO_3 on a discrete analyzer (AQ2; Seal Analytical, Mequon, WI).

Gas fluxes were measured from four of the six replicates on days 1, 2, 8, 29, and 30. Days 1 and 29 served as baseline fluxes to compare to fluxes following amendment application on days 2 and 30. We used five-gallon screw-top buckets (Uline, Pleasant Prairie, WI) that were modified with a bucket lid gasket ring and rubber septum installed in the lid as gas sampling chambers. After watering, the pots and leachate trays were placed into the gas sampling chambers. After closing the lids, air samples were taken at 0, 30, 60, and 90 minutes. Samples were analyzed for N_2O, CO_2, and CH_4 using a gas chromatograph equipped with a63Ni electron-capture detector (ECD). The ECD operated at 325 °C. N2 (99.999%) was used as the carrier gas. Standard curves were prepared

using four standard gas solutions with N_2O concentrations of 305, 693, 1092, and 1885 ppb as described previously (Bressler & Blesh, 2023). Timed data points for each sample were screened for nonlinearity and removed. The remaining points were used to calculate the N_2O flux using linear regression.

Soil DNA isolation and N functional genes

DNA was extracted from four of the five replicate soil samples using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) and stored at -20℃. N functional gene abundances were quantified using real-time quantitative polymerase chain reaction (qPCR). Genes were chosen to evaluate the soil microbial community's potential for ammonia oxidation (ammonia monooxygenase - amoA in archaea (AOA) and bacteria (AOB)) and denitrification (nitrite reductase - nirS and nitrous oxide reductase - nosZ). qPCR was performed on QuantStudio 3 (Applied Biosystems, Foster City, CA). Each reaction consisted of 5 µL of iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), 0.4-0.9 µL of each primer (Table 4-3), 0.4 µL of 25 mg/mL bovine serum albumin (Thermo Fisher Scientific, Waltham, MA), 1 µL of 25 µM ROX reference dye (Biotium, Fremont, CA), 1 µL of template DNA, and ultra-pure water to reach a total reaction volume of 10 µL. The qPCR amplification conditions were 95 ℃ for 5 minutes, 40 cycles of 95 ℃ for 15 seconds, an annealing temperature specific to the gene (Table 4-3) for 30 seconds, and 60 ℃ for 30 seconds. Melt curve analysis was performed from 65 ℃ to 95 ℃. The primer specificity was verified by comparing the peak temperature of the melt curve to the expected temperature for each target amplicon and randomly-selecting qPCR-amplified samples for Sanger sequencing to confirm that they align with the target gene sequence on GenBank. Standards for each gene were purchased as gBlocks. gBlock sequences were the consensus sequences generated by aligning the gene sequence from reference organisms that the primers have

successfully detected in previous studies. Each qPCR run included a 6-point standard curve between 10^1 and 10^6 copies of each gBlock, no-template controls, and samples. All standards, controls, and samples were run in duplicate. All no-template controls were at least 3.5 Ct above the highest Ct in the standard curve, except for AOA which was at least 1.1 Ct above the highest Ct in the standard curve. We calculated the limit of quantification (LOQ) as the Ct value at which 95% of 10 replicates were detected and the coefficient of variation was less than 25% (Kralik & Ricchi, 2017). All data below the LOQ were set to the LOQ. The amplification efficiencies of the standards were 75% for AOA and 80-96% for the other genes. The results were analyzed using the QuantStudio Design and Analysis Software v1.5.2 (Applied Biosystems) and gene abundances were reported as the number of gene copies per gram of dry soil.

Table 4-3. qPCR primers and amplification conditions

Gene	F primer	R primer	Length of amplicon (bp)	Primer concentration (µM)	Annealing temperature (°C)	References
AOB (AOB amoA1F, 2R)	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC	491	0.5	60	Rotthauwe et al., 1997
AOA(AOA amoAF, amoAR)	STAATGGTCTGGCTTAGACG	GCGGCCATCCATCTGTATGT	635	0.5	56	Francis et al., 2005
nirS (Cd3aF, R3cd)	GTSAACGTSAAGGARACSGG	GASTTCGGRTGSGTCTTGA	425	0.4	56	Throback et al., 2004
nosZ (nosZ 2F, 2R)	CGCRACGGCAASAAGGTSMSSGT	CAKRTGCAKSGCRTGGCAGAA	267	0.5	56	Henry et al., 2006

Soil bacterial community composition

16S rRNA (V4 region) Illumina sequencing was used to determine the relative abundances of bacteria by genus in each soil sample (Kozich et al., 2013). Amplicons were paired-end sequenced with Illumina MiSeq (MiSeq Reagent Kit V2 500 cycles, Illumina Inc., San Diego, CA). Sequence data was analyzed using DADA2 (Callahan et al., 2016). Sequences were trimmed

to 70 bp for reverse reads and 190 bp for forward reads, paired-end joined, and screened. Sequences were clustered into ASVs and aligned to the SILVA database (Quast et al., 2013). We used R package phyloseq (version 3.17) to calculate the alpha and beta diversity and relative abundance of genera present in the sample.

Data analysis

We calculated an estimate of cumulative N_2O emissions and leachate as the sum of N_2O emissions and leachate on the days we measured them due to the limited number of sample points. Although this is likely an underestimate of the total emissions and leachate, our measurement on day 8 of the experiment shows that N_2O emissions returned to levels similar to day 1. To calculate NUE, we divided the N harvested by the total N input. We calculated the N loss to harvested ratio by dividing the total mass of N lost as leachate or N_2O emissions by the mass of N in the aboveground biomass. Significant differences among treatments were identified with one-way Welch's ANOVA at $\alpha = 0.05$ for plant yield, N harvested, soil health indicators (e.g., PMN, C and N contents in the POM, MAOM, and total soil), and N cycling indicators (e.g., N_2O emissions, amount of N in leachate, NFG abundances). Pairwise comparisons were performed with Tukey's Honest Significant Difference test for homoscedastic variables and Dunnett's T3 test for non-homoscedastic variables. Relative abundances of bacteria were compared across fertilizer treatments using multivariate analysis of variance (MANOVA) with the adonis R package.

4.3 Results

Plant yield and soil health indicators

Plant biomass and N harvested

Plant biomass was the highest in the compost treated sample (CT; 3.16 +/- 0.792 g) and lowest in the control sample (CL; 0.556 +/- 0.118 g) (Figure 4-1). Compared to CL, all N

treatments significantly increased plant biomass ($p < 0.01$) with CT improving plant yield by an average of 5.68-fold. On average, treatments with compost (CT, IC, and UC) had slightly higher plant mass than without compost (I and U), but the difference was not statistically significant ($p > 0.2$). N content in the aboveground biomass was higher in CT (28.5 +/- 4.80 mg N/g dry biomass, $p = 0.02$), UDF (29.8 +/- 5.90 mg N/g dry biomass, $p = 0.004$), and UC (29.3 +/- 3.61 mg N/g dry biomass, $p = 0.009$) than in CL (20.9 +/- 4.12 mg N/g dry biomass) (Figure B-1). The mass of N in the plant was highest in CT (91.3 +/- 25.5 mg), which was significantly greater than UDF (70.2 +/- 14.0 mg, $p = 0.02$), I (65.2 +/- 18.5 mg, $p = 0.002$), and IC (71.5 +/- 7.55 mg, $p = 0.005$) (Figure 4-1).

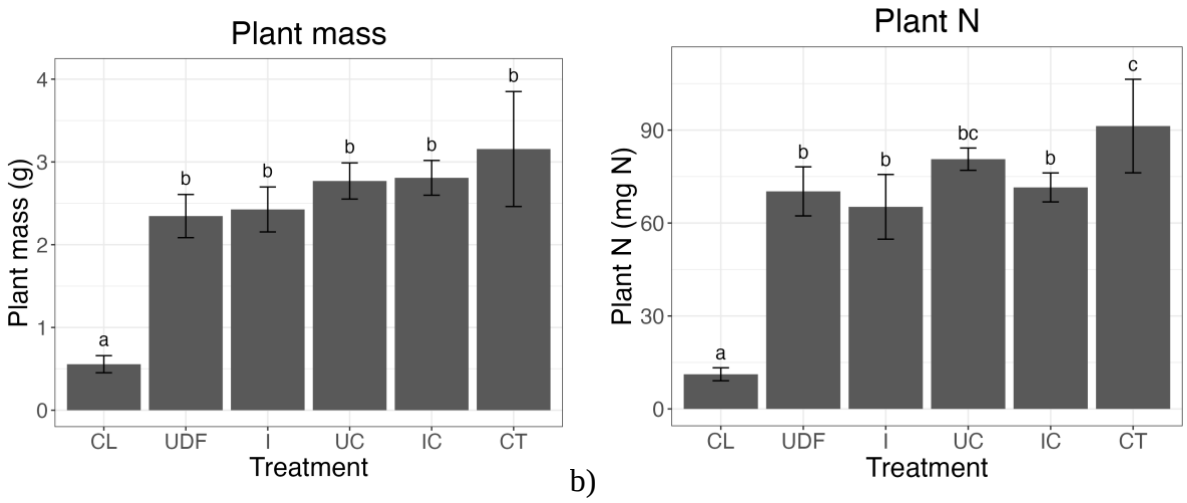


Figure 4-1. a) Plant mass for each treatment. b) plant N mass for each treatment. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups.

Soil chemical composition

The C:N in the POM and total soil were not significantly different across treatments (Figure 4-2). The C:N in MAOM was statistically different between CL and IC, but the difference is small and does not have physical significance. The average C:N in POM, MAOM, and total soil across all treatments were 6.31 +/- 0.709, 10.3 +/- 0.240, and 9.80 +/- 0.360, respectively.

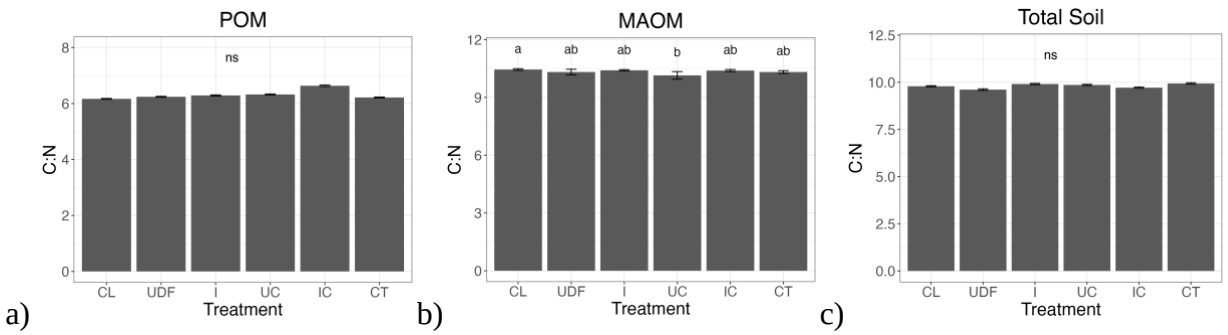
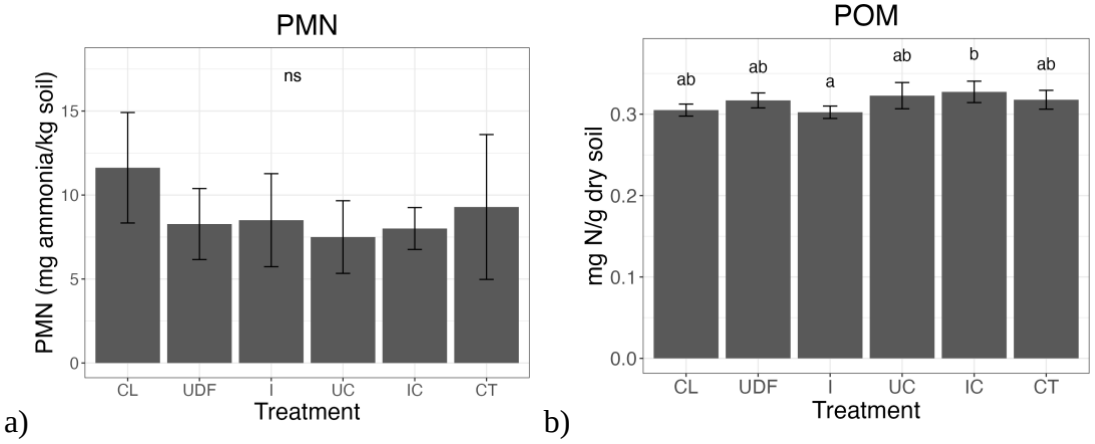


Figure 4-2. C:N ratio in the a) POM, b) MAOM, and c) total soil. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups and "ns" is non-significance among the treatments.

There were no significant differences in PMN, but PMN in CL was higher than all the N treatments (Figure 4-3). For total soil N content, IC (0.709 +/- 0.0453 mg N/g dry soil) was higher than CL (0.649 +/- 0.0385 mg N/g dry soil, p = 0.05). In POM, the N content was higher in IC (0.327 +/- 0.0212 mg N/g dry soil) than I (0.302+/- 0.0123 mg N/g dry soil, p = 0.04). Although statistically significant, the differences are small and do not have physical significance.



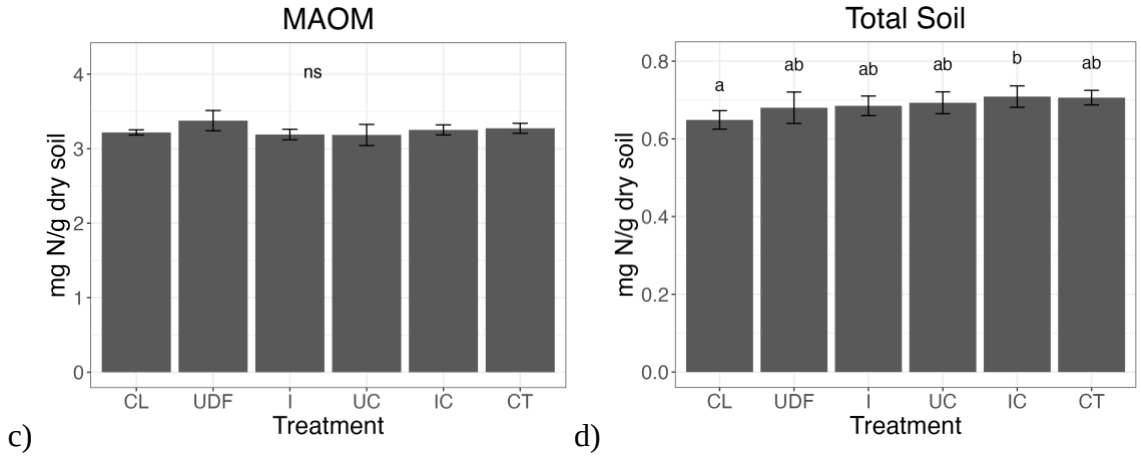


Figure 4-3. a) PMN and % N in the b) POM fraction, c) MAOM fraction, and d) total soil. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups and "ns" is non-significance among the treatments.

Soil enzyme activity

For β-glucosidase (BG), NAGase (NAG), and phosphatase (PHOS), there were no statistically significant changes in enzyme activity (Figure 4-4). However, there was a consistent increase in enzyme activity when compost was added to the control and to inorganic fertilizer. On average, BG activity increased 50% between CL and I as compared to CT and IC, respectively. The differences were even higher for NAG activity, with 100% increase between I and IC. In contrast, compost added with UDF either reduced or did not change enzyme activity. On average, PHOS and NAG activities decreased by approximately 40% between UDF and UC.

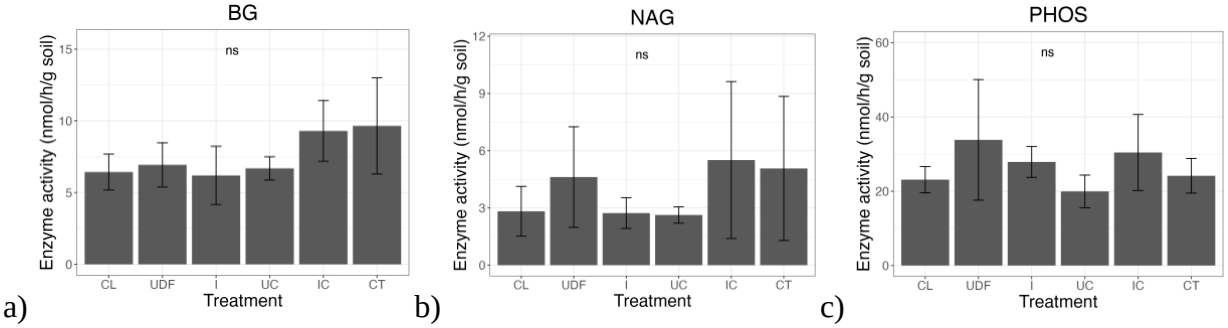


Figure 4-4. Enzyme activity (nmol/h/g soil) for a) β-glucosidase (BG), b) NAGase (NAG), and c) phosphatase (PHOS). Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic

and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. “ns” is non-significance among the treatments.

Soil N cycling

N_2O emissions

Taking a sum of the N_2O emissions across our sampling days (1, 2, 8, 29, 30), all N treatments, except I, significantly increased N_2O emissions (Figure 4-5). Compared to CL (62.2 +/- 9.87 g N_2O/ha/day), the largest difference was observed for CT (387 +/- 46.7 g N_2O/ha/day, $p = 3 \times 10^{-8}$), which had 6.22-fold greater emissions. The UDF treatment (176 +/- 43.2 g N_2O/ha/day) also increased emissions as compared to CL by 2.20-fold ($p = 0.01$). Among the N treatments, UDF and I (144 +/- 26.8 g N_2O/ha/day) had similar total N_2O emissions, but all treatments with compost (CT, IC, UC) were higher. On average, IC had a flux of 277 +/- 30.7 g N_2O/ha/day, which was 1.92-fold higher than I ($p = 0.003$). UC had a flux of 315 +/- 68.4 g N_2O/ha/day, which was 1.79-fold higher than UDF ($p = 0.002$).

The N_2O emissions over time were different for the treatments. N_2O emissions were low immediately after fertilizer application on days 1 (initial application) and 29 (side-dressing) (Figure 4-5) and increased within 24 hours after application. Emission levels on day 8 suggest that the plants reached baseline levels within one week of fertilizer application, but CT emissions remained slightly higher than those of the other treatments. On day 30, one day after side-dressing UDF and inorganic fertilizer to I, IC, U, and UC, N_2O emissions increased, but CT, which did not receive additional N, had as high or higher emissions than those that received additional N.

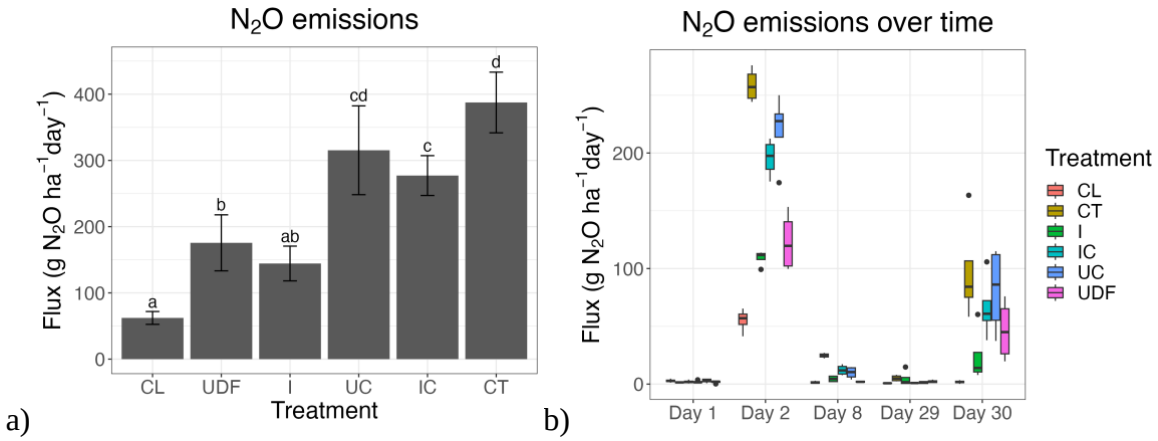


Figure 4-5. N_2O fluxes a) as a sum of all days N_2O was measured and b) for each day. Fertilizer was applied on days 1 and 29; N_2O measurements were taken after treatment on those days. Flux is calculated as a sum of the fluxes for days sampled. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups.

N leachate

The sum of NO_3-N and NH_3-N in the leachate was significantly higher in CT (7.23 +/- 0.574 mg N) than CL (2.14 +/- 0.551 mg N) by 3.38-fold ($p = 1 \times 10^{-4}$) (Figure 4-6). There were no differences among the N treatments, but UDF had the highest leachate at 9.54 +/- 2.60 mg N (1.27-1.50-fold greater than the other treatments, $p > 0.8$) and 4.46-fold higher than CL ($p = 0.07$).

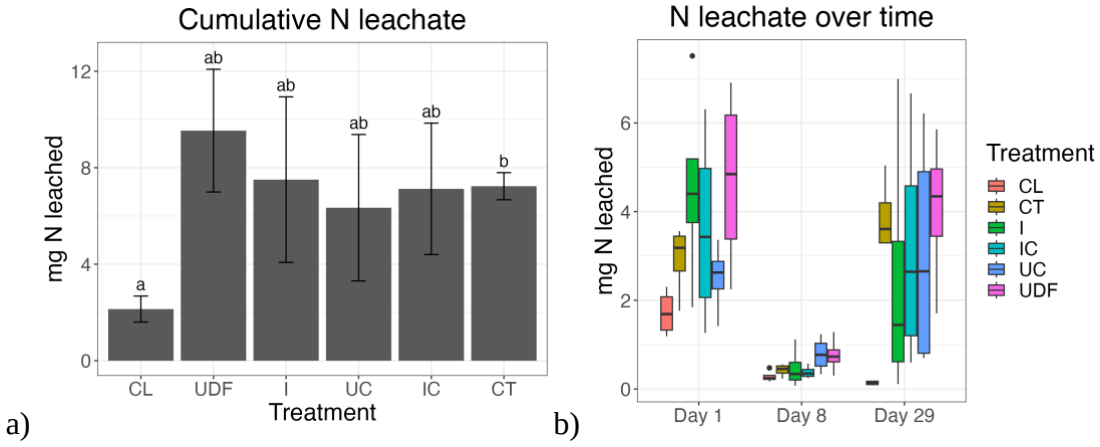


Figure 4-6. Mass of N leached a) as a sum of all days leachate was measured and b) for each day. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each

treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups.

N use efficiency

NUE, calculated as the N harvested divided by the total N applied, was the highest in UDF (0.617 +/- 0.0486) and I (0.627 +/- 0.196) and decreased with additions of compost (Figure 4-7). The NUE for UC (0.490 +/- 0.0233) was higher than IC (0.428 +/- 0.0286) by 1.14-fold ($p = 0.004$), both of which were not significantly higher than CT (0.393 +/- 0.0971). Since we did not apply N to CL, we did not calculate NUE for that treatment.

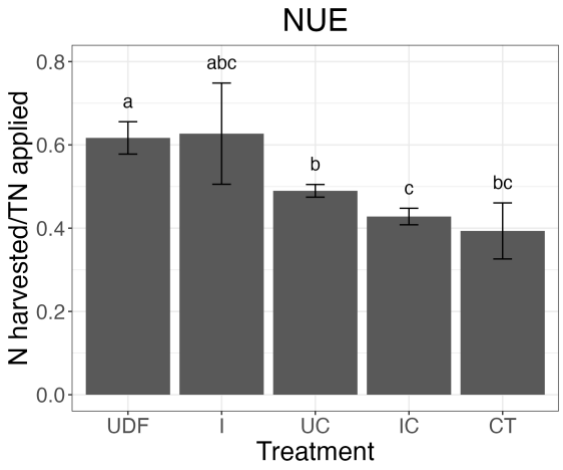


Figure 4-7. NUE calculated with total N applied. Treatments are CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups.

N losses

We report total N loss as the sum of the mass of N in the leachate and as N_2O emissions on all days sampled. Total N loss was not significantly different among N treatments (Figure 4-8), but the proportion of N loss as leachate and N_2O were different between treatments. The proportion of N loss as N_2O was significantly higher in all the compost treatments (CT, IC, UC) than the others (CL, I, U). Compost treatments had greater than 7.49% of N losses as N_2O emissions whereas the other treatments had less than 4.78% of their losses as emissions. Among the compost

treatments, UC had the highest percentage of loss as N_2O (9.44% +/- 2.37%), which was greater than UDF, the treatment with the lowest percentage (3.78% +/- 1.19%) by 2.50-fold ($p = 1 \times 10^{-4}$). The opposite pattern was seen with leachate. UDF had the highest percentage loss as leachate (96.2% +/- 1.19%) (Figure B-2). Additionally, the ratio of N loss to harvested was higher in all treatments, except CT (9.57×10^{-2} +/- 1.94×10^{-2}), as compared to UC (8.54×10^{-2} +/- 3.67×10^{-2}). Adding compost to the UDF significantly reduced the N loss to N harvested ratio by 1.87-fold from 1.60×10^{-1} +/- 5.30×10^{-2} to 8.54×10^{-2} +/- 3.67×10^{-2} ($p = 0.02$) (Figure 4-8).

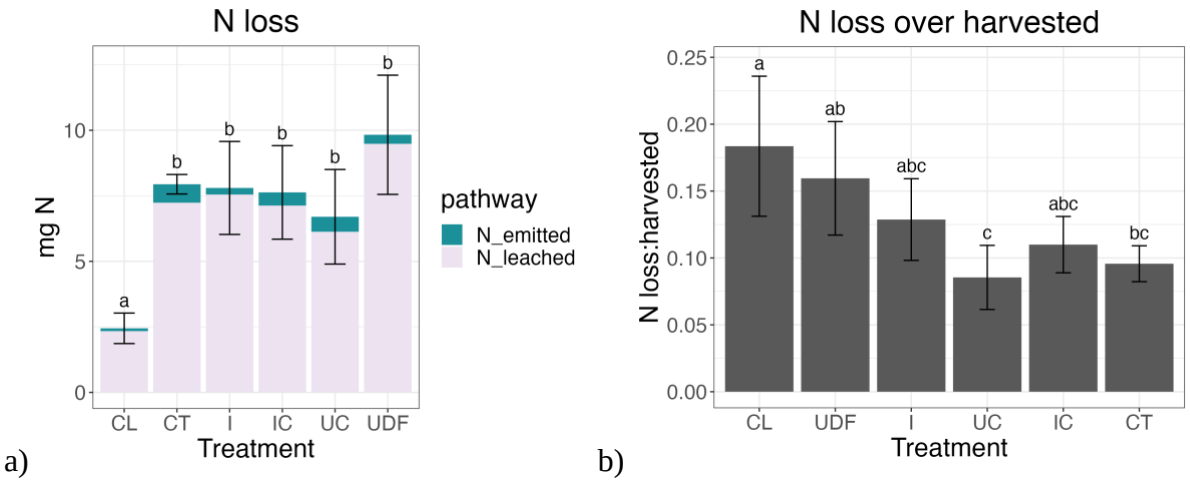


Figure 4-8. a) Mass of N_leached (lost as leachate) and N_emitted (N_2O emissions), b) ratio of mass of N lost to mass of N in the aboveground biomass. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups. For N loss, the error bars and letters are 95% intervals and significance groups for total N loss as a sum of N emitted and N leached.

<u>Soil microbial community and N cyclers</u>

N cycling genes

For nitrification genes, there were no significant differences in AOA abundance across all treatments, but AOB abundance was significantly higher in all N treatments compared to CL ($p < 0.03$) (Figure 4-9). CT, I, IC, and UDF treatments had similar AOB abundance (5.00-5.27 $\log_{10}$-gc/g dry soil). AOB abundance in UC (4.74 $\log_{10}$-gc/g dry soil) was significantly lower than CT

(p = 0.02). In both AOA and AOB, compost combined with UDF and I resulted in lower abundances, but the differences were not statistically significant (p > 0.6). For denitrification genes, nirS and nosZ abundances were significantly higher in CT (4.90 log_{10}-gc/g dry soil for nirS, 5.88 log_{10}-gc/g dry soil for nosZ) than CL (4.28 log_{10}-gc/g dry soil for nirS, 5.36 log_{10}-gc/g dry soil for nosZ, p = 0.04 for both), but they were not significantly different for other treatments (Figure 4-9).

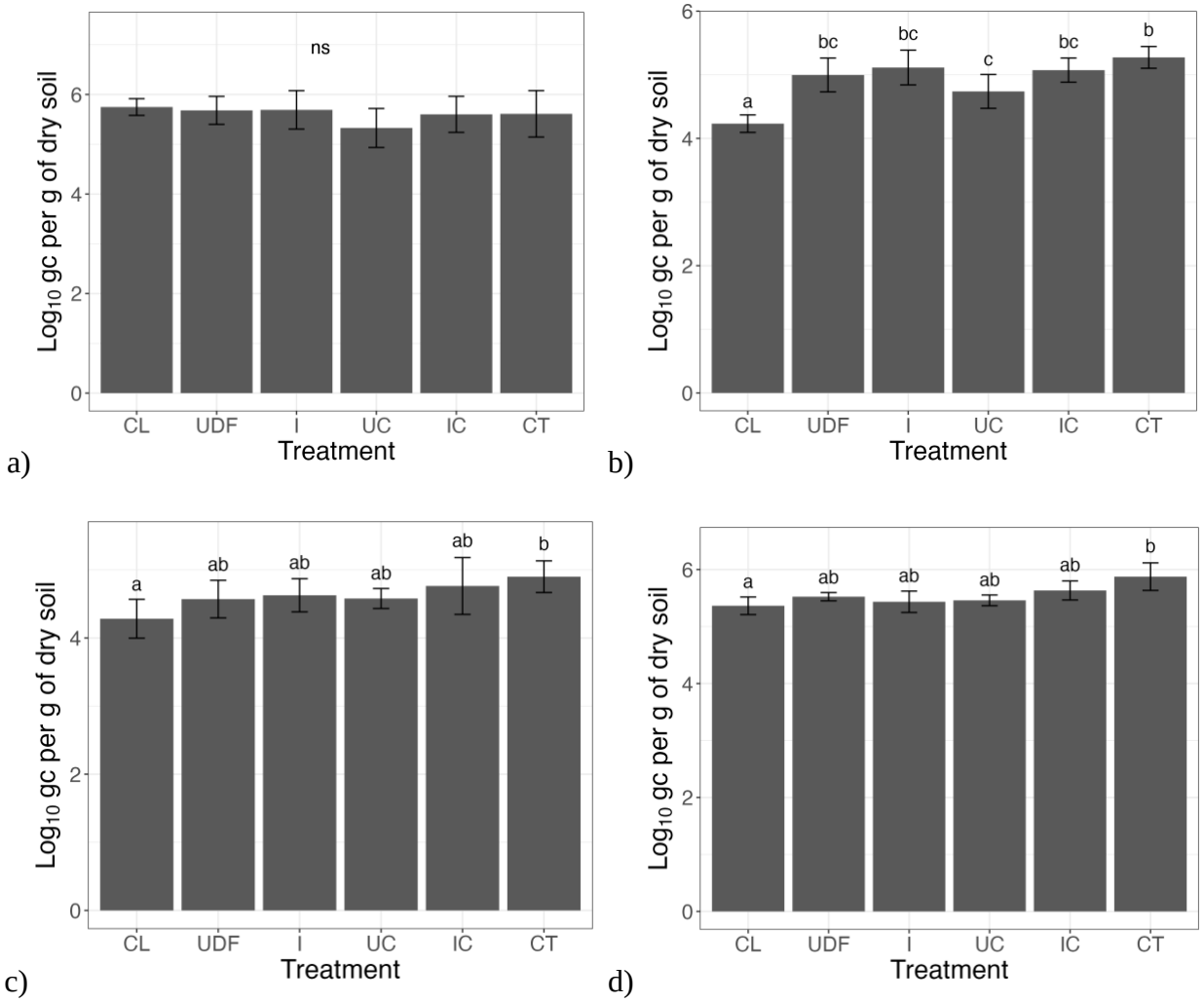


Figure 4-9. Log_{10} gene abundance for a) AOA, b) AOB, c) nirS, and d) nosZ. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups and "ns" is non-significance among the treatments.

Microbial community structure

Analysis of the 16S rRNA sequencing data showed no differences in Shannon and Simpson diversity indices between treatments (Figure 4-10). Additionally, MANOVA of the relative abundance of each genus were not significantly different.

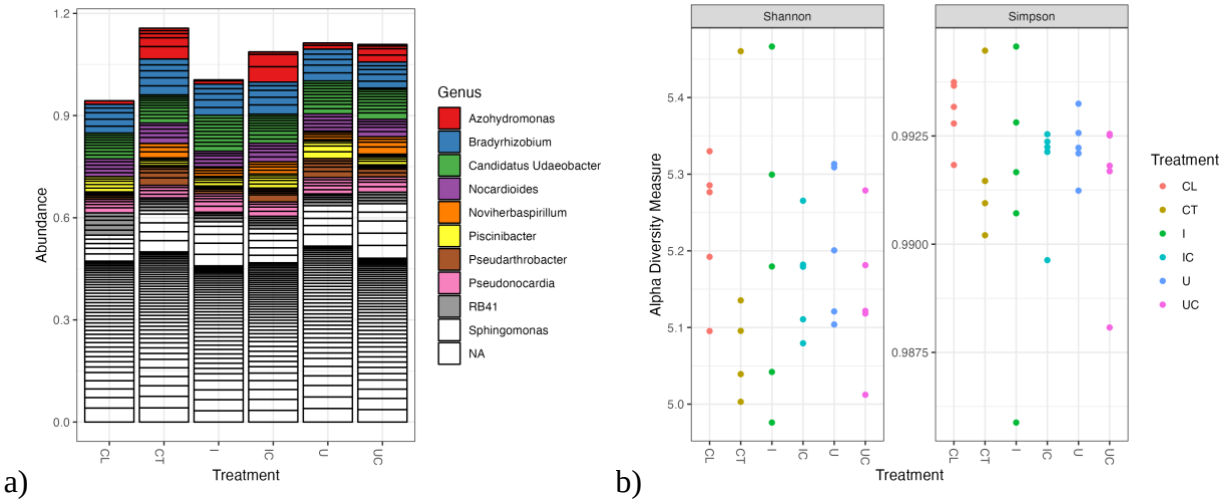


Figure 4-10. a) Relative abundances of the top ten genera in each treatment. b) Shannon and Simpson diversity indices where each dot represents one replicate sample for each treatment. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost.

4.4 Discussion

<u>UDF performed similarly to the inorganic fertilizer</u>

The UDF and inorganic fertilizer had similar effects on all soil health indicators and N cycling processes measured in this experiment. As we hypothesized, the two treatments led to similar increases in yield, NUE, and leachate N as compared to the control. The similarity suggests that UDF performed as well as inorganic fertilizers with no compromise or benefits to soil health in the short term. Other studies have also reported similar N harvested and plant yield between UDF and inorganic fertilizers (Martin et al., 2020; Martin et al., 2021), but UDF has also led to lower N harvested at the same application levels (Pradhan et al., 2010; Martin et al., 2021). The

similar forms of N found in UDFs and inorganic fertilizers may explain why they have similar effects on plant yield, soil health, and soil N cycling.

Although the UDF and inorganic fertilizer are highly soluble N fertilizers, the N leached was not significantly higher than the organic fertilizer and did not support our hypothesis that the UDF and inorganic fertilizer would result in higher N losses as leachate. Leaching is a physicochemical process and the stability of the soil physical properties in the short term suggests that there would be no difference in leaching potential when soluble N fertilizers were applied. However, the UDF treatment tended to have the highest leaching losses across all treatments, although it was not statistically significant. In a previous study, a similar N application rate (111-133 kg N/ha) of stored urine (pH > 9) was compared to inorganic fertilizer (blend of ammonium nitrate, triple super phosphate, and potassium chloride), and there were also similar or higher amounts of N leaching from urine (Pandorf et al., 2019).

Other studies report significant loss of N as ammonia volatilization from UDFs as compared to inorganic fertilizers. Visual Minteq, a chemical equilibrium model, estimated that 67% of NH_4^+ is lost from UDFs at pH 9 (Rumeau et al., 2023). However, field measurements of NH_3 volatilization after applying stored urine showed that less than 10% is lost and differed significantly by application method and amount (Rodhe et al., 2004). Although we did not measure NH_3 volatilization in our experiment, we adjusted the pH of the UDF to 7.7 to process it with activated carbon prior to application, suggesting much lower values than previously reported. For reference, Visual Minteq estimated that a decrease of 0.2 units in pH from 8.7 to 8.5 can reduce the % of NH_4^+ in soils from 21% to 14% (Rumeau et al., 2023).

N_2O emissions from compost were higher than from UDF and inorganic fertilizer

We hypothesized that the compost treatment would result in lower plant yields, but the compost treatment tended to have the highest yield. Additionally, as we hypothesized, NUE was lowest for the compost treatment. In previous studies, UDF also had higher NUE than cattle slurry (Martin et al., 2021) and a variety of organic wastes (Gomez-Munoz et al., 2017), which was attributed to the higher level of soluble N in the urine. These results likely depend on the compost or organic fertilizer composition. The compost used in this study was derived from chicken litter and had a high N content compared to many composts from other sources, which can explain the higher plant yield and N harvested. However, most of the N in the compost was organically-bound and not readily available to plants, resulting in a lower NUE. To achieve similar yields, farmers need to apply more N as compost than as UDF or ammonium sulfate.

Compost also resulted in higher fluxes of N_2O and a higher proportion of N lost as N_2O emissions than that of the UDF and inorganic fertilizer treatments. Our measurements of the NFGs suggest that the input of compost resulted in higher AOB gene abundance, which is as an indicator of nitrification activity, and higher nirS and nosZ abundances, which are indicators of denitrification activity. Higher rates of nitrification and denitrification could explain the higher N_2O emissions we observed with compost. The addition of organic C provided a source of energy for heterotrophic denitrifiers, increasing denitrification activity. Additionally, higher N content in the compost we used may have provided NH_4^+ and NO_3^- as substrates for nitrification (Xia et al., 2020). However, from our data, we are not able to determine the relative contributions of nitrification or denitrification activity to the elevated N_2O emissions.

There are relatively limited measurements of N_2O emissions from UDFs from previous studies, but one study performed an aerobic incubation of soil amended with different fertilizers

and found that human urine and cattle slurry had lower cumulative N_2O emissions than inorganic N, phosphorus, and potassium fertilizer. Other organic waste-derived fertilizers (e.g., sewage sludge, composted organic household waste, cattle deep little) had higher N_2O emissions than human urine and inorganic fertilizer (Gomez-Munoz et al., 2017).

Combined used of compost and UDF and inorganic fertilizer had mixed effects

Soluble N fertilizers like UDFs can benefit from co-application with a C-rich amendment (Fatunbi, 2009; Shrestha et al., 2013), but we observed potential trade-offs for greenhouse gas emissions. In our experiment, compost applied with UDF or inorganic fertilizer had similar effects as compost and led to significant differences between compost treatments (CT, IC, UC) and non-compost treatments (CL, UDF, I). For example, the proportion of N lost as N_2O was higher for all three treatments with compost, whereas N lost via leaching was higher for the CL, UDF, and I treatments. All treatments with compost also tended to have higher plant biomass and N harvested, lower NUE, significantly higher N_2O emissions, and significantly lower N loss to harvested ratios. These results support our hypothesis that adding compost to UDFs and inorganic fertilizers would increase plant yield and increase N loss as gaseous emissions by providing a source of organic carbon.

Previous studies have shown that combined urine and compost had positive short-term effects on yield, but limited research has been done to assess the effects on soil properties (Fatunbi, 2009; Shrestha et al., 2013). It can take years to detect changes in total SOM with ENM practices (Drinkwater & Snapp, 2022), so we also measured POM and MAOM fractions of SOM, which can be more sensitive to short-term management changes (Cotrufo et al., 2019). However, we did not observe significant differences in POM and MAOM fractions of SOM or any soil health indicators. In terms of N cycling, we observed a significant decrease in N loss to harvested ratio

for co-application of UDF with compost. Although there are overall ecological benefits of reducing N loss, different N loss pathways have different environmental implications. In the UDF with compost treatment, N_2O emissions were higher than in the UDF alone, which has a negative effect on global warming potential. It is worth noting that this treatment tended to have lower N_2O emissions than compost alone although not statistically significant. Other ENM practices such as increasing crop diversity can be coupled with UDFs to mitigate potential ecological risks associated with their use.

Compost can also benefit from co-application with UDFs, which can provide short-term needs of soluble N whereas compost can provide organic C and slow-release organic N. The benefits that UDFs can provide to compost can happen in a shorter time frame as short-term N availability is related to inorganic N application (Gomez-Munoz et al., 2017). When inorganic fertilizers have been applied with organic amendments, there were improved yields and soil properties (Wu & Ma, 2015). Interestingly, we observed different effects of compost application with UDF and compost application with the inorganic fertilizer. The UDF with compost treatment tended to have lower losses as leachate than UDF alone, which supports our hypothesis, whereas there were no differences between the inorganic with compost and inorganic treatments. NUE was also higher for the UDF with compost treatment than for inorganic fertilizer with compost.

Extracellular enzyme activities were also different when compost was added with UDF versus inorganic fertilizers. There were slightly higher activities of β-Glucosidase and NAGase, but no change in phosphatase activity, with compost plus fertilizer as compared to inorganic fertilizer alone. However, NAGase and phosphatase activities were lower in the UDF and compost treatment than in UDF alone, and there was no change in β-Glucosidase activity. β-Glucosidase activity is used as an indicator of changes in organic matter and its activity increased with various

compost and straw mulch applications in previous studies (Adetunji et al., 2017), which may explain why its activity was slightly higher in our inorganic and compost treatment. Phosphatase activity increases when there is reduced inorganic P in the soil, causing increased solubilization of phosphate. Increased microbial activity associated with organic amendments and combined vermicompost or solid waste compost with inorganic N fertilizer have also been linked to higher phosphatase activity (Adetunji et al., 2017), but we didn't see this pattern with the inorganic plus compost treatment. NAGase activity is correlated to organic C and N and may play a role in N mineralization (Tabatabai et al., 2010). Although it is unclear which mechanisms caused the differences between inorganic and UDF applied with compost, it is important to consider that other compounds in UDF can affect biological processes. For example, hippuric acid and ammonium bicarbonate in urine can inhibit denitrification (Kool et al., 2006) and nitrification (Clough et al., 2003), respectively.

4.5 Implications

Implications for using UDF in an ENM context

ENM practices are needed to mitigate Nr emissions when using UDFs. Our results, taken with previous studies, demonstrate that UDFs perform just as well as inorganic fertilizers in yield and NUE, but provide no benefits or compromise to short-term soil health or agricultural N losses. Unlike inorganic fertilizers, UDFs reduce resource-consumption for waste management and fertilizer production and mitigate N emissions from waste management. However, it is critical to consider UDF use within an ENM framework to reduce agricultural N emissions from UDF application. Our results are particularly relevant to farms that are transitioning from long-term use of inorganic N fertilizer to UDFs, compost, UDF and compost, or inorganic N combined with compost. For farmers, our results show that yield and N harvested are not compromised when

changing from inorganic fertilizer to the treatments we studied. On the contrary, the three treatments with compost tended to have higher biomass suggesting that there are opportunities for increased yields if compost is used on its own or combined with inorganic N and UDFs. From an environmental health perspective, our results suggest that there are short-term changes with soil N cycling. N losses were not improved with any treatment, but UDF application with compost reduced N loss to harvested ratio. Although N_2O emissions were higher for the UDF and compost treatment, they tended to be lower than compost alone. Ultimately, we found that UDF application with organic amendments may contribute to ENM goals by increasing soil organic matter without reductions in plant yield.

Limitations

One limitation of this study is that it was conducted with just one growing season. Although some of the indicators we evaluated are relevant in the short-term, changes in soil organic matter and their impacts on soil health need to be assessed over years or even decades (Drinkwater & Snapp, 2022). Additionally, we conducted our experiment in the greenhouse to study N fluxes such as leachate and N_2O emissions, but they may not be representative of field conditions.

Future work

Future work will include exploration of relative abundance differences between specific genera using DESeq2, patterns between physicochemical and community structure using correlation analyses (e.g., PCA, CCA, NMDS), and community function using PICRUSt2. A long-term study on soil health and N cycling, particularly N losses as leachate and gaseous emissions for UDFs and UDFs applied with a C-rich amendment can help address questions about the role of UDFs in ENM. Additionally, different combinations and types of organic amendments applied

with UDFs can give insight into biochemical soil and fertilizer interactions and identify more beneficial combinations and best management practices.

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Chapter 5 Quantifying Environmental Impacts and Suitability of Recovering Nitrogen and Phosphorus From Waste in New York City

5.1 Introduction

Anthropogenic use of nitrogen (N) and phosphorus (P) have increased reactive N and P in the environment that can disrupt natural processes and habitats. The Stockholm Resilience Center reports that N and P are two out of three systems that are at high risk of irreversible environmental damage (Rockstrom et al., 2009). The release of reactive nitrogen (Nr) and phosphorus (Pr) to the environment results in harmful algal blooms and reduced air quality, which result in biodiversity loss and negative public health impacts (Galloway et al., 2002). Reducing Nr and Pr in the environment is an increasingly urgent challenge for protecting public and environmental health.

Urban nutrient use is an important contributor to Nr and reactive Pr in the environment. In 2018, more than 55% of the global population lived in urban environments, and it is expected to reach 68% by 2050 (United Nations, 2018). Due to the high levels of human activities in cities, urban activities contribute up to 78% of carbon emissions and 60% of residential water use (Brown, 2001). Nutrients, particularly N and P, are also heavily used in cities. Urban nutrient use is tied to food and waste management systems. N and P predominantly enter cities in the form of food, but most of it ends up in waste. Detroit imports 83% and 94% of total N and P inputs, and 64% and 75% of it is discharged to the local environment as wastewater and other losses in the food system (Liang et al. 2019). Other cities such as Phoenix (Baker et al., 2001), Toronto (Forkes, 2007), and Bangkok (Faerge et al., 2001) also report high levels of N and P losses to the environment. In 2016, wastewater discharge was the largest source of N into the East River, most of which

originated in New York City (NYC) (Vaudrey, 2017). To minimize nutrient losses to the environment from urban environments, we need an approach to waste management that allows circularity and returns nutrients back to agriculture communities.

Waste generated in a city requires energy-intensive processes to convert Nr and Pr back into inert forms. Alternatively, the Nr and Pr can remain in waste and be used in agriculture to offset fertilizer production. The urban waste flows with the highest potential for nutrient recovery are food waste, sewage sludge, and human urine (Haan & Geel, 2013). Other waste streams such as black and greywater and gaseous emissions contain N and P but are not considered in this study (Figure 5-1). Depending on the waste management processes, these waste streams are typically not as concentrated and recovery from them can be more resource intensive. In contrast, urine contributes approximately 80% of the N and 55% of the P, despite making up less than 1% of the wastewater volume (Höglund, 2001). With current wastewater operations, energy-intensive processes are needed to convert wastewater N and P into non-reactive forms, which can account for up to 35% of a municipality's energy usage (DOE, n.d.). Instead, urine can be separated at the toilet to keep most of the nutrients in wastewater as a relatively clean and concentrated solution and can then be processed into a urine-derived fertilizer (UDF) with relatively minimal processing. Another component of wastewater with high N and P recovery potential is sewage sludge, which has high organic carbon content in addition to N and P but contains heavy metals and other contaminants sent down the drain. Diverting food waste and processing it into a soil amendment can also yield a carbon-rich product for agriculture (Santagata et al., 2021). Globally, about 40% of food that is available for consumption is wasted (NRDC, 2017). Most food waste ends up in landfills and contributes to 14.3% of the national methane emissions in 2021 (EPA, n.d.). These

waste streams are promising opportunities to recover nutrients from urban communities and improve the sustainability of waste management.

If urban waste was processed with the focus to return them to food production, waste management can become more sustainable, improving overall urban sustainability. A material flow analysis (MFA), which takes a mass balance approach at evaluating flows and stocks, can be used to quantify the nutrient needs and nutrients wasted in a given system. Previous studies have used this approach to track nutrient flows, but it is particularly important for quantifying nutrient recovery potential. In Bangkok, an urban nutrient balance demonstrated that only 7% and 10% of N and P is recovered, respectively, and 97% and 41% of N and P is lost to the Chao Phraya River, respectively (Faerge et al., 2001). The most promising pathways for nutrient recovery from waste are recovering food waste, land application of sewage sludge, and source separation and processing of urine into a fertilizer. By doing a nutrient MFA, we can evaluate the amount of nutrients recoverable from these three waste streams and quantify how much of the nutrient needs for the city can be met with recovered nutrients or returned for food production. These numbers can then be used to quantify the environmental impacts of nutrient recovery and return.

Despite potential environmental benefits of nutrient recovery, challenges exist in public infrastructure and human interactions with nutrient recovery technologies. Before implementing these technologies, we need to identify the scale of implementation and key parameters that are most important for identifying where these technologies can be implemented. A suitability raster analysis weighs components of suitability and maps them to identify areas that are most suitable for a particular purpose (Banai-Kashani, 1989). This type of analysis is important for spatially- or resource- constrained cities and technologies that require a consideration of infrastructural and user-related challenges. Nutrient recovery technologies can require significant changes in human

behavior and infrastructure and thus it is important to consider suitability components that encompass socioeconomic and technological aspects of using the technology. For cities considering implementing nutrient recovery, a suitability analysis can identify where nutrient recovery is most feasible.

In this study, we used a material flow analysis to quantify recoverable nutrients and the potential environmental benefits for returning nutrients to regional agriculture. Using a MFA framework, we compared the recoverable nutrients from food waste, sewage sludge, and source-separated urine to identify which source of nutrients has the highest potential for recovery. After quantifying recoverable nutrients, we compared the environmental benefits of diverting the waste and returning nutrients for agriculture for the three waste streams and performed a suitability analysis to identify suitable locations for urine separation. We focused on urine separation in the suitability analysis because it requires significant infrastructural and behavioral changes. We demonstrated these methods in NYC, the most populous city in the US, to quantify the potential benefits that nutrient recovery can provide for waste management agencies and regional agriculture. Additionally, we provided a suitability analysis framework and identified the suitability criteria that other cities or regions may use for considering urine separation.

5.2 Waste Management in New York City

Due to increasingly stringent nutrient discharge standards, aging infrastructure, and space constraints in NYC, it is an important city for demonstrating the potential benefits of nutrient recovery and circularity for other cities. The city has five boroughs, spanning an area of 306.2 miles^2. In 2021, the US Census estimates that the population in NYC was 8,467,513 people (US Census, n.d.), making it the most populous and one of the densest cities in the United States. The city generates up to 8 million pounds of food and organic waste (DSNY, n.d.-a) and 1.3 billion

gallons of wastewater per day (DEP, n.d.). All of the wastewater generated within the city boundaries is treated at 14 WRRFs by the NYC DEP. Solid waste generated in the city is managed by the Department of Sanitation (DSNY) or private commercial waste haulers.

NYC has adopted stringent waste management goals. The OneNYC plan to reduce 50% of the city's GHG emissions by 2030 and 100% by 2050 requires zero waste to landfills (DEP, 2023). DSNY expanded curbside composting service and organic waste drop-off sites throughout the city while regulations became more stringent for commercial waste generators (Tisch, 2023; NYC Mayor's Office of Sustainability, 2017). DEP reports that 30% of sewage sludge was diverted from landfills for beneficial reuse in 2019, but they have goals to reach 90% non-landfill use of sewage sludge by 2030 (DEP, 2023). In addition to solid waste management, NYC has a goal to prevent wastewater Nr discharge into the Long Island Sound. Over $1 billion was invested into four NYC WRRFs, resulting in a 61% reduction in N discharge. Despite the improvement, wastewater discharge from NYC into the sound is still a significant source of N inputs affecting water quality and the projected rise in NYC population presents a challenge for WRRFs to further reduce its contributions to Long Island Sound (Vaudrey, 2017).

Amid projected population growth, limited capacity to treat wastewater, and increasingly stringent N discharge regulations, NYC has limited solutions for processing projected increases in wastewater N loads. Specific WRRFs are already at capacity for their BNR processes and any increase in N loads will directly be discharged into the receiving water body. Rezoning efforts to convert existing buildings into new, larger developments that will increase population are underway in some of these sewersheds, requiring a technology such as urine separation to prevent increased N loading at the WRRF.

5.3 Methods

Nutrient flow analysis

MFA, sometimes referred to as substance flow analysis, is a mass balance model of the material or substance of interest as inputs, stocks, and outputs for a given system. The MFA framework has been used extensively to identify hotspots for material use and evaluate scenarios to improve efficiency and sustainability in waste management (Clift & Druckman, 2016) and urban metabolism (Barles, 2009). In this study, we used a MFA to quantify the recoverable N and P in waste generated in a city. Results from the MFA were used to quantify their impacts on material needs for downstream waste management and sustainability benefits if they were returned to regional agriculture.

Building the MFA

For our MFA, the system boundaries are confined to the political boundaries of NYC which includes the five boroughs Bronx, Brooklyn, Manhattan, Queens, and Staten Island. Within the city, we considered three major systems that extensively impact N and P: food, solid waste, and wastewater (Figure 5-1). We used a time scale of one year to quantify flows entering and exiting, and the stocks remaining in the city. Figure 5-1 illustrates the flows entering and exiting each system.

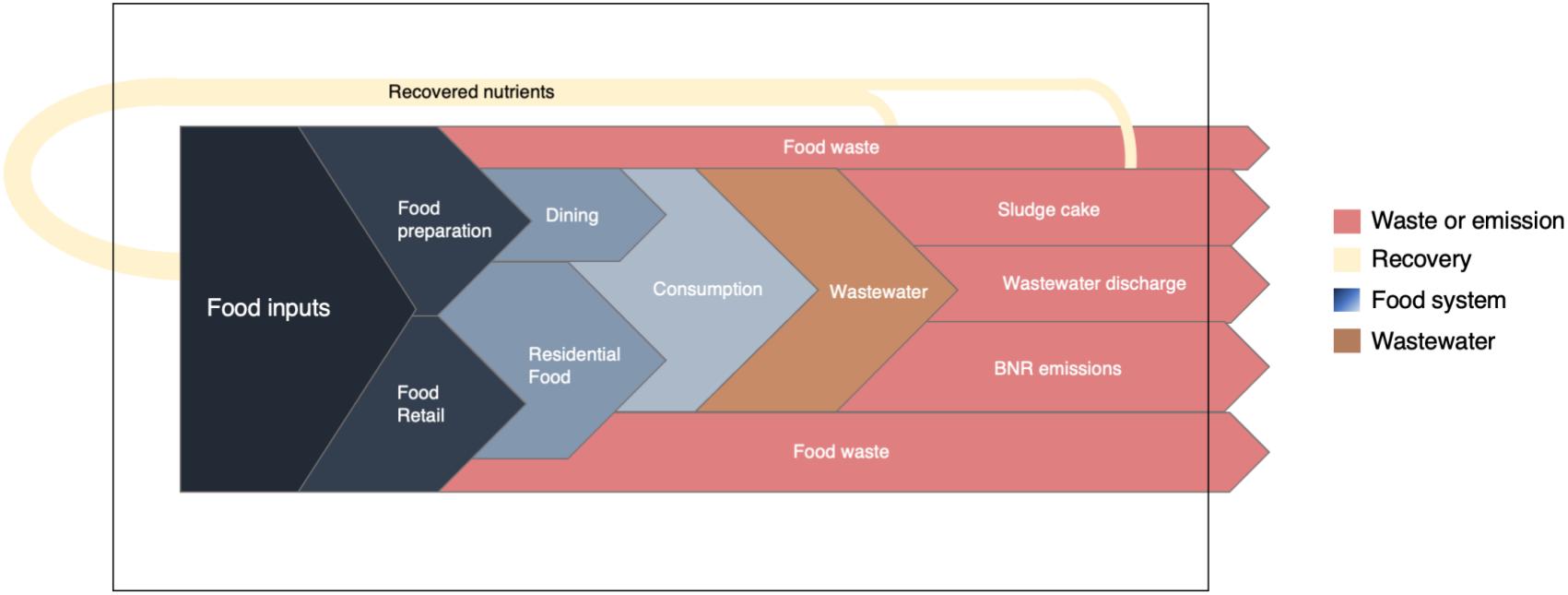


Figure 5-1. Flows in the Material Flow Analysis with the food and waste systems. The black boundary represents the boundary of the system, which is the boundaries of New York City in this study. Subsystems include food inputs, food preparation, food retail, food consumption, wastewater, and solid waste.

System flows and data sources

Food

In a 2016 study on NYC food flows, the NYC Economic Development Corporation traced food from outside of the city into distribution centers and finally to point-of-sale (POS) outlets in the city. We assumed that all imported food that is consumed or wasted in the city goes through a POS outlet. POS outlets include bodegas, chain convenience stores, chain quick service restaurants, chain supermarkets, drug stores, food markets, hotels, and independent restaurants and cafes. Outlets were categorized into food retail where packaged food is sold and food preparation where food is prepared and then sold. Bodegas, which are small convenience stores that sell prepared and packaged food, are categorized as both. Food inputs at retail outlets are either wasted or purchased for residential consumption. Food inputs at preparation outlets are either wasted during preparation and consumption or consumed in residences as takeout and onsite, at the outlet. Food flows were converted from wet mass to mass of N and P based on data from the USDA Food Availability Data System (FADS) (USDA, n.d.). Flow N and P calculations can be found in Table C-1.

Solid waste

Solid waste in NYC is divided into municipal and commercial waste. Municipal waste consists of residential and institutional sources of waste that are collected and managed by DSNY. Commercial waste that is generated by commercial activities are collected and managed by private waste haulers. We used DSNY data from 2019 (DSNY, n.d.-b) and estimated the commercial waste generated from a 2022 DEP food waste feedstock assessment. Almost all of the waste generated in the city is transported outside of the city and usually outside of the state for landfilling or incineration. NYC waste can be landfilled as far as Ohio (DSNY, 2017). A small fraction of the

organic waste is recovered through various efforts. Waste flows were converted from wet mass to mass of N and P based on the 2017 DSNY waste characterization report (DSNY, 2017). N and P calculations can be found in Table C-1.

Wastewater

Wastewater in NYC is conveyed to 14 WRRFs throughout the city that are managed by the DEP. Eight WRRFs have biological nitrogen removal (BNR) processes to meet N discharge standards for the city. Sludge generated from the WRRFs are dewatered at six WRRFs. Wastewater N and P data was collected from DEP and 2021 data was used in our analysis. Sludge flows were converted from dry mass to mass of N and P based on a 2022 DEP study that characterized the sludge from each of the WRRFs. N and P calculations can be found in Table C-1.

Compare recoverable nutrients for different scenarios

We used the MFA model to compare different scenarios of nutrient recovery that represent various waste management goals. For each scenario, we compared recoverable N and P, which were calculated from food waste and wastewater outputs from the MFA according to Equation 5-1. Recovery implementation is the extent of recovery from the recoverable waste. At maximum recovery implementation, we assume that all recoverable waste is recovered, but not all waste is recoverable. For example, some of the N in urine must remain in the wastewater influent to allow adequate removal of chemical oxygen demand at WRRFs. Therefore, 100% implementation of urine separation is approximately equivalent to source-separating 65% of the influent N in NYC. Recovery efficiency is the proportion of N and P in the waste that is recovered after losses from collection and processing. For the results we present in this study, we assumed nutrient recovery efficiency is 80%, but this is a parameter that can be adjusted in the model.

Equation 5-1. N and P recovery potential = recoverable waste N and P * recovery efficiency * recovery implementation

We compared 11 scenarios that include targeted and combined recovery from waste (Table 5-1). The baseline represents nutrient recovery as it was implemented at the time of analysis. For the remaining scenarios, N and P recovery potential is not additive to the baseline. To represent the maximum recovery potential, the total recovery scenario is the sum across the three waste streams at 100% recovery implementation. We also compared targeted scenarios that represent the maximum recovery potential from each of the individual waste streams at 100% implementation. To vary the degree of implementation, we included targeted scenarios under specific policy or implementation circumstances. For example, we defined two additional food waste scenarios to target recovery from commercial-only and DSNY-only food waste. In NYC, commercial waste is collected by private haulers whereas residential and institutional waste is collected by DSNY. The two sources of food waste have different challenges and regulations for collection and recovery. For urine separation, a more feasible approach is to target only waterless urinals, which would approximately account for 50% of the urine generated in NYC. To represent this, we defined a targeted recovery scenario of 50% source-separated urine. Finally, we defined scenarios to combine waste streams that align with waste management goals: wastewater focused recovery (source-separated urine plus sewage sludge) for DEP, landfill diversion (food waste plus sewage sludge) for diverting organic waste from landfills, and recovery from urine plus food waste.

Table 5-1. Summary of the recovery scenarios. The recovery for each scenario is the sum of percent recovered across each waste stream.

Scenario	Source-separated urine	Food waste	Sewage sludge
Baseline	0%	10%	30%
Total recovery	100%	100%	100%
Food waste	0%	100%	0%
Commercial food waste	0%	53%	0%
DSNY food waste	0%	36%	0%
Urine separation	100%	0%	0%
50% urine separation	50%	0%	0%
Sewage sludge	0%	0%	100%
Urine plus food waste	100%	100%	0%
Urine plus sludge	100%	0%	100%
Food waste plus sludge	0%	100%	100%

Estimate the environmental benefits of local and regional nutrient circularity

Using model outputs from the MFA, we calculated the potential environmental benefits of recovering nutrients from waste and returning it for regional agriculture. Environmental benefits were considered for reduction in fertilizer production and diversion of waste from landfills and WRRFs. For fertilizer production, we calculated the reductions in energy demand, water consumption, and GHG emissions associated with N and P fertilizer production. For waste management, we calculated the reductions of GHG emissions of landfilling food waste and sewage sludge and the reductions of energy demand, chemicals needed, and GHG emissions of BNR at WRRFs.

Suitability analysis

A raster suitability analysis is a commonly used method to compare and rank locations based on a set of suitability criteria. We used a raster suitability analysis to identify locations in the city where urine separation can help DEP meet N discharge limits in the context of increasing population growth.

Identify components of suitability

The suitability of urine separation was determined by sociotechnical factors including N reduction needed at WRRFs, urine generation, nutrient density, operational likeliness, and projected population growth. N reduction needed at WRRFs was determined by evaluating the WRRFs BNR technology, capacity to process higher N loads, ability to modify BNR processes, the sensitivity of the discharge body to N, current N discharged, and projected N increase based on projected population growth. Urine generation is a numerical parameter that represents the volume of urine generated in a community district for each land use category. Nutrient density is a qualitative parameter that represents the density of urine generation in a given land use category. Finally, operational likeliness is how likely a land use category will allow the installation of waterless urinals or urine-diverting toilets.

Generate component raster files

Urine generation was calculated using water consumption data. Automated water reading (AMR) data was gathered from DEP and consolidated at the community district level for each land use category (gallons per day). We converted AMR data into urine production using a list of factors determined by making assumptions of urine production in different building classes: 0.5% in residential buildings, 5% in commercial and institutional buildings, and 1% in industrial and outdoor settings.

For operational likeliness and nutrient density, we used the MapPLUTO database from NYC Department of City Planning (NYC DCP, n.d.) to map the land use category and area at the tax lot scale. We then assigned an amount of urine generated to each tax lot based on land use category, community district, and the proportion of the tax lot area of the total area of that land use category in that community district. WRRF suitability criteria were determined with data from DEP for each sewershed, except the projected N increase, which was calculated from the population change between 2010 and 2020 at the neighborhood level (NYC DCP, 2023). Vector files for each suitability component were joined either with MapPLUTO or the sewershed map and converted into raster files with a cell size of 0.001 degrees using the Global Coordinate System World Geodetic System 1984 (GCS WGS 84).

Weighed suitability model

Each criterion was reclassified as a score from one to nine that represents how important the criteria are to the suitability analysis goal of meeting wastewater N discharge limits amid population growth. For urine separation and WRRF effluent N, we assigned a nine-step scale to the continuous range of values where the largest amount of urine generation and WRRF effluent N are most suitable. For nutrient density, we considered how dense human activity and thus, urine generation is in a given land use category. For example, we ranked commercial & office buildings as high and open space & outdoor recreation as low. For operational likeliness of installing waterless urinals and urine-diverting toilets, we assigned higher rankings to land use categories with higher commercial and institutional activity. In these buildings, toilet and urinal maintenance can be more easily monitored and specific maintenance rules can be enforced. For the criterion that represent WRRF N reduction needs, we gave higher rankings to WRRFs that discharge into a water body that is sensitive to N inputs, do not have BNR technology, do not have capacity to

accommodate higher N loads, do not have the ability to modify BNR to meet higher N loads, but have a higher projection of N increases. Reclassification scores were determined with the Saaty scale of relative importance (Saaty, 1977), as outlined in Table 5-2. We then used the analytical hierarchical process (AHP) to perform pairwise comparisons between suitability criteria and calculated weights for each criterion. Based on our discussions with DEP, we determined that the primary motivation for implementing urine separation is to help WRRFs meet N discharge limits and prioritized the criteria that affect this goal (Table C-3). The final suitability score was calculated by weighing each component.

Evaluate suitability and calculate benefits to WRRF(s)

Using ArcGIS Pro, we generated a heat map of suitability. Based on this map, we identified the sewershed(s) where urine separation can significantly impact the receiving WRRF(s). For this shed(s), we quantified the energy, chemical, and GHG reductions using the same equations to calculate the environmental benefits from the MFA outputs.

Table 5-2. Ranking used to reclassify suitability criteria in the suitability analysis. A reclassification ranking of 9 is high in suitability and 1 is low. H indicates a high value for the suitability criteria, MH for medium high, M for medium, ML for medium low, and L for low.

Reclassification	Urine generation (10^5 gallons per day)	Nutrient density	Operational likeliness for urinals	Operational likeliness for urine-diverting toilets	WRRF - BNR	WRRF - effluent N (10^6 lbs/day)	WRRF - capacity for higher N loads	WRRF - ability to modify BNR	WRRF - discharge body sensitivity to N	WRRF - projected N increase (10^4)
9	1.6 – 1.8	H	H	H	No, with dewatering	8.6 – 9.6	None	No	Jamaica Bay, East River	28.4
8	1.4 – 1.6				No	7.6 – 8.6				
7	1.2 – 1.4	MH				6.5 – 7.6	L			13.7
6	1.0 – 1.2				Yes, without supplemental carbon	5.5 – 6.5				10.5 – 10.6
5	0.8 – 1.0	M		M	Yes, with dewatering	4.5 – 5.5	M			9.0 – 10.0
4	0.6 – 0.8					3.5 – 4.5				
3	0.4 – 0.6	ML	ML	ML	Yes	2.5 – 3.5				7.3 – 7.9
2	0.2 – 0.4					1.5 – 2.5				
1	0 – 0.2	L	L			0.48 – 1.5	H	Yes	Hudson River, NY Bay, Kill Van Kull	

5.4 Results and Discussion

Nearly all inputs of N and P exit as waste or emissions from waste management

Within the food and waste systems, 2.0×10^8 lbs N entered NYC and 91% of the N inputs, or 1.3×10^8 lbs N exited as waste or emissions from waste management (Figure 5-2). We estimated that 2.7×10^8 lbs N is applied as fertilizers in conventional agriculture for food entering the city. With existing nutrient management strategies, 11% of N contained in food waste and sludge cake is diverted from landfills. Of the N that exited the city, 16% are in WRRF discharge, 65% in BNR gaseous emissions, 13% in landfilled food waste, and 6% in landfilled sludge cake. Most of the N inputs end up in wastewater and is converted to N_2 and N_2O gaseous emissions from BNR. In 2001, Toronto also only recovered 4.7% of N in waste and most of the losses were from wastewater management (Forkes, 2007), whereas N recovery was 7% in Bangkok in 2000 (Færge et al., 2001).

P flows are approximately 1/10 of N flows, which is similar to the average N:P ratio of terrestrial plants (Gusewell, 2004). 2.1×10^7 lbs P entered NYC and 93% of the P inputs, or 1.9×10^7 lbs P exited as waste or losses from waste treatment (Figure 5-2). We estimated that 2.9×10^7 lbs P is applied as fertilizers in conventional agriculture for food entering the city. About 22% of the P inputs is diverted from landfills and recovered for beneficial use. For comparison, P recovery in Bangkok was 10% in 2000 (Færge et al., 2001). Of the P that exited NYC, 24% was in WRRF discharge, 66% in landfilled food waste, and 10% in landfilled sludge cake. Although most of the P ends up in landfilled food waste, a significant portion still remains in the WRRF effluent. In Gothenburg, Sweden, P outputs were primarily in sewage sludge (40%) and incineration ash (40%) in 2009. Although different solid waste management strategies are used, there is a similar pattern of higher P in solid waste (Kalmykova et al., 2012). Soluble Pr is not the limiting nutrient in the

water bodies where NYC WRRFs discharge into, and P management is not a common process for DEP (Ammerman, 2018).

The per capita N and P inputs in NYC are 10.8 kg N/capita and 1.1 kg P/capita. These values are in the upper range of inputs compared to other cities in previous years (4.1-13.4 kg N/capita and 0.4-1.1 kg N/capita) including Beijing, Paris, the Chaohu watershed, Phoenix, the Detroit metropolitan area, Toronto, Vienna, Linkoping, Harare, Busia, and the Thachin basin from 1817-2012 (Liang et al., 2019). While NYC had the third highest N inputs per capita and the highest P inputs per capita among other cities, this study only included flows in the food system and does not include agricultural or landscaping inputs which may further increase the per capita N and P inputs. Furthermore, the values reported are for 2017 and previous studies were completed prior to 2013. With current waste management strategies, NYC discharged 1.7 kg N/capita and 0.3 kg P/capita as wastewater effluent, which are low compared to 0.6-4.0 kg N/capita and 0.05-2.1 kg P/capita for other cities from 1818-2012 (Liang et al., 2019). NYC emissions into water bodies are lower partially because our MFA boundaries were the city boundaries of NYC whereas previous studies included agricultural areas surrounding the urban center. When agricultural areas are included, N and P emissions can be higher from the large agricultural contributions to emissions flows into water bodies.

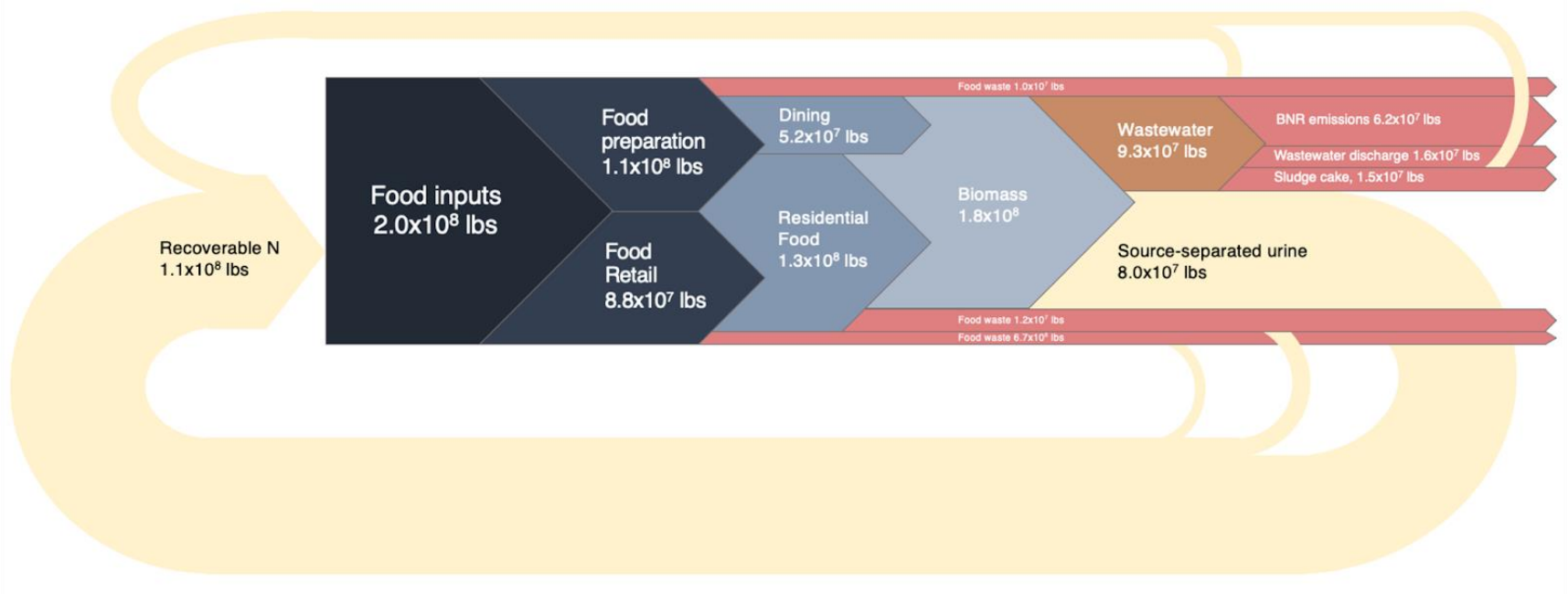


Figure 5-2. N flows for NYC. Flows include food inputs, the internal processes of food preparation, consumption, retail, and waste they undergo, and the outputs as solid waste and wastewater with maximum recovery. Flows in blue are part of the food system and include inputs, preparation, retail, and consumption. After food is consumed, N is excreted in the brown mixed wastewater flow. Food waste exits the city in the red flows. Nutrient recovery is represented by the yellow flows from source-separated urine, recovered food waste, and sewage sludge that is diverted for beneficial use.

Urine and food waste have high potential for N and P recovery, respectively

While waste prevention and reduction in N- and P- heavy diets are two of the five most promising nutrient reduction strategies (Houlton et al., 2019), NYC can advance their waste management and nutrient emission reduction goals by increasing nutrient recovery implementation. Specific waste streams or a combination of the waste streams can be targeted to meet the waste management and sustainability goals of the city. We calculated the recoverable N and P via food waste, separated urine, and sewage sludge in NYC, not including blackwater, greywater, or gaseous emissions. Of the total mass of 2.0×10^8 lbs N and 2.1×10^7 lbs P entering NYC as food, we determined that the contributions are distributed as follows, based on existing infrastructure and population: 18%, 74%, and 9% of recoverable N and 68%, 21%, and 11% of recoverable P in food waste, urine, and sewage sludge, respectively. Therefore, separated urine has the highest proportion of recoverable N in the food and waste systems in NYC, while food waste has the highest proportion of recoverable P. However, NYC currently only captures 11% of recoverable N and 22% of recoverable P via food waste and sewage sludge management. This scenario serves as the baseline to compare other scenarios to.

Nutrient recovery potential

If all recoverable food waste, sewage sludge, and urine were recovered, as much as 1.1×10^8 lbs of N and 1.9×10^7 lbs of P can be recovered, which is approximately equal to 72% and 93% of the N and P inputs, respectively. Since source-separated urine contains the largest fraction of recoverable N, targeted recovery from urine has the highest potential N recovery, at 5.42×10^7 lbs N or 37% of the inputs. Even at 50% urine separation, the potential N recovery is 2.71×10^7 lbs, which is slightly larger than the food waste scenario (2.56×10^7 lbs) and slightly lower than the sewage sludge scenario (2.94×10^7 lbs). When waste streams are recovered together,

significant N recovery can be achieved with the urine plus sewage sludge (8.29 x 10^7 lbs or 57% of the inputs) and urine plus food waste (7.70 x 10^7 lbs or 53% of the inputs) scenarios (Figure 5-3). For P, food waste contains the largest fraction of recoverable P. Targeted recovery from food waste can recover 1.25 x 10^7 lbs or 52% of the inputs. Recovery from commercial only and DSNY only food waste are similar, with moderate levels of P recovery (31% and 21% of the P inputs, respectively). P recovery from urine and sewage sludge are much lower, at 3.54 x 10^6 lbs or 17% of the inputs and 5.35 x 10^6 lbs or 25% of the inputs, respectively. When waste streams are recovered together, significant P recovery can be achieved with the urine plus food waste (1.47x 10^7 lbs P or 69% of the inputs), and food waste plus sewage sludge diversion (1.65 x 10^7 lbs or 77% of the inputs) scenarios. Comparing the recovery potential of all scenarios, N and P recovery can be best achieved by incorporating urine separation and food waste diversion, respectively. Consequently, high recovery of both N and P can be achieved by the urine plus food waste scenario without the need to recover from sewage sludge.

Recovering multiple waste streams can advance waste management goals and agriculture goals. The stoichiometry of the recovery product can impact nutrient use efficiency and the value of the crop (van der Wiel et al., 2019). Therefore, it is important to consider the ratio of total N and P recovered. The N:P ratios for the scenarios are summarized in Table C-2. High N:P ratios were found for the targeted recovery for urine separation (15.4) and urine plus sewage sludge (9.5) scenarios. The targeted recovery for food waste and urine plus food waste had low N:P ratios (<5), but all scenarios had ratios greater than one. The ideal N to P ratio in a fertilizer depends on the N and P levels of the soil and the crop. To meet specific needs of the soil and crop, recovered waste streams can be combined to achieve the appropriate N:P ratio.

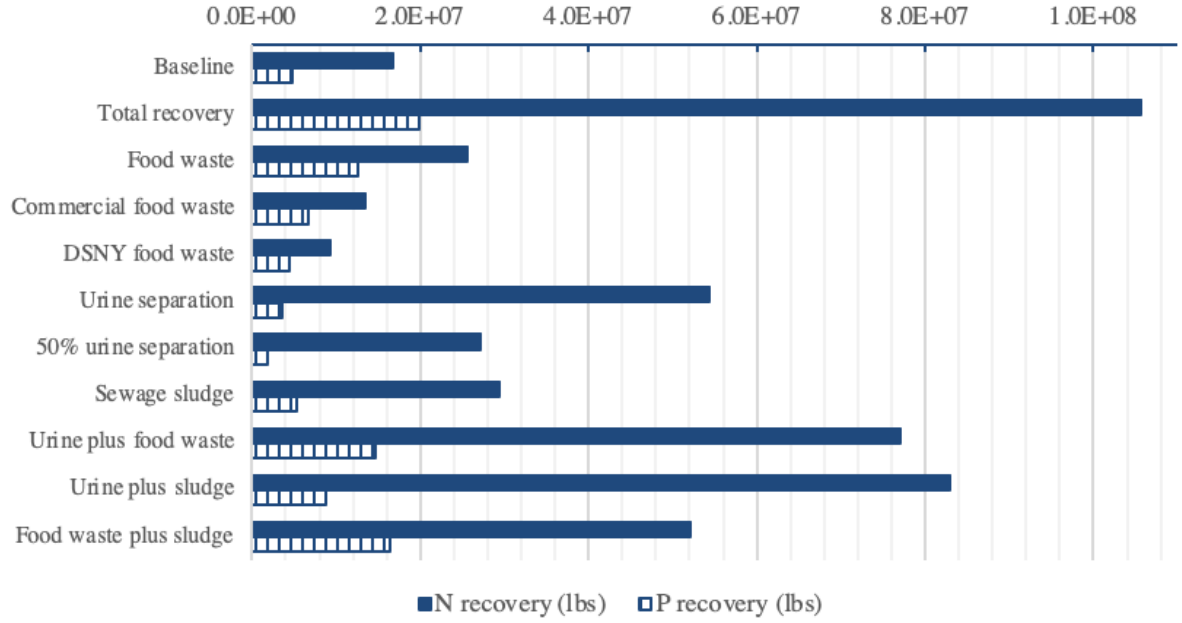
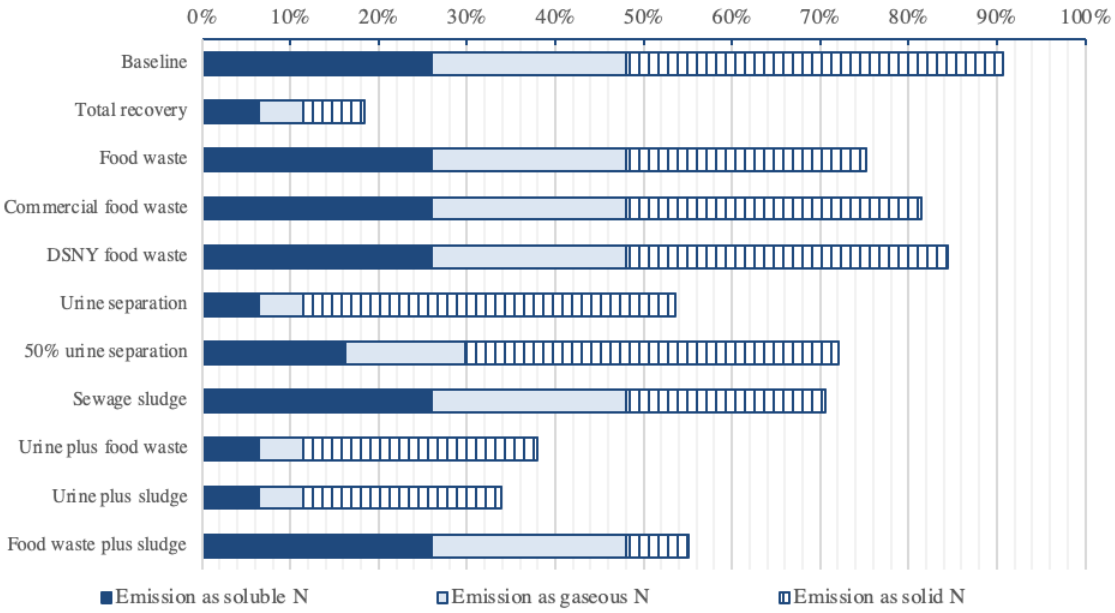


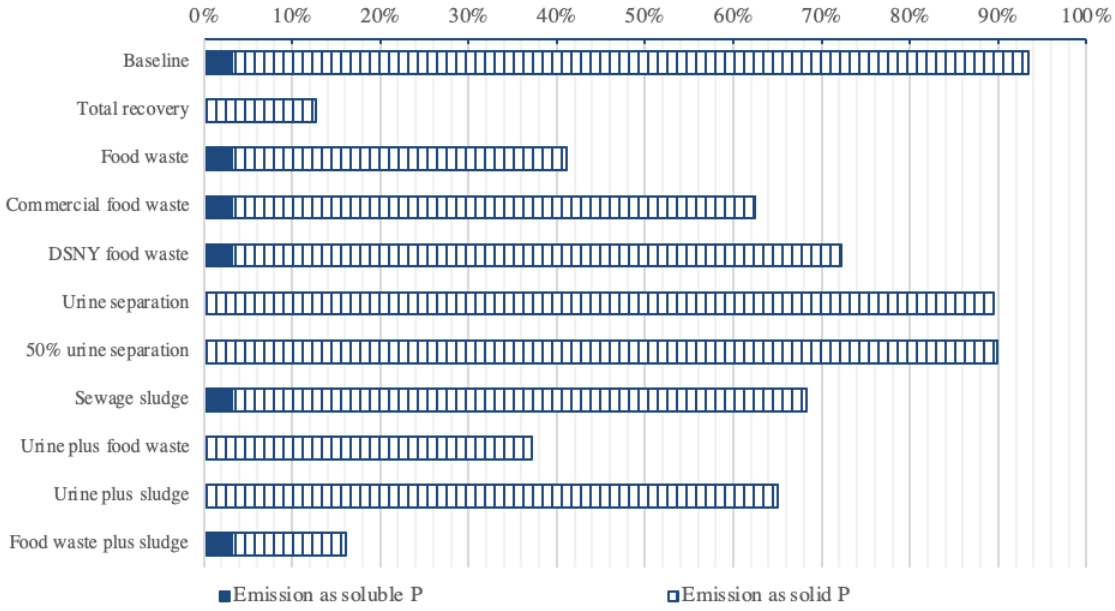
Figure 5-3. Mass of N and P recoverable from each scenario.

Nutrient emissions

At baseline, 91% of N and 93% of P inputs are lost to the environment in any form. When all nutrients are recovered, N and P loss is reduced to 18% and 0% of the inputs, respectively (Figure 5-4). Reducing N emissions to below 50% of the inputs can be achieved in the urine and food waste (38%) and wastewater-focused (34%) scenarios. These scenarios reduce soluble N entering WRRFs, which result in lower soluble and gaseous N emissions from wastewater treatment (Figure 5-4). For P, emissions below 50% can be achieved in the food waste (41%), urine and food waste (37%), and landfill (16%) scenarios. These scenarios are effective because total P emissions are primarily made up of solid P particularly in food waste (Figure 5-4). To reduce both N and P emissions, recovery from source-separated urine and food waste can reduce emissions to 38% for N and 37% for P. Urine separation and the urine plus sewage sludge scenarios can advance DEP's goals to reduce N discharge into Long Island Sound and other nearby water bodies.



a)



b)

Figure 5-4. % of the N and P inputs emitted to the environment for (a) N and (b) P. Each section of the bar represents the proportion of the total emissions that are soluble, gaseous, or solid.

Fertilizer offset potential

In addition to reducing nutrient emissions, recovered nutrients can be used to offset fertilizer production. At maximum recovery, $1.1x10^8$ lbs of N and $4.8x10^6$ lbs of P can be recovered and offset 52% and 69% of the external N and P needs, respectively, to produce the food inputs for NYC. Regional agricultural fertilizer needs can also be met with recoverable nutrients from the city. In 2017, an estimated $2.2x10^8$ and $1.3x10^8$ lbs of N and P commercial fertilizer were purchased in New York State (EPA, 2023). The total recoverable N and P can offset 47% and 16% of the purchased commercial N and P fertilizers. At the estimated cost of $509 per ton of ammonia fertilizer and $425 per ton of diammonium phosphate fertilizer (Schnitkey, 2017), the recovered nutrients are worth as much as $32.6M for N and $18M for P per year. However, the market value of recovered nutrients as fertilizer is variable and depends on many factors including their nutrient use efficiency and consumer acceptance of the product that need to be further evaluated.

<u>The environmental impact of nutrient reduction is small for NYC overall as GHG, but significant for eutrophication potential</u>

Nutrient recovery benefits the environment by offsetting fertilizer production and reducing nutrient emissions. Offsetting fertilizer production reduces the needed energy demand and GHG emissions associated with fertilizer production, while reducing nutrient emissions results in lower environmental Nr and Pr and reduces the need for waste management agencies to convert them back into inert forms. Here, we compare the environmental benefits for reducing nutrient emissions and offsetting fertilizer production for each scenario without making assumptions about the waste processing technology and the environmental impacts associated with them.

Greenhouse gas emissions

At maximum recovery from the three waste streams, $1.6x10^9$ lbs of CO_2 equivalent of GHG emissions are reduced at the landfill, at WRRFs, and from offsetting fertilizer production at 76%, 1%, and 23%, respectively (Figure 5-5). GHG emissions from waste management are primarily reduced at landfills by diverting organic waste that would decompose and generate GHG (NYC Mayor's Office of Sustainability, 2017). Subsequently, the scenarios with food waste recovery yield the highest reductions in GHG emissions. For food waste recovery, we estimated a reduction of $1.1x10^9$ lbs CO_2 equivalent, 7% of which is attributed to offsetting fertilizer production and 93% to diversion from landfills. If nutrient recovery efforts are expanded to the food waste plus sewage sludge scenario, $1.4x10^9$ lbs of CO_2 equivalent can be reduced with 87% from landfill diversion and 13% from fertilizer offset (Figure 5-5).

Urine separation has moderate GHG reduction. We estimated $5.2x10^8$ lbs of CO_2 equivalent reduction, of which 36% is for fertilizer production offsets and the rest is for reduced emissions at landfills (61%) and WRRFs (3%) (Figure 5-5). The reduction in GHG emissions at WRRFs is attributed to reduced N_2O emissions from nitrification and denitrification processes. We assumed that 0.1% of N that undergoes BNR at WRRFs are emitted as N_2O (Kampschreur, 2009) and contributed to GHG emissions from WRRFs. By reducing $5.4x10^7$ lbs N in the influent, $6.0x10^4$ lbs of N_2O emissions are also reduced (Figure 5-5). GHG emissions are also reduced with urine separation by reducing $4.3x10^7$ lbs of sewage sludge generated at WRRFs that would have been landfilled. In the urine plus food waste scenario, there is a reduction of $1.6x10^9$ lbs of CO_2 equivalent, with 1% from WRRFs, 82% from landfills, and 17% from fertilizer offset (Figure 5-5).

Although GHG emissions from landfills and WRRF N_2O emissions only make up 3% and 0.1% of citywide annual emissions (NYC Mayor's Office of Sustainability, 2017), respectively,

reducing them will still be important for meeting the OneNYC Plan to cut GHG emissions by 100% by 2050 (DEP, 2023).

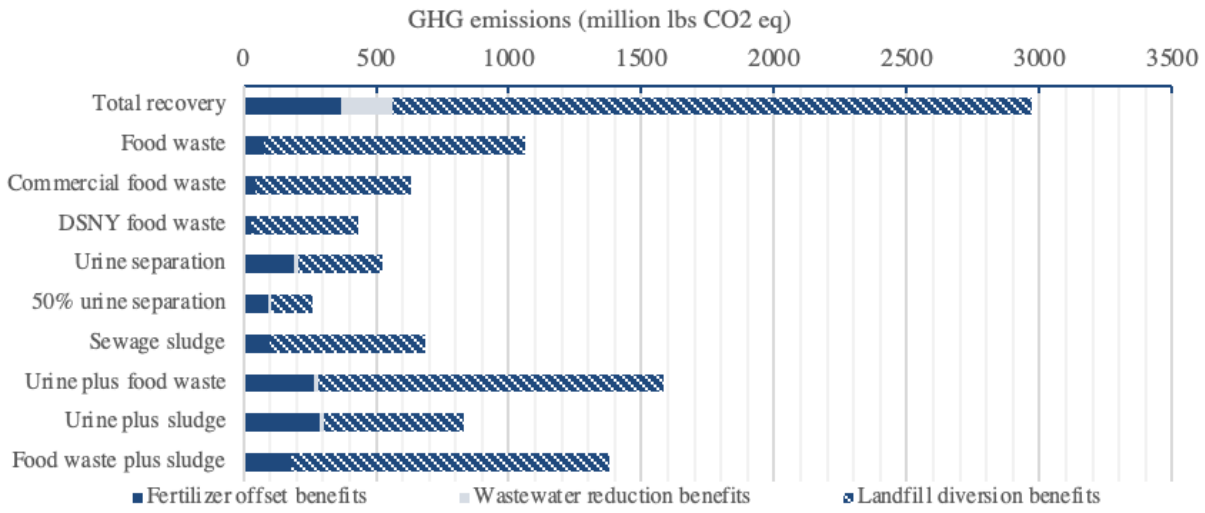


Figure 5-5. GHG emissions that are reduced with each recovery scenario in million lbs of CO_2 equivalent. Each section of the bar represents the proportion of the GHG reductions from offsetting fertilizer, reducing N at WRRFs, and diverting organic waste from landfills.

Energy

At maximum recovery, energy is reduced at the WRRF and from offsetting fertilizer at 40% and 60%, respectively of the $7.9x10^5$ GJ of energy reduced (Figure 5-6). Energy reduction primarily occurs at WRRFs as lower N loads at WRRFs significantly reduces the O2 demand for nitrification. Subsequently, the scenarios that involve sewage sludge and urine yield the most benefit (Figure 5-6). Among the four scenarios that affect WRRFs, the urine plus sewage sludge and urine plus food waste scenarios have similar energy reduction at $6.3x10^5$ and $7x10^5$ GJ of energy, 75% and 68% at WRRFs and 25 and 32% from offsetting fertilizer production, respectively (Figure 5-6). Urine separation alone accounted for the bulk of the reduction in energy demand.

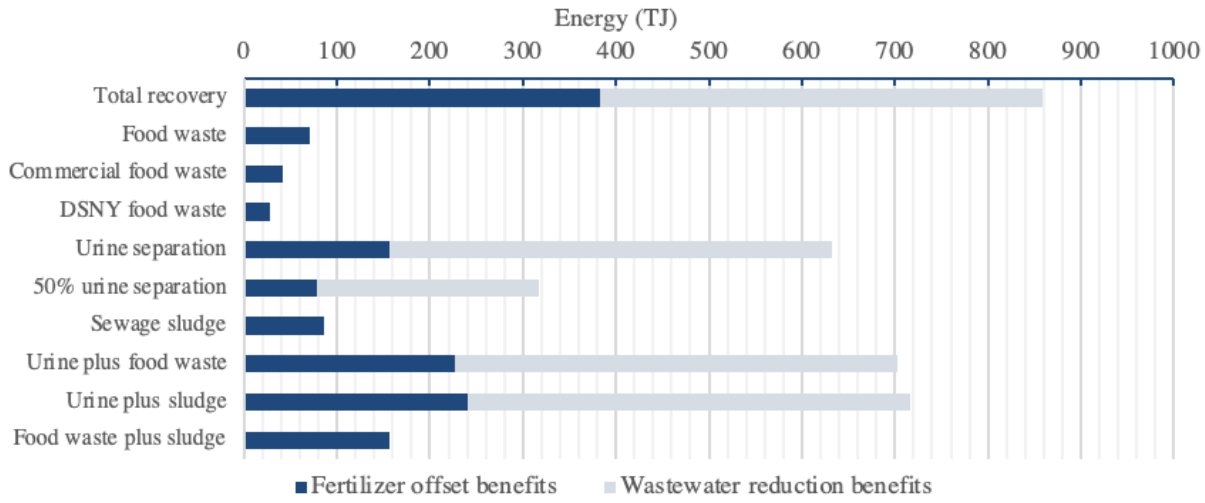


Figure 5-6. Energy demand that is reduced with each recovery scenario in TeraJoules. Each section of the bar represents the proportion of the GHG reductions from offsetting fertilizer and reducing N at WRRFs.

Chemical inputs

For scenarios including urine separation, other benefits include reducing $1.1x10^8$ lbs $CaCO_3$ of alkalinity and $7.0x10^7$ lbs of glycerol as supplemental carbon for BNR, which is 100% and 99% of the baseline alkalinity and carbon inputs, respectively. Reduction of alkalinity and glycerol lowers costs for DEP to maintain BNR processes at WRRFs to meet stringent N discharge needs in addition to the environmental impacts of producing these chemicals.

Comparing scenarios in the context of sociotechnical challenges

In all scenarios, the largest GHG and energy reduction is from diverting food waste from landfills and source-separating urine, respectively. Subsequently, the urine plus food waste scenario can capture a majority of the N and P and reduce their emissions. However, there are significant sociotechnical challenges in collecting food waste and urine. Upon separation and collection, all waste streams require different technologies to process them into recovered nutrient products.

Food waste recovery is challenging due to barriers in collection and processing. Recovering food waste requires source-separation of food waste from the rest of municipal solid waste, which requires significant behavioral and infrastructural changes to ensure safe and effective recovery for food production. Efficient food waste recovery relies on consumers or waste generators to source separate food and organic waste from other solid waste. In 2019, NYC spent $32 million on an organics curbside collection program, but there was low participation. During the program's peak, only 3.7% of organic waste was collected and diverted from landfills (NYC IBO, 2021). Food waste that is collected can have significant contamination. In a previous study, physical contamination of non-compostable waste was found in 57% of source separated food waste samples in addition to PFAS, pathogens, and antibiotic resistance genes (Thakali et al., 2022), which can require additional waste processing technologies that reduce the environmental benefits of recovery from this waste stream.

Urine separation is challenging due to barriers in collection, but processing is relatively simple. For large-scale implementation of urine separation, urine-diverting toilets and waterless urinals are required. Commercially available urine-diverting toilets have been designed to minimize changes to user experience, but implementation requires additional plumbing and adequate space to store or process the collected urine. Waterless urinals have fewer plumbing challenges since major changes to plumbing throughout the building are not necessary, but buildings still need adequate storage space for collected urine. To overcome some of the infrastructural challenges, only targeting urinals, at approximately 50% of the urine, would require traditional urinals to be replaced with waterless ones. Other challenges include modifying toilet maintenance to prevent toxic cleaning agents from entering the urine collection system and to minimize user behaviors that minimize the efficiency of urine collection (e.g., blocking the urine

diverter with toilet paper). However, processing source-separated urine is relatively simple as it is a liquid waste stream with available technologies to remove contaminants and pathogens (Udert & Wächter, 2012).

Although sewage sludge can be easily collected from WRRFs, there are significant challenges in processing this waste stream. Due to the presence of heavy metals, organic contaminants, pathogens, and other constituents that can negatively impact human and soil health, there are many restrictions on using sewage sludge as a soil amendment, limiting its potential to circularize urban nutrient flows (Reilly, 2001; Forkes, 2007). Consequently, significant processing is needed to reduce public health risks for using sewage sludge as a soil amendment. For NYC, sewage sludge is digested and then dewatered into a sludge cake. However, not all of the sludge is processed to the standards of biosolids (as defined by the Environmental Protection Agency under the Clean Water Act) and may not be suitable for land application. In 2019, DEP land applied less than 1% of their sewage sludge but has plans to increase this method of beneficial use of sewage sludge to 10% by 2030 (DEP, 2023).

The different sociotechnical challenges of each waste stream suggest different approaches to advancing their technologies. Technology that requires behavioral change will require communication and outreach; infrastructural challenges require regulations and/or new technologies; and pathogen and contaminant challenges require innovative processes to reduce their risks. For urine separation, local infrastructure is needed for source separation, collection, and processing of urine into a fertilizer. We further explored urine separation as a nutrient recovery technology with high potential to improve wastewater treatment, but with significant challenges to adoption. We used a suitability analysis to identify the WRRFs where urine separation can have

the most impact and quantify the scale at which this technology needs to be implemented to have desired benefits for DEP.

Suitability of urine separation is dependent on WRRF needs and operational feasibility

The MFA results demonstrate that urine separation can be used to advance several waste management goals: meet increasingly stringent N discharge permits, reduce chemical and energy inputs at WRRFs, and reduce the need to expand BNR at WRRFs. For NYC, urine separation can also reduce N loads from new developments, helping DEP maintain N discharge limits. We used this goal to inform our suitability analysis and developed a framework that is applicable to other cities interested in using urine separation to reduce N loads at WRRFs, particularly from new developments. We focused on new developments because it can be more challenging to retrofit existing buildings (e.g., difficulty of working with old plumbing systems, lack of built-in space for urine collection and storage, inconvenience of construction for existing building inhabitants, and social acceptance among existing building habitants) and it likely will be the first option for implementing urine separation. Additionally, retrofitting a number of buildings may be more difficult to do than expanding or modifying BNR and other WRRF processes at just one facility. We used the analytical hierarchical process (AHP) approach to define relevant suitability criteria, assign weights to them based on their importance for implementing urine separation, and identify suitable sewersheds in NYC for implementing urine separation.

AHP suitability criteria weights

We performed a pairwise comparison of the suitability criteria (Table C-3) with the goal of implementing urine separation to help DEP meet N discharge limits amid growing population while considering some challenges of implementation. Our suitability criteria weights are summarized in Table 5-3. Based on our pairwise comparisons, the AHP weights are highest for

discharge body sensitivity to N, capacity for increasing N loading at the WRRF, projected increases in N loading at the WRRF, and nutrient density of the land use category. Our suitability analysis primarily focused on the impacts of urine separation on WRRFs processes, but we also considered the operational feasibility for installing collection units in buildings. The AHP weights we used reflect the importance of the sensitivity of N loading to the discharge body of the sewershed, capacity of the WRRF to accommodate growth in the sewershed, and the need for dense areas for collecting urine. While we evaluated some operational considerations for implementing urine separation, other socioeconomic/sociopolitical criteria such as impacts on users and communities, plumbing code, and building operation require more research before they can be incorporated into the analysis. Additionally, pairwise comparisons are subjective and can yield different results for different stakeholders, especially if they have different goals for the suitability analysis. For example, stakeholders who have more expertise in building operations can have different rankings of the suitability criteria and may even include different criteria that we have not considered due to our focus on wastewater management.

Table 5-3. AHP weights that were calculated from our pairwise comparison of the suitability criteria.

Category	Suitability criteria	Weight
Need for N reduction at WRRF	Discharge body sensitivity to N	31%
	Presence/type of BNR	1.6%
	Ability to modify BNR	2.3%
	Capacity for increase in N loading with existing infrastructure	16%
	Effluent N mass	3.5%

	Projected N load increase	13%
Land use feasibility	Nutrient density	10%
	Operational likeliness for waterless urinals	5.9%
	Operational likeliness for urine-diverting toilets	8.3%
	Urine generation mass	8.7%

Suitability analysis results

Using our weights, the most suitable sewersheds are Newtown Creek, Bowery Bay, and Jamaica Bay with average suitability scores of 6.6, 6.4, and 6.2, respectively (Figure 5-7). For context, the maximum score is 9 based on our reclassification method. These three sewersheds discharge into the East River and Jamaica Bay, which are the most sensitive estuaries to N loading in NYC. Additionally, population growth dynamics in the last ten years suggest that Newtown Creek has the highest projected growth and N increase. While the projected growth was not particularly high in Bowery Bay and Jamaica Bay, these WRRFs have no capacity for N increase and any increase in N would be directly discharged into the receiving body. For other sewersheds, suitability will be higher if there are projected N increases and changes to land use towards commercial and institutional activity.

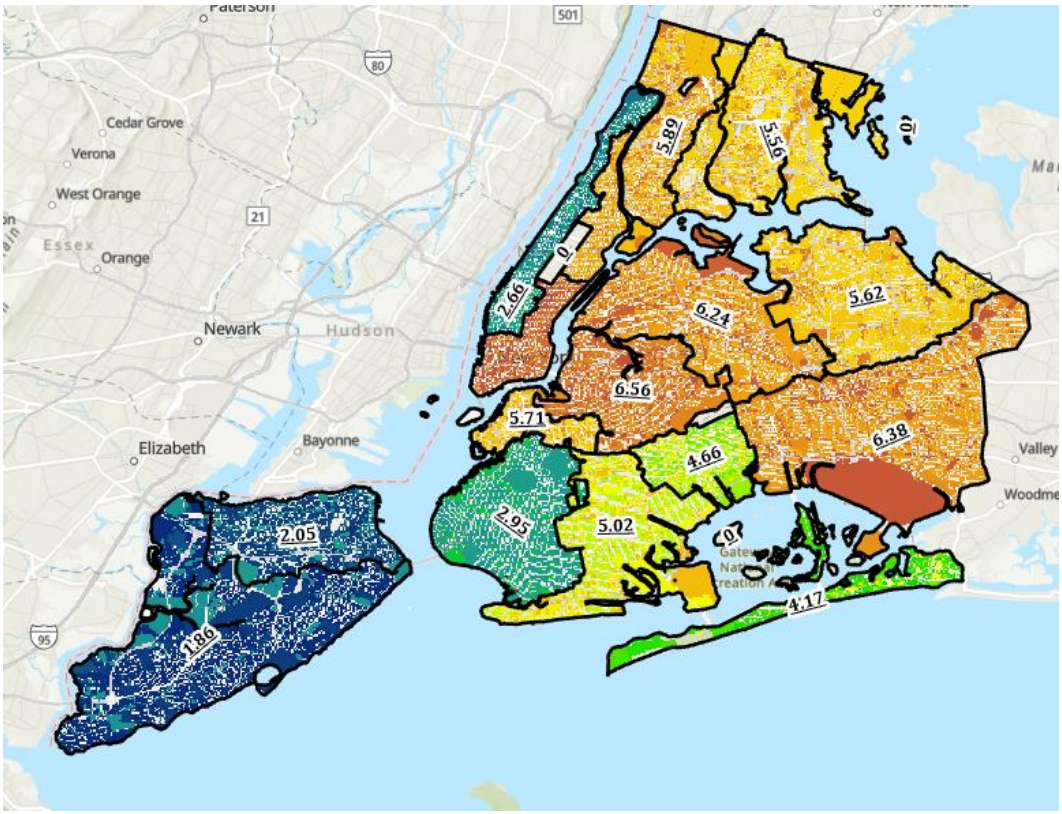


Figure 5-7. Heat map of suitability with the mean suitability score for each sewershed in NYC. The maximum score is 9.

Scale of urine separation needed to have an impact on WRRFs

For the Newtown Creek, Bowery Bay, and Jamaica sewersheds, we quantified the impacts of urine separation at the WRRFs. At 100% implementation of urine separation, there will be a reduction of 3.5×10^7 lbs of alkalinity, 2.8×10^7 lbs of supplemental carbon, 4.5×10^7 GJ of energy, and 9.6×10^6 lbs of N discharged into the receiving water body across the three WRRFs. The land use categories with the highest nutrient density and operational likeliness for waterless urinals and urine-diverting toilets are commercial and office buildings and public facilities and institutions. We estimated the number of toilets or urinals needed to collect all the recoverable urine in these settings. Assuming 11 g N/person/day is excreted in urine (Rose et al., 2015), and following OSHA guidelines to have one toilet and one urinal for every 50 people, each toilet or urinal can divert 443 lbs N per year. Installing 1,000 urine-diverting toilets or waterless urinals can divert 5% of the N

contributions from urine in Newtown Creek. To achieve 100% of urine separation in all three of the three sewersheds, 43,000 urine-diverting toilets or waterless urinals would need to be installed. This calculation provides a rough estimate of toilets or urinals needed assuming that they are only installed in commercial and office buildings. Including residential buildings will increase the number of toilets and urinals needed since residential units have a smaller ratio of toilet or urinal to people.

5.5 Conclusion

Key findings

In this study, we quantified and compared the nutrient recovery potential of different waste streams. There is a high potential for nutrient recovery from waste to circularize N and P flows, with opportunities to combine waste streams to meet various waste management goals. Although waste management contributes a significant amount of GHG emissions, it is a small fraction of the citywide GHG emissions. A stronger motivator for nutrient recovery is the reduction of wastewater effluent and gaseous emissions and the potential to offset fertilizer production for regional agriculture. N and P emissions can be reduced by recovering source-separated urine and food waste, respectively, and recovery from both can yield high levels N and P recovery.

Limitations

Our results are limited by the quality of the available data and simplifying assumptions we made to model the N and P flows. We assumed an efficiency value of 80% for the nutrient recovery processes and assumed that recovered nutrients can be used to offset 100% of the nutrients in fertilizers. Further research is needed to identify the nutrient use efficiency and market value of recovered nutrients. While NYC has high quality data on solid waste and wastewater, the food

input data was generated by surveying a selection of food point-of-sale outlets and it may not be representative of the actual food inputs entering the city.

From our suitability analysis, we identified three sewersheds that have the highest suitability score for implementing urine separation. For these sewersheds, urine separation can significantly reduce N emissions into the N-sensitive Long Island Sound and Jamaica Bay estuaries while reducing chemical inputs, energy demand, and GHG emissions for DEP. The suitability analysis was conducted from the perspective of maximizing benefits for wastewater management, but other sociotechnical factors involved in implementing urine separation will also need to be considered in future suitability analyses.

Implications and relevance of analysis

The MFA results can be used to inform waste management and sustainability goals for NYC waste management agencies. Although nutrient recovery does not have a large impact on energy demand and greenhouse gas emissions for the city, it is still critical for achieving sustainable waste management goals of reducing GHG emission and energy demand. More importantly, there are significant benefits in reducing Nr and Pr emissions and recovering N and P to offset fertilizer demand, which will be critical as nutrient pollution and inefficiency become increasingly urgent challenges for city agencies. The suitability analysis identified the most suitable sewersheds for implementing urine separation and can be used to help DEP target specific locations where urine separation can benefit the treatment processes at the receiving WRRF. The MFA model and suitability analysis that we developed can be applied to other cities that are considering urine separation and can be used as frameworks for spatializing nutrient recovery and considering sociotechnical barriers to implementation.

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Chapter 6 Conclusions, Significance, and Future Research Directions

6.1 Overview

A circular nutrient economy is critical for maintaining public and environmental health by mitigating nutrient pollution and optimizing the efficiency with which resources are used. Separating human-derived wastes at the source of generation can enhance this efficiency. Urine separation is one form of source separation that can be used to optimize nutrient capture by preventing its introduction into wastewater and processing the captured urine into beneficial products for reuse through what is called circular nutrient management. Creating a circular nutrient economy requires deepening our understanding of the risks and benefits at the source of collection, at the point of product use, and of how the technology fits within a community's broader nutrient cycle.

This dissertation assesses public health risks during urine collection (Chapter 3), nitrogen (N) losses from urine-derived fertilizer (UDF) application (Chapter 4), and the role of urine separation in a circular nutrient economy (Chapter 5). In Chapter 3, we measured virus emissions from toilet flushing to compare emission levels between a urine-diverting toilet (UDT) and mix flush toilet. To assess the impacts of using the collected urine, Chapter 4 describes a greenhouse experiment that compared the impacts of UDF on soil health and N losses. When considering nutrient cycling at a city-scale, Chapter 5 describes a nutrient flow and suitability analysis we performed using New York City (NYC) as a model city to determine the role of urine separation in urban nutrient circularity and identify potential locations for implementing urine separation there. Together, the findings of this dissertation suggest that collecting and processing urine into a UDF can reduce risk to public health and is critical for achieving a circular nutrient economy.

6.2 Main Findings and Significance

Urine separation is critical for achieving a circular nutrient economy

Source separating urine for nutrient recovery is critical for a circular nutrient economy. While previous studies have quantified the potential benefits of urine separation in reducing water and energy consumption for conventional wastewater management and fertilizer production (Hilton et al., 2021, Ishii and Boyer, 2015), we compared the potential of nutrient recovery from urine to other nutrient-dense urban waste streams. Results from Chapter 5 demonstrated that urine contains the largest proportion, as much as 74%, of recoverable N from urine, food waste, and sewage sludge. Consequently, we found that urine separation is critical for N recovery, which can help NYC reduce energy use and greenhouse gas emissions at WRRFs and reduce N discharge into nearby N-limited estuaries. The importance of urine separation for reducing nutrient pollution and improving resource efficiency of wastewater treatment depends on wastewater treatment technologies and the biogeochemistry of the receiving water bodies. For other watersheds, P may be the limiting nutrient and reduction of P in the wastewater influent can reduce resource needs associated with P removal or recovery. We found that food waste contains the largest proportion, as much as 68% of the recoverable P in NYC. Thus, N and P recovery involve different waste streams and require different technologies. Simultaneous recovery from different waste streams can have synergistic benefits. For example, urine separation reduces N loading at WRRFs which leads to lower sludge production and associated resources for sludge processing and landfilling. Additionally, recovery from different waste streams can be used to meet various waste management goals in NYC: recover nutrients for specific N to P ratios in recovery product, divert waste from landfills to reduce greenhouse gas (GHG) emissions, and divert nutrients from WRRFs to reduce energy consumption. For other cities, the relative proportion of recoverable N and P in

different waste streams depends on how much food is consumed and wasted in addition to other N and P flows, such as agriculture and manufacturing. Identifying the nutrient recovery potential in different waste streams can help waste management agencies and policy makers make informed decisions about waste management and resource recovery practices tailored to their city.

While reducing N lost from waste can contribute to more efficient waste management, inefficiency in N management primarily occurs in agriculture. To the best of our knowledge, we conducted the first study to quantify N losses from UDF application and compare it to inorganic, organic, and co-application with organic fertilizer (Chapter 4). We found that UDF applied with compost led to improvements in plant yield similar to what an inorganic fertilizer achieved, and a lower ratio of N lost for every unit of N harvested. These results suggest that UDFs can replace inorganic fertilizers without compromising plant yield. While the overall N loss to uptake ratio decreased when UDFs were used with compost, N_2O emissions increased. Results can vary for a different soil type, plant, and farm management practice, but findings from Chapter 4 suggest that it is critical to consider different approaches to using UDFs, especially those within an ecological nutrient management framework, to mitigate N losses for more efficient nutrient management in agriculture. Understanding the role of UDFs in ecological nutrient management can help farmers and policy makers in agriculture determine how UDFs should be used and regulated. Overall, urine separation provides an opportunity to reduce urban and agricultural nutrient pollution while reducing resource consumption for both agricultural and waste management processes.

Urine separation reduces risk to public health

Urine separation can improve public health by reducing virus emissions from flushing UDTs. Due to the frequency of urination and toilet bowl design, UDTs can significantly reduce emissions of urine-associated viruses as compared to mix flush toilets (Chapter 3). Virus

emissions vary by flush pressure and toilet bowl design. In some cases, UDTs divert urine without a flush, which reduces emissions of urine-associated viruses entirely. A flushing cistern UDT, which has lower flush pressure, represents the lower end of emission levels for viruses that are suspended in the toilet water. An institutional mix flush toilet yields higher levels of emissions due to the high flush pressure and lack of urine diversion. The potential reduction in urine-associated virus emissions from UDTs suggests that more research is needed on their emissions from mix flush toilets. Users interacting with UDTs may be exposed to fewer virus emissions and potentially lower risks of infection. To quantify risk, emission levels from Chapter 3 can be used in a quantitative microbial risk assessment.

Urine separation can also benefit public health by reducing the amount of reactive N in the environment. Reactive N such as NO_x, N_2O, and NH_3 gases, and NO_3 and NH_4 in solution, can have negative consequences for public health. Lower N_2O and other GHG emissions improve air quality and mitigate global warming potential and its associated public health consequences: rising sea level, higher frequency and intensity of natural disasters, and higher temperatures. In Chapter 5, we found that diverting N from WRRFs through urine separation results in lower N_2O and other GHG emissions from reducing nitrification and denitrification activities, decreasing sludge production and landfilling, and offsetting fertilizer production. In Chapter 4, we found that UDF-treated plants generated lower N_2O emissions than compost-treated plants, suggesting a potential advantage of using UDF instead of compost as a waste-derived fertilizer to replace inorganic nitrogen fertilizer needs. Displacement and increases in soluble N in the environment can cause harmful algal blooms that lead to biodiversity loss and human exposure to toxins. In Chapter 5, we demonstrated that urine separation results in lower soluble N emissions to nearby water bodies. However, in Chapter 4, we found that land

application of the captured urine had similar N losses as leachate as inorganic fertilizers. There are opportunities to mitigate N losses by applying UDFs within an ecological nutrient management framework, but results from Chapters 4 and 5 suggest that there is still a net reduction in soluble N emissions when considering the whole N management chain. By comparing reactive N emissions with urine separation to conventional nutrient management practices, we demonstrated the potential public health benefits of urine separation.

6.3 Future Research Needs

Results from this dissertation can allow toilet users, farmers, agricultural policymakers, and waste management agencies to make informed decisions about urine separation. However, unanswered questions remain about urine separation as an approach toward nutrient circularity and additional research is needed. For example, we need to better understand and address barriers to scaling up the technology such as regulatory challenges with collection and application, risk communication, and scenario-specific implementation.

Regulatory barriers to urine separation exist for urine collection and UDF application. Urine collection at the building-scale requires UDTs or waterless urinals. Existing plumbing codes were not established to allow building-scale implementation of urine separation. Additional research is needed to identify the impacts of UDTs and waterless urinals on building operations that can inform necessary changes to plumbing codes. There can also be challenges with required behavior changes when using and maintaining UDTs that affect the efficiency of urine collection. For example, improper maintenance (such as use of cleaning agents) of a UDT can introduce toxic chemicals into the UDF product. A better understanding of the user interactions with UDTs can inform more accurate estimations of the risks and benefits. There are also regulatory challenges with applying UDFs as they are human-derived waste products. More data

is needed to identify safe use of UDFs and establish guidelines for different types of applications including edible and non-edible plants. The generated data can be used within existing frameworks for regulating waste-derived fertilizers such as manure and biosolids; however, identifying a separate regulatory pathway for "source separated materials" may help to characterize UDFs more accurately as a product that is quite distinct in composition from manure and biosolids.

There is a significant component of risk communication about microbial and chemical risks of collecting urine and applying UDFs. As technologies around collection and processing of urine for beneficial use are developed, they should be evaluated for their ability to mitigate these risks. Furthermore, comprehensive risk assessments are needed to understand the reasonable range of risks and how it compares to those of other exposure routes. Results generated from this and future research require careful dissemination as it can impact stakeholder perception and ultimately, the implementation of the technology.

Finally, urine separation can be implemented in different communities to serve their specific needs. In Chapter 5, we explored some sociotechnical factors that should be considered from a wastewater management perspective when deciding where to implement urine separation. However, additional factors can garner the attention of developers and other regulatory agencies. For example, we anticipate that source separation is best implemented in multi-use buildings but then developers would need to assess how to implement it using existing building codes that do not consider the practice. So, they would need to take additional, deliberate steps towards implementation by involving and educating the building permitting office. A framework is needed to tailor implementation in different contexts ranging from commercial and institutional buildings to single family homes and rural to urban communities. Factors such as space

constraints for storing and processing urine as well as transportation distance between urine generation and application should be assessed to determine how urine separation can be implemented to achieve different goals. There is tremendous potential in future research to advance widespread adoption of urine separation for a circular nutrient economy and more sustainable food, energy, and water systems.

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Appendices

Appendix A: Supplementary Information for Chapter 3

Recovery experiments from polyethylene film

We conducted triplicate bench scale experiments to quantify the recovery rate of the method we used to capture viruses from the toilet. The polyethylene film recovery surface used in the toilet flush experiments were cut into 5 x 5 cm^2 squares and ten 1 µL droplets of diluted virus stock at 10^6 plaque forming unit (pfu) mL^{-1} were added randomly onto the squares. A sterile cotton gauze pad (Dukal 2283) that was soaked in a phosphate buffer saline (PBS) solution containing 1% bovine serum albumin (BSA) was used to recover virus-laden droplets from the recovery surface. The gauze pad was then placed into ten mL of 1% BSA in PBS after wiping the film. Viruses were eluted into a 1% BSA in PBS solution from the gauze by vortexing at maximum speed for 1 minute. This method yielded average recovery rates of 88.8% for T3 and 130.1% for MS2.

Table A-1. Log_{10} pfu mL^{-1} in virus stocks and samples in all experiments. Data below the LOD are italicized.

	MFT		UDT	
T3	**Virus stock**	**Samples**	**Virus stock**	**Samples**
Protein	9.20	3.15	9.20	1.30
	9.06	2.54	9.06	1.30
	8.26	2.70	8.26	1.60
	8.81	2.73	8.81	1.95
	8.52	1.30	8.52	1.60
	8.57	2.11	8.57	1.78
No protein	9.17	2.87	9.17	*1.00*
	9.60	1.85	9.60	2.26
	8.69	1.48	8.69	*1.00*
	8.82	2.66	8.82	*1.00*
	8.55	2.29	8.55	*1.00*

	8.24	3.04	8.24	*1.00*
MS2	**Virus stock**	**Samples**	**Virus stock**	**Samples**
Protein	9.41	3.22	9.41	2.11
	9.59	3.02	9.59	2.29
	9.34	3.48	9.34	2.67
	9.25	3.17	9.25	2.48
	9.86	2.69	9.86	2.82
	9.51	3.38	9.51	3.38
No protein	9.47	2.59	9.47	*1.00*
	9.25	2.67	9.25	3.16
	9.54	2.22	9.54	1.30
	9.61	3.09	9.61	1.60
	9.35	2.74	9.35	*1.00*
	9.40	4.08	9.40	1.78

qPCR

qPCR was used to evaluate how gene copy (gc) emissions differed from pfu emissions using two of the experimental replicates. To improve experimental detection limits, experiment samples were concentrated approximately 14-fold with 100 kDa centrifugal ultrafilters (MilliporeSigma UFC901024). The samples were then processed with a Zymo viral DNA/RNA kit (Zymo D7020) to extract MS2 RNA and T3 DNA. Extracted MS2 RNA was synthesized into cDNA using a BioRAD advanced iScript advanced cDNA synthesis kit (iScript 1725037) and stored at -20^{o}C. T3 DNA and MS2 cDNA in samples were quantified with a Biotium FastEva Green mastermix (Biotium 31003) with 0.5 μM primers, 1x ROX, 0.125 μL BSA, and 1 μL template on the realplex^{2} Mastercycler epgradient S automated real-time PCR system (Eppendorf). We used MS2 primers (99 bp; forward, TGG CAC TAC CCC TCT CCG TAT TCA CG; reverse, GTA CGG GCG ACC CCA CGA TGA C) (Rolfe et al., 2007) and T3 primers (351 bp; forward, CCA ACG AGG GTA AAG TGA TAG; reverse, CGA CGA TAG CGA ATA GGA TAA G) that were previously developed and validated. T3 qPCR assays consisted of an initial denaturation step at 95^{o}C for 5 min, 40 cycles of denaturation at 95 ^{o}C for 15s, annealing at 56 ^{o}C for 30s, and extension at 72 ^{o}C for 45s. MS2 qPCR assays were similar to the T3 assay but without the initial

denaturation step and an annealing temperature of 60°C. Standard curves were prepared from 100 to 10^4 gc μL^{-1} with the extracted DNA and RNA from purified T3 and MS2 stocks. Limit of quantification (LOQ) levels of 148 gc μL^{-1} for T3 and 8530 gc μL^{-1} for MS2 were determined with ten replicates of the standard curves (Kralik & Ricchi, 2017). All plates included an ultrapure nuclease-free water negative control and a positive control of DNA or cDNA extracts from the virus stocks; all negative control results were below the assay detection limit. qPCR calibration curves resulted in efficiencies greater than 86% and R^2 values greater than 0.995.

Table A-2. gc μL^{-1} in virus stocks and concentrated samples in two experimental replicates. Data below the LOQ are italicized.

	MFT		**UDT**	
T3	**Virus stock**	**Samples**	**Virus stock**	**Samples**
Protein	7.98×10^5	*0.86*	7.98×10^5	*0.72*
	8.70×10^5	*1.82*	8.70×10^5	*2.29*
No protein	1.29×10^6	*3.90*	1.29×10^6	*0*
	6.58×10^5	20.3	6.58×10^5	*0.33*
MS2	**Virus stock**	**Samples**	**Virus stock**	**Samples**
Protein	8.58×10^5	*12.6*	8.58×10^5	*15.0*
	2.77×10^6	*36.0*	2.77×10^6	*30.1*
No protein	1.60×10^5	*22.0*	1.60×10^5	*0*
	6.50×10^6	*358*	6.50×10^6	*7.51*

Table A-3. gc pfu^{-1} ratios for virus stocks and concentrated samples in two experimental replicated.

	MFT		**UDT**	
T3	**Virus stock**	**Samples**	**Virus stock**	**Samples**
Protein	2.42	4.14	2.42	1.70
	2.35	1.03	2.35	2.25
No protein	3.64	1.39	3.64	0.00
	3.75	1.47	3.75	2.05
MS2	**Virus stock**	**Samples**	**Virus stock**	**Samples**
Protein	0.12	2.44	0.12	2.12
	0.86	1.11	0.86	0.74
No protein	0.07	2.78	0.07	0.00
	2.56	2.33	2.56	7.89

Appendix B: Supplementary Information for Chapter 4

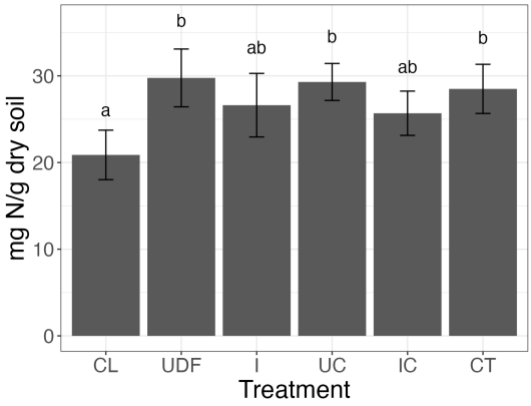

Figure B-1. N content in the aboveground plant mass for each treatment. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups.

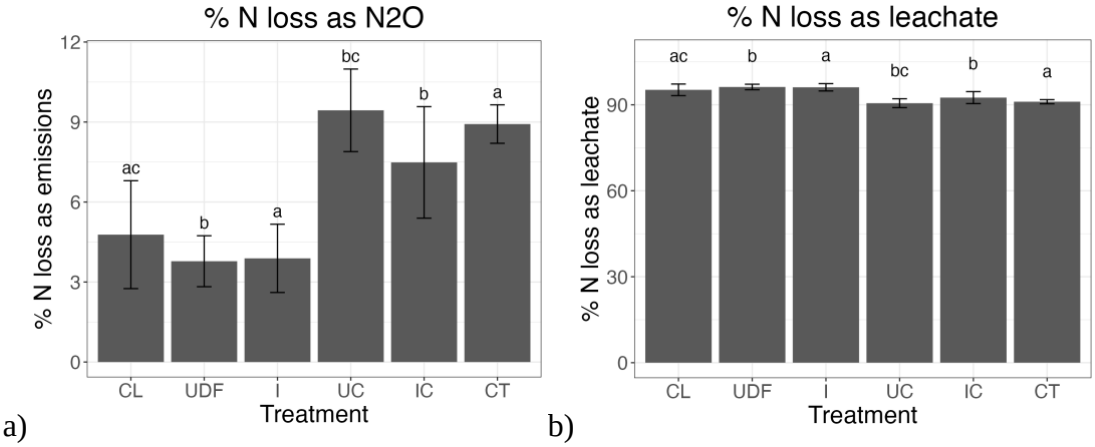

Figure B-2. a) % of N loss as N_2O emissions. b) % of N loss as leachate. Treatments are CL: control, CT: compost, I: inorganic, IC: inorganic and compost, UDF: UDF, UC: UDF and compost. The bar is the average value for five replicates within each treatment and the error bars are 95% confidence intervals. Letters above the bars represent statistical significance groups.

Appendix C: Supplementary Information for Chapter 5

Table C-1. Description and calculations for each flow

Flow	Description	Calculation
Food inputs	Food arriving at point-of-sale outlets	Food mass at point-of-sale outlets * % of food in category * N/P of food category
Commercial food retail	Food that is sold in supermarkets, convenience stores, drug stores, food markets, and bodegas	Food inputs * % in food retail
Commercial food prep	Food that is prepared at bodegas, restaurants, hotels, and cafes	Food inputs * % in food prep
Residential consumption	Food that is consumed at home	Food in retail * (1 - % waste at retail) + Food in prep * (1 - % waste for prep) * % takeout
Commercial consumption	Food that is consumed at bodegas, restaurants, hotels, and cafes	Food in prep * (1 - % waste for prep) * (1 - % takeout)
Wastewater	Wastewater influent	City population * N/P generated per person
DSNY	Food waste collected by municipality	Food waste collected * N/P in food waste
Commercial food waste	Food waste collected by private collectors	Food waste collected * N/P in food waste

Table C-2. N to P ratios for all recovery scenarios.

	N:P ratio
Total recovery	5.31
Food waste	2.04
Commercial food waste	2.04
DSNY food waste	2.04
Urine separation	15.35
50% urine separation	15.35
Sewage sludge	5.50
Urine plus food waste	5.25
Urine plus sludge	9.48
Food waste plus sludge	3.16

Table C-3. Pairwise comparison values for AHP

	Discharge body N sensitivity	**Presence/type of BNR**	**Ability to modify BNR**	**Capacity for increase in N loading**	**Effluent N mass**	**Projected N load increase**	**Nutrient density**	**Operational likeliness - toilet**	**Operational likeliness - urinal**	**Urine generation**
Discharge body N sensitivity	1	8	6	5	6	4	4	5	4	4
Presence/type of BNR	0.125	1	0.25	0.166667	0.2	0.166667	0.2	0.25	0.2	0.2
Ability to modify BNR	0.166667	4	1	0.142857	0.25	0.2	0.166667	0.2	0.166667	0.166667
Capacity for increase in N loading	0.2	6	7	1	4	1	3	4	3	3
Effluent N mass	0.166667	5	4	0.25	1	0.2	0.2	0.25	0.2	0.2
Projected N load increase	0.25	6	5	1	5	1	2	2	2	2
Nutrient density	0.25	5	6	0.333333	5	0.5	1	3	2	1
Operational likeliness - toilet	0.2	4	5	0.25	4	0.5	0.333333	1	0.5	0.5
Operational likeliness - urinal	0.25	5	6	0.333333	5	0.5	0.5	2	1	1
Urine generation	0.25	5	6	0.333333	5	0.5	1	2	1	1