UNIVERSITY OF CALIFORNIA, MERCED

ACHIEVING NUTRIENT PROVISION IN THE ANTHROPOCENE: SOLUTIONS FROM A CIRCULAR APPROACH TO SANITATION AND AGRICULTURE

DISSERTATION

submitted in partial satisfaction of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in Environmental Systems

by

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Bischak E, Ryals R, Ghezzehei TA. Nitrogen and phosphorus mineralization dynamics in human excreta-derived fertilizers. (In preparation).

Bischak E, Ward P, Jeliazovski J, Remington C, Philas F, Bazil Y, Daceus E, Janvier JC, Joachim L, Joseph F, Predinor S, Felix-Jean W, Kramer S, Ryals R. Enhancing soil health through feces-derived compost application: a case study in Northern Haiti. (In preparation).

Trondsen, L., **Bischak E**., McNicol G., Ryals R., Reid M., Russel K. Climate change mitigation potential of container-based sanitation and emerging non-sewered sanitation services. (In preparation).

Published journal articles:

Harrison BP, Gao S, Gonzales M, Thao T, **Bischak E**, Ghezzehei TA, Berhe AA, Diaz G, Ryals RA (2022) Dairy Manure Co-composting with Wood Biochar Plays a Critical Role in Meeting Global Methane Goals. *Environmental Science and Technology*, 56 (15):10987–10996. https://doi.org/10.1021/acs.est.2c03467

Ryals R, **Bischak E**, Porterfield KK, Heisey S, Jeliazovski J, Kramer S, Pierre S (2021) Toward Zero Hunger Through Coupled Ecological Sanitation-Agriculture Systems. *Frontiers in Sustainable Food Systems*, 5:341. https://doi.org/10.3389/fsufs.2021.716140

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Bischak, E. Introduction to Ecological Sanitation, **Invited guest lecture** at Sustainability of Agricultural Ecosystems ESS/BIO 172 Fall 2022, School of Natural Sciences, University of California, Merced, CA, USA

Bischak E, Fusi SC, Jeliazovski J, Beheshtian K, Ryals R. 2022. Urine Storage Conditions Influence Nitrogen Adsorption and Plant Uptake Dynamics in Urine-Enriched Biochars. **Contributed talk** at American Geophysical Union Conference 2022; Chicago. Available at https://ui.adsabs.harvard.edu/abs/2022AGUFM.B22A..04B. Accessed 2023 Jun 26.

Bischak E, Fusi SC, Jeliazovski J, Beheshtian K, Ryals R. 2022. Urine-enriched biochar: coupling sanitation and agricultural for a more sustainable food system. **Contributed poster** at Ecological Society of America Conference 2022; Montreal.

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Bischak, E. Demonstrating the Efficacy of Ecological Sanitation Practices in Haiti by Assessing the Net Climate Change Mitigation and Restoration Potential of Human-Waste Derived Compost Application. **Contributed talk** at UC Global Health Day 2020 (Virtual).

Trondsen, Lauren Haroff. **Bischak, E**. McNicol, Gavin. Reid, Matthew. Ryals, Rebecca. Russel, Kory. Calculating the climate change mitigation potential of container-based sanitation systems. UNC Water and Health Conference 2020 (Virtual).

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ABSTRACT OF THE DISSERTATION

Achieving Nutrient Provision in the Anthropocene: Solutions from a Circular Approach to Sanitation and Agriculture

by

Elena Bischak

Doctor of Philosophy in Environmental Systems

University of California, Merced, 2023

Dr. Rebecca Ryals, Chair

Fertilizer production and application to global agricultural land, along with incomplete nutrient removal during human excreta treatment, exerts a considerable strain on global biogeochemical cycles. There is increasing interest in the safe recovery of nutrients in human excreta for reuse in agriculture, often termed Ecological Sanitation (EcoSan). Often employed in EcoSan systems is the source-separation of urine and feces, which allows for distinct treatment processes that may reduce greenhouse gas emissions and water use compared to traditional sewerage, while conferring high nutrient recovery efficiencies. EcoSan may also provide a local source of plant-available nutrients to smallholder farmers, who are a foundational, yet underserved, part of the global food system. While EcoSan is gaining traction in terms of both research and operation, there is limited information on the biogeochemical outcomes of EcoSan end-use product fertilizer application. Particularly, soil and plant nutrient dynamics following the application of urine and urine-enriched biochar is understudied. Additionally, agronomic research on the application of feces-derived compost on agricultural land near EcoSan systems that produce such compost, is lacking. In this dissertation, we address these research gaps to further the field of EcoSan. In the first project, we investigated urine-enriched biochar as a nitrogenous fertilizer for tomato growth. We prepared urine-enriched biochar with three types of biochar combined with human urine stored in three realistic conditions. We found that the < 500-μm biochar particle size fraction retained significantly more nitrogen (N) than larger particles across biochars, and that urine-N in fresh urine had higher sorption affinities for > 500-μm biochar particles compared to urea-hydrolyzed urines. We also showed that urine-N applied alone is more immediately plant-available than urine-N sorbed to biochar. In our second project, we investigated the agronomic relevance of a suite of EcoSan fertilizers by assessing nitrogen (N) and phosphorus (P) mineralization of urine, urine-enriched biochar, and feces-derived compost in a 90-day amended soil incubation. We showed that urine applied alone is an excellent source of immediately plant-available N, while urine-enriched biochar application supplied approximately half of the N applied. We observed that feces-derived compost application stimulated substantial mining of native soil-P and led to a moderate, slow release of plant-available N. In the third project, we investigated the effect of repeated feces-derived

compost application on sorghum production and soil health indicators over two consecutive cropping cycles in an agroecosystem in northern Haiti. We found that feces-derived compost, particularly when applied at 150% of the sorghum N demand at the start of the growing season, led to significant increases in bioavailable soil macro- and micronutrients by the second cycle of management. One application of feces-derived compost resulted in decreased soil bulk density. Moderate increases in soil carbon (C) and N in the topsoil were observed for compost applied at 150% of the N demand by the end of the second cropping cycle. We also provide a chapter reflecting on the unique challenges and opportunities inherent to EcoSan research. This dissertation shows that human excreta is an important, largely untapped resource that can provide nutrients to agricultural soils and improve their health. EcoSan is a powerful tool that can help ameliorate the negative impact of human activity on global biogeochemical cycles, which is urgent to preserve our Earth system.

Chapter 1.
Introduction

Human activity has already exceeded the planetary boundaries for the disturbance of the global nitrogen (N) and phosphorus (P) cycles, which may lead to the destabilization of the Earth system as we know it (Richardson et al., 2023). The main source of this disruption is from fertilizer application (Richardson et al., 2023). Nutrient flows from incomplete treatment in sanitation systems place further stress on these biogeochemical cycles. It is estimated that sewage flows into coastal ecosystems contribute an additional 6.2Tg N yr^{-1} to coastal waters, approximately 40% of the total N contribution from agriculture (Tuholske et al., 2021). Human excreta-derived P generation is estimated at 3.3 Tg P yr^{-1} (Yuan et al., 2018). This is largely due to the fact that once humans reach adulthood, we excrete most of the plant-essential N, P, and potassium (K) that we consume in food products (Jönsson et al., 2004). Humanity is faced with the massive challenge of redefining our role within these global biogeochemical cycles. However, these two separate stressors to these cycles present an opportunity for a circular approach to human excreta-derived nutrient management. In this work, we refer to the recoupling of sanitation and agriculture through the safe recovery and reuse of human excreta-derived nutrients in agroecosystems as Ecological Sanitation, or EcoSan. This dissertation studies the biogeochemical outcomes of the application of EcoSan fertilizers in both the lab and field.

Current modes of fertilizer provision and production are largely linear, meaning that most fertilizers rely on the single use of nutrient sources. Mineral N fertilizer is produced through the energy-intensive Haber-Bosch process, by which inert atmospheric N is reduced to reactive N (Zhang et al., 2015). It is estimated that 17% of N applied to crops is consumed by humans, with the rest lost to watersheds and the atmosphere (Fowler et al., 2013). This contributes to the eutrophication of surface water and contamination of groundwater, as well as global climate change and stratospheric ozone depletion (Fowler et al., 2013). Phosphorus fertilizers are mined from non-renewable, spatially heterogeneous mineral reserves (Cordell and Neset, 2014). The global demand for P is expected to increase by up to 100% by 2050, though rock P reserves may be depleted in the next 50 to 100 years (Cordell et al., 2009). Most European and North American agricultural soils have been over applied with P and thus require minimal inputs, while the majority of new demand is expected to come from developing economies (Cordell et al., 2009). As reserves are exhausted, low income countries with growing populations will be faced with the consequences. Though K reserves are likely to meet projected demand for centuries to come, K reuse should also be prioritized (Sardans and Peñuelas, 2015). Waste produced from potash mining contributes to the salinization of rivers, a major disturbance to global freshwater resources (Cañedo-Argüelles et al., 2017).

While fertilizer nutrient management is a massive global problem, so is the provision of sanitation to the global population. 4.2 billion people lack access to safely managed sanitation, of which 2 billion have no access to basic sanitation (United Nations, 2020). United Nations Sustainable Development Goal (SDG) 6 aims to ensure access to clean water and sanitation for the global population by 2030 (United Nations, 2020). Unless

current rates of implementation increase considerably, SDG 6 will not be met by the target date (United Nations, 2020). Managing sanitation in a changing climate is a challenge, particularly when systems rely on large quantities of fresh water and centralized, extensive infrastructure that requires trained management (Kohlitz et al., 2017). The WHO has identified the need for climate-resilient and adaptive sanitation systems that preserve and reuse water and nutrients embedded in waste, encouraging a movement away from centralized sewerage (WHO, 2009).

Many prevailing sanitation paradigms also adopt a linear approach to nutrient management, meaning that most of the nutrients in human waste are ultimately released back to the environment (Cornejo et al., 2013). This leads to eutrophication, groundwater contamination, and the spread of enteric disease (Heinonen-Tanski et al., 2010). Nearly 2 billion people rely on pit latrines, a form of on-site sanitation that collects feces and urine in a subterranean basin to eventually be emptied (Reid et al., 2014). Though this approach provides inexpensive, low water sanitation access, pits are infrequently emptied leading to groundwater contamination, and often overflow during floods (Sherpa et al., 2014). Additionally, anaerobic decomposition in pit latrines accounts for up to 1% of global anthropogenic methane emissions (Reid et al., 2014). Though Western approaches to sanitation may confer better treatment outcomes, large amounts of freshwater are used to convey urine and feces to a centralized treatment facility (Guo et al., 2014). Up to 4% of total US energy expenditure can be attributed to wastewater conveyance and treatment (Guo et al., 2014), of which 80% of that energy is used to remove nutrients introduced by urine (Wilsenach and Van Loosdrecht, 2003). This process often renders large quantities of precious freshwater unusable, and incomplete treatment leads to eutrophication and groundwater contamination (Heinonen-Tanski et al., 2010).

A paradigm shift in which excreta is treated as a resource rather than pollutant could lead to a transformation in nutrient management (Bouwman et al., 2009). EcoSan is a potential solution to the two immense global problems of nutrient and sanitation access (Langergraber and Muellegger, 2005). EcoSan may reduce public health risks while creating locally accessible fertilizer that improves soil health through addition of organic matter and nutrients, thus increasing food security and bolstering local economies (Langergraber and Muellegger, 2005). This is further salient as while fertilizer demand and production increases annually, over 1 billion people remain undernourished (Zhang et al., 2015). EcoSan could provide fertilizers to smallholder farmers, vital actors in the global food system who often lack access to fertilizers (Rapsomanikis, 2015). There is particularly high potential for the implementation of EcoSan in emerging urban environments with dense populations located near cropland, which may help offset mineral fertilizer imports (Trimmer and Guest, 2018). A form of EcoSan, Container-based Sanitation (CBS), is an emerging sanitation technology in which human excreta is source-separated in containers and transported to a local facility for waste treatment and processing (Kramer et al., 2013). CBS is a decentralized, low-to-no-water sanitation option that can be managed for resource recovery, particularly in densely populated peri-urban communities (Russel et al., 2019).

In Chapter 2 of this dissertation, we investigated the efficacy of urine-enriched biochar as a nitrogenous fertilizer. We conducted a tomato growth experiment in a greenhouse with urine-enriched biochar made from three types of biochar and urine from three storage conditions. To our knowledge, this work is the first to compare plant growth with multiple preparations of urine-enriched biochar in the same study. In Chapter 3, we incubated soils amended with a suite of urine-derived and feces-derived EcoSan

fertilizers to assess their N and P mineralization. We extracted amended soils for available N and four biologically-based P pools at multiple points over a simulated cropping cycle. This work contributes to a nascent body of research on the mineralization of novel human excreta-derived fertilizers. In Chapter 4, we conducted a sorghum growth study in northern Haiti to assess the effect of successive feces-derived compost application on crop production and soil health indicators. This work is the first to our knowledge to study the effect of repeated feces-derived compost application on a suite of soil health indicators, particularly in an agroecosystem proximate to a prominent CBS EcoSan organization. In Chapter 5, we present a reflection on EcoSan research methods in both the field and lab, as this body of work presented unique challenges and opportunities that can be of interest to other EcoSan researchers. In Chapter 6 of this dissertation, we present general conclusions from this work. This dissertation contributes to an important and growing understanding of the potential of EcoSan fertilizers to provide nutrients to underserved communities and mitigate climate change. This research addresses multiple SDGs, particularly SDGs 2, 3, 6, and 13 (United Nations, 2020).

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Chapter 2.
Urine-enriched biochar: coupling sustainability in sanitation and agriculture

Abstract

Linear models of fertilizer production and application are environmentally harmful. Predominant approaches to waste management treat human excreta as a pollutant rather than a source of nutrients for agriculture. Container-based sanitation (CBS) systems safely contain and transport excreta for treatment and reuse, though urine is often contained but not treated. A major challenge of urine-nutrient recovery is the shift in nitrogenous species in urine during storage, from urea to ammonia (NH_3) and ammonium (NH_4^+), due to urease activity. This can lead to gaseous NH_3 losses from urine that depletes its fertilizer potential. Urine-enriched biochar (UEBC) may act as a slow-release fertilizer of urine nutrients. We quantified the adsorption of nitrogen (N) in fresh, stored, and CBS-style urine to wood waste, sewage sludge, and walnut shell biochars. These UEBCs were compared to urine-only treatments and fertilized and unfertilized controls in a greenhouse growth experiment. We found that the < 500-μm biochar size fraction retained significantly more N than larger particles across biochars. Urine-N adsorption to biochar and uptake into plant tissue varied across biochar type and urine condition. The quantity of urine applied in urine-only treatments, regardless of type, was positively correlated with plant N uptake. Plant biomass did not differ significantly across treatments. These findings emphasize the need to optimize urine-enriched biochar application for different urine and biochar conditions, particularly for CBS and other urine-diverting operations.

2.1 Introduction

2.1.1 Overview

Nitrogen fertilizers are frequently over applied to agricultural fields, leading to the pollution of water systems, the release of the powerful greenhouse gas (GHG) nitrous oxide, and stratospheric ozone depletion (Zhang et al., 2015). Additionally, fertilizers are not always accessible to smallholder farmers, critical actors of the global food system (Rapsomanikis, 2015), and fertilizer prices are subject to volatility (Crespi et al., 2022). These global issues highlight the need for a circular approach to nutrient management that prioritizes recovery and reuse, particularly in a world with a changing climate.

One largely unused resource for nutrient recovery and reuse is human excreta. Once humans reach adulthood, we excrete nearly all the N, phosphorus (P), and potassium (K), that we consume (Jönsson et al., 2004). Human urine is an important potential waste stream for resource recovery, as it contains most of the N, and approximately half of the

P and K that we excrete (Harder et al., 2019). A paradigm shift in which human excreta is treated as a resource rather than pollutant could lead to a transformation in nutrient management (Bouwman, Beusen and Billen, 2009). Container-based Sanitation (CBS) is an emerging technology in which human excreta is source-separated in containers and transported to a local facility for waste treatment, processing, and reuse (Russel et al., 2019). This study explores urine-N recovery scenarios relevant to CBS systems. We use biochar, a low-cost carbon-rich adsorbent, to recover urine-N from human urine stored in conditions realistic to CBS and other urine-diverting systems. To the best of our knowledge, this research is the first to examine multiple types of urine-enriched biochar (UEBC) made with various kinds of urine and biochar in a single greenhouse growth trial. Figure 2-1 summarizes our research approach and how it relates to CBS systems.

2.1.2 Background

As of 2020, 4.5 billion people lack access to safely managed sanitation, of which 2 billion have no access to basic sanitation (United Nations, 2020). United Nations Sustainable Development Goal (SDG) 6 aims to ensure access to clean water and sanitation for the global population by 2030 (United Nations, 2020). Unless current rates of implementation increase considerably, SDG 6 will not be met by the target date (United Nations, 2020). CBS has the potential to help bridge this gap in global sanitation provision by providing resilient, low-to-no water, decentralized sanitation with the added benefit of nutrient recovery and reuse (Tilmans et al., 2015). CBS is commonly employed in areas where conventional sewerage systems face challenges, such as densely populated low-income urban settlements and regions prone to water contamination from flooding or high water tables (Russel et al., 2019). CBS systems typically collect feces and urine in separate, above-ground containers that can be sealed and transported for treatment and/or recovery.

Management in CBS systems usually focuses on the safe treatment and reuse of feces for various outcomes (Figure 2-1). Feces-derived end-use products include compost, animal feed, and biomass/biogas fuels (Russel et al., 2019). However, recovering nutrients from both urine and feces would better close local nutrient cycles (Ryals et al., 2021). Nitrogen in urine is plant-available, present as mainly urea (75-90%), and the remaining N as NH_4^+, creatine, or nitrate (Jönsson et al., 2004; Rose et al., 2015). The P and K in urine are excreted in plant-available ionic forms. Notably, these are some of the most common forms of N, P, and K found in synthetic fertilizers (Jönsson et al., 2004). Though urine has been used as a fertilizer, both in ancient agricultural practices (Angelakis et al., 2018) and in recent scientific research, its adoption in modern agricultural systems is slow (Karak and Bhattacharyya, 2011). Proper urine treatment in CBS systems is also important to reduce nitrate and pharmaceutical leaching to groundwater, particularly in places with high water tables (Russel et al., 2019).

A major barrier to urine-nutrient recovery in CBS systems is the difficulty and cost of transporting large quantities of liquid (Russel et al., 2019). Additionally, while the mass of excreted nutrients is higher in urine than feces, the concentration of these nutrients is lower since urine is 97% water (Senecal and Vinnerås, 2017). This barrier points to the need to concentrate urine-nutrients at a decentralized scale into a solid product that would be easier to transport. While methods like NH_3 stripping, struvite precipitation, and electrochemical technologies have demonstrated successful nutrient recovery from urine (Kabdaşlı and Tünay, 2018), they may have high operational costs and may not be suitable for implementation at a decentralized scale (Kundu et al., 2022). In our research,

we explore an alternative approach for urine-N recovery: adsorption to biochar. Biochar, produced through biomass pyrolysis (Weber and Quicker, 2018), offers a potentially low-cost and widely available adsorbent option that can be produced and utilized in resource-constrained settings. Although other adsorbents may exhibit higher N removal efficiency (Tarpeh, Udert and Nelson, 2017), we specifically investigate biochar as an adsorbent for urine-N due to its potential to repurpose wasted biomass, sequester carbon in soil when applied, and mitigate climate change (Masrura et al., 2020). Field trials have already demonstrated the efficacy of UEBC as a fertilizer (Schmidt et al., 2015, 2017; Sutradhar et al., 2021). Biochar could potentially be used to recover urine-N at the individual toilet scale (i.e. an attached filter) or the neighborhood scale (i.e. communal soak pit) (Ryals et al., 2021).

Many areas in UEBC research are underexplored. There is substantial variation in the chemical composition of urine due to storage conditions in CBS systems. Urease, a ubiquitous bacterial enzyme, hydrolyzes urea to NH_4^+ and carbon dioxide (Krajewska, 2009). It is typically present in large quantities in urine storage systems and rapidly hydrolyzes urea in fresh urine (Tarpeh et al., 2018). Tarpeh et al. (2018) found lower NH_4^+ concentration in open containers compared to closed containers in a CBS system, attributed to NH_3 volatilization. This has implications for the value of urine-derived fertilizers produced with urine from open containers, as gaseous loss of N as NH_3 may be significant. NH_3 emissions from open urine containers may also have consequences for human health, as NH_3 can react with N and sulfur oxides to form $PM_{2.5}$, which can cause severe respiratory and circulatory problems (Stokstad, 2014). Additionally, NH_3 emissions cause an unpleasant odor in urine storage systems (Hashemi and Han, 2017). Understanding how the various solution chemistry created by different urine storage conditions influences N adsorption to biochar is an important part of practical urine-nutrient recovery research. The mechanisms, kinetics, and adsorption affinity of organic and inorganic molecules to biochar have also been shown to differ across biochar feedstocks and pyrolysis temperatures (Ambaye et al., 2020).

The objective of this research was to assess the effect of urine storage conditions on N adsorption to different biochars, and the effect of these UEBCs on tomato growth and plant N uptake. We sought to identify how different urine-biochar combinations may impact agricultural outcomes. We produced UEBC from three urine types: fresh (hereafter Fresh); stored, covered urine (hereafter Stored); and CBS-style urine (hereafter CBS), and three types of biochar (sewage sludge, wood waste, and walnut shell), for nine UEBC combinations. These UEBC combinations represent a range of realistic urine storage conditions combined with a range of biochar feedstocks and production conditions. These UEBCs were compared with a synthetic and organic fertilizer, an unfertilized control, and urine-only treatments. We expected UEBC treatments to outperform urine-only treatments due to the adsorption and subsequent slow release of nutrients, as opposed to the gaseous and leaching N losses that likely reduce fertilization quality in urine-only treatments. We expected CBS treatments to lead to lower yield due to initial N losses as NH_3 during storage, and all treatments to outperform unfertilized controls.

2.2 Methods

2.2.1 Urine collection and urea hydrolysis

Human urine was collected from consenting volunteers over the age of 18 from the University of California (UC), Merced. Collection protocols were approved for expedited review by the Institutional Review Board of UC Merced (IRB # UCM2020-171). Urine was provided by volunteers in 100 mL containers and immediately refrigerated until use, at most 1 week later. Three urine treatments were prepared: Fresh urine; Stored urine; and CBS urine. CBS urine was completely urea-hydrolyzed and left uncovered for 1 day in a CBS-style container to mimic storage conditions at a leading CBS provider, Sustainable Organic Integrated Livelihoods (SOIL) in Haiti.

Fresh urine was combined into multiple 1 L polypropylene containers. It was confirmed to be in the electrical conductivity (EC) range of fresh urine (~11 mS cm^{-1}) based on prior experimentation and work by Ray et al. (2018). Urine to be urea-hydrolyzed for Stored and CBS treatments was combined in multiple 1 L containers. To account for differences in environmental enzyme loading in CBS systems, this urine was allowed to urea-hydrolyze completely before it was either refrigerated until use (Stored) or left uncovered (CBS). All containers were filled nearly completely to mitigate volatilization within the headspace while covered. 0.533 g L^{-1} of urease (CAS 9002-13-5, Fisher Scientific) was added to each container based on methods used by Ray et al. (2018), and shaken for 30 minutes at 180 oscillations/minute (Eberbach E6010.00). All containers were kept sealed during urea hydrolysis. Urine was considered completely urea-hydrolyzed when the EC values stabilized at ~24 mS cm^{-1}, consistent with Ray et al. (2018) and prior experimentation, after approximately 36 hours at room temperature. After urea ghydrolysis, 3.18 L of urine was transferred to a 3.78 L CBS-style urine collection container (**Figure S2-1**) and left uncovered for a day to mimic CBS urine storage conditions at SOIL. This volume was chosen based on expert opinion at SOIL, stating that urine containers are typically allowed to fill approximately 3/4 of the way before being emptied (J. Jeliazovski pers. comm). We recognize other urine storage conditions were dissimilar to SOIL's CBS conditions, such as temperature and humidity, in our lab-scale study. A subsample of each urine type was frozen until later NH_4^+ analysis using the microplate colorimetric salicylate-nitroprusside method (Mulvaney, 1996) (Agilent BioTek Gen5 Microplate Reader, Agilent Technologies, Santa Clara, CA, USA). Samples were thawed at room temperature immediately before analysis, and 0.533 g L^{-1} urease was applied to half of the Fresh urine sample and shaken for 30 minutes at 180 osc min^{-1} to determine urea content. All analyses were run in triplicate.

2.2.2 Urine-enriched biochar preparation

Three biochar types were used in this study: a walnut shell biochar produced at 350 °C by pyrolysis (hereafter Walnut Shell) (NextChar, Amherst, MA), a sewage sludge biochar produced at 550 °C by pyrolysis (hereafter Sewage Sludge) (UK Biochar Research Centre, SS550), and a wood waste biochar produced at 593.3 °C by gasification (hereafter Wood Waste) (Aries GREEN™ All Natural Soil Conditioner). Biochar pH and EC were measured using methods recommended by Balwant et al. (2017). Briefly, biochar was prepared at a 1:10 biochar: deionized water ratio, shaken for 1 hour at 150 rpm, and allowed to settle for 30 minutes. Readings were taken in triplicate from each sample. Other physiochemical properties were obtained from the supplier or research

partners. Biochar properties are shown in Table 2-1. All urine types were combined with each biochar for a total of 9 UEBCs. UEBC was prepared at a 200 g: 1 L ratio. This ratio was chosen based on preliminary experimentation that found this ratio favorable for urine-N adsorption. Respective urine and biochar types were added to 1 L containers, briefly agitated, and allowed to soak for 48 hours. All containers remained sealed for the duration. UEBCs were then allowed to drain freely over a 500-µm sieve and refrigerated until use. An effluent sample from each was filtered through a 0.45 µm polypropylene filter and frozen for later NH_4^+ analysis. The effluent N concentration was used to calculate the total sorbed concentration (Q_T) as follows:

$$Q_T = \frac{C_{in} - C_f}{M} * V$$

$$Q_T = Q_{coarse} + Q_{fine}$$

Where C_{in} and C_f are the initial and final N concentrations in solution (mg L^{-1}), M is the mass of biochar (g), and V is the volume of urine (L). Q_T includes all N removed from the urine, in both the > 500-µm and < 500-µm size fractions. A subsample of each UEBC was allowed to air dry for elemental analysis using a Costech 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA). These data were used to determine the N adsorbed to biochar (mg urine-N g-biochar^{-1}) (Q_{coarse}) in the > 500-µm size fraction, as follows:

$$Q_{coarse} = (\%N_{UEBC} - \%N_{raw}) * 10$$

Where $\%N_{UEBC}$ is the percentage total N in the UEBC, and $\%N_{raw}$ is the percentage total N in the unenriched biochar. The mass of N retained by the biochar particles < 500-µm (mg-N) (Q_{fine}) was calculated as follows:

$$Q_{fine} = \left(C_{in} * V - C_f * V\right) - Q_{coarse}$$

The portion of N potentially lost to volatilization is neglected. Separate subsamples of UEBC were oven-dried at 105 °C until a stable weight was reached to determine moisture content.

2.2.3 Plant production using urine-enriched biochar

A five-week growth experiment was conducted to test the efficacy of UEBCs as fertilizers and investigate urine-N plant availability. Tomato (Burpee Early Girl) was chosen as a globally important crop with relatively rapid growth rates (FAO, 2023). 15 treatments were tested: 9 UEBCs (Sewage Sludge-Fresh, Sewage Sludge-Stored, Sewage Sludge-CBS, Wood Waste-Fresh, Wood Waste-Stored, Wood Waste-CBS, Walnut Shell-Fresh, Walnut Shell-Stored, Walnut Shell-CBS), 3 urine-only treatments (Fresh, Stored, and CBS), a synthetic fertilizer (Miracle-Gro® Water Soluble Tomato Plant Food), an organic fertilizer (Jobe's Organics Vegetable & Tomato Granular Plant Food), and an unfertilized control. All UEBCs were tested at application rates of 1%, 2%, 6%, and 10% (w/w), on a dry mass basis. For urine-only treatments, the volume of urine applied aligned with the corresponding UEBC application rate. This allowed us to test the same amount of urine with or without biochar. For example, at a 1% UEBC application rate,

0.107 g biochar and 0.535 mL urine were applied in the UEBC mixture. Thus, for the 1% urine-only application rate, 0.535 mL urine was applied alone. 1.07 mL of urine was applied at the 2% application rate, 3.21 mL at the 6% application rate, and 5.35 mL at the 10% application rate. These urine-only treatments are hereafter referred to as "Fresh 1%", for example. The organic and synthetic fertilizers were applied at the start of the study at a rate of 150 kg-N/ha, the upper N fertilization rate suggested for tomatoes (FAO, 2023.). All other N application rates were determined post hoc (Table 2-2). Amendments were incorporated into a soilless substrate (Premier Tech Pro-Mix BX Mycorrhizae) at the appropriate ratio. The soilless substrate is 79-87% sphagnum peat moss and 10-14% perlite mixture inoculated with *Glomus intraradices* (Premier Tech Horticulture, Québec, Canada). 10.7 g of amendment + soilless substrate was applied to 90-cm^3 plug flat pots, with 9 replicates per treatment, for a total of 459 plants. Trays were brought to 90% of field capacity, determined by weight and preliminary experimentation. One tomato seed was sown into each pot. Pots were maintained at 90% of field capacity by misting with an automated sprayer or watering with a watering can, typically daily. Deionized water was used to prevent the introduction of nutrients from tap water.

Plant height was measured every 4 days starting 8 days after first emergence for 6 randomly selected plants per treatment. Height was measured with a ruler from the soil surface to the petiole of the highest leaf. The number of leaves > 1 cm was also recorded. Final plant height, number of leaves > 1 cm, and leaf area of the largest leaf was measured for all plants after five weeks. Leaf area was measured with a Tamaya Technics Planix 5 Digital Planimeter. In the case that the largest leaf had very irregular margins, the second largest leaf was measured. After final plant growth monitoring, all plants were destructively harvested. Above-and-belowground biomass was separated at the soil surface. Soil was removed from belowground biomass using deionized water. Biomass samples were dried in an oven at 65 °C until a stable weight was reached. Above-and-belowground biomass for each treatment (n = 5) were ground separately in a ball mill (SPEX SamplePrep 8000M Mixer/Mill®, SPEX SamplePrep, Metuchen, NJ, USA). All replicates were ground together for each treatment for one analysis per treatment (above or below). Samples were subsequently analyzed for elemental C and N content.

2.2.4 Statistical analyses

All statistical analyses were performed in R version 4.1.3 (R Core Team 2022). All tests used a significance level of 5%. Separate three-way ANOVAs were performed for total biomass, aboveground biomass, belowground biomass, final plant height, final number of leaves > 1 cm, leaf area, and final leaf number as the response variable and biochar type, urine type, and application rate as factors. Data were log transformed prior to analysis. Post hoc Tukey's Honestly Significant Difference (Tukey HSD) tests were performed with the "HSD.test" function in the *agricolae* package. A one-way Kruskal-Wallis test with a Bonferroni correction was performed to compare N content of all UEBCs with the "kruskal" function in the same package. A general linear model was used to compare final germination percentage across treatments. One-and two-way ANOVAs were also performed to compare grouped data, with post hoc Tukey's Honestly Significant Difference tests using the "TukeyHSD" test in the *stats* package. Separate one-way ANOVAs were performed for urine, biochar, and application rate as predictor variables and total biomass as the response variable. To compare the difference between UEBCs and urine-only treatments, we performed a two-sample Wilcoxon Rank Sum test using "biochar/no biochar" as grouping terms, using the "compare_means" function in the *ggpubr* package. Two-way ANOVAs were performed with total biomass as a response

variable and all combinations of biochar type, urine type, and application rate as factors to test for interaction effects. Linear regression models were fitted to N data within each biochar type (or no biochar, referred to as "urine-only") with mg urine-N applied per pot as the predictor variable and mg-N uptake per plant as the response variable, using the *ggpmisc* package. A two-sample Wilcoxon Rank Sum test was used to compare Q_{fine} and Q_{coarse}, within biochar and across urine types.

2.3 Results and Discussion

2.3.1 Nitrogen adsorption on biochars

Across biochar types, Q_T indicates that 85-98% of the initial N in urine regardless of type was removed from solution (Figure S2-2). Q_{fine} was significantly higher than Q_{coarse} across UEBCs ($p < 0.001$, Wilcoxon Rank Sum test) (Figure 2-2). This discrepancy is likely due to Q_{coarse} being determined from analysis of biochar particles > 500-µm. Previous studies have shown that larger biochar particle size fractions retain significantly less NH_4^+ and take longer to reach equilibrium with NH_4^+ (Kizito et al., 2015; Bai et al., 2018). Across biochars, Q_{fine} was the highest for Stored urine (Figure 2-2). The principle chemical differences between Fresh urine and Stored/CBS urine are their nitrogenous species, pH, and EC. Urea is the primary solute in Fresh urine, constituting 75-90% of N in solution (Rose et al., 2015). Most N in Stored or CBS urine is present as NH_3/NH_4^+, due to the complete activity of the urease enzyme in these urine types prior to UEBC preparation. The decrease in sorption capacity with increasing particle size may be due to the higher surface area and shortened diffusion paths of smaller particles. Specifically, small size fractions have a higher concentration of oxygen-containing surface functional groups, and more sorption sites relative to larger particles (Nocentini et al., 2010). Thus, the difference between Q_{fine} (14-20 mg-N g-biochar^{-1}) and Q_{coarse} (1-4 mg-N g-biochar^{-1}) may be explained by particles < 500-µm having more surface functional. Future work should determine N capacity in < 500-µm particles via elemental analysis. The feasibility of recovering this small UEBC size fraction for use should also be explored. Future work should also investigate the role of pH in urine-N sorption to biochar. While monitoring the urea hydrolysis process, we found that the initial pH of Fresh urine averaged at 6.06 ± 0.28 and pH after urea hydrolysis averaged at 9.00 ± 0.15. The EC prior to urea hydrolysis was 11.40 ± 0.79 mS cm^{-1}, and EC after completion was 24.58 ± 0.63 mS cm^{-1}. These parameters are typical of fresh and urea hydrolyzed urine found in the literature (Karak and Bhattacharyya, 2011).

A different trend was seen with Q_{coarse}, where Fresh led to the highest removal of N (Figure 2-2). Q_{coarse} was significantly higher in UEBCs prepared with Fresh urine, compared with CBS or Stored urine for Sewage Sludge and Wood Waste ($p < 0.001$, one-way Kruskall-Wallis test) (Figure S2-3). Q_{coarse} for Sewage Sludge-Fresh was 571% and 357% higher than Q_{coarse} for Sewage Sludge-CBS and Sewage Sludge-Stored, respectively. Q_{coarse} for Wood Waste-Fresh was 1377% and 473% higher than Q_{coarse} for Wood Waste-CBS and Wood Waste-Stored, respectively. The same trend held for Walnut Shell but was not significantly higher when compared across all UEBCs. Q_{coarse} for Walnut Shell-Fresh was 27% and 60% higher than Walnut Shell-CBS and Walnut Shell-Stored, respectively. Q_{coarse} was similar in Fresh UEBCs across biochar types. Sewage Sludge-Fresh averaged 3.76 ± 0.95 mg-N g-biochar^{-1}, Wood Waste-Fresh averaged 3.36 ± 0.21 mg urine-N g-biochar^{-1}, and Walnut Shell-Fresh averaged 3.94 ± 0.16 mg urine-N g-biochar^{-1}. Walnut Shell biochar had the highest Q_{coarse} across urine

types, with an average of 3.09 ± 0.28 mg urine-N g-biochar^{-1} for Walnut Shell-CBS, and 2.47 ± 1.73 mg urine-N g-biochar^{-1} for Walnut Shell-Stored.

The differences between Q_{coarse} and Q_{fine} may also be attributed to preferential binding of urea molecules in Fresh urine to larger biochar particles. Alternatively, it is possible that urea has similar affinities for biochar particles in either size fraction. If this is the case, it may indicate a stronger affinity for urea molecules than NH_3/NH_4^+ molecules for biochar surfaces, or that solution conditions in Fresh urine, such as lower pH and EC, are generally more favorable for adsorption to biochar compared to the higher pH and EC of Stored/CBS urine. It is possible that the lower EC in Fresh urine results in less competitive sorption of urea with other molecules, and thus a greater mass of bound urea compared to NH_3/NH_4^+ in the Stored/CBS UEBCs. Solanki and Boyer (2019) studied the removal of pharmaceuticals from urine using biochar and found that adsorption of paracetamol from real urine treatments was significantly lower than from synthetic urine, which they attributed to the presence of metabolites in real urine. Though urea and paracetamol are vastly different molecules, this research suggests that competition between urine metabolites may reduce organic molecule adsorption to biochar.

Multiple studies have suggested urea uptake onto biochar or activated carbon is dominated by physisorption (Ganesapillai and Simha, 2015; Ganesapillai et al., 2016; Kameda, Ito and Yoshioka, 2017). These studies agree that urea sorption to these adsorbents is governed by pseudo-second order kinetics. Simha et al. (2016) propose that urea adsorption to biochar is limited and controlled by intra-particle and surface diffusion. NH_4^+ adsorption to biochar is believed to be controlled by cation-exchange, surface complexation with oxygen-containing functional groups, hydrogen-bonding, precipitation, or electrostatic interaction (Cai et al., 2016; Cui et al., 2016). Tarpeh et al. (2017) found that ion exchange was the dominant adsorption mechanism for NH_4^+ in real, undiluted, urea hydrolyzed urine to biochar, among other adsorbents. Future research should develop adsorption isotherms and investigate adsorption mechanisms of urea, NH_4^+, and NH_3 in Fresh, Stored, and CBS urine.

2.3.2 Nitrogen uptake in plant tissue

There was differential uptake of N in plant tissue across treatments (Figure 2-3). There is a strong positive linear relationship between urine-N applied and N uptake in plant tissue in the urine-only treatments, regardless of urine type ($R^2 = 0.98$) (Figure 2-3). This indicates that N was potentially still a limiting nutrient, as no plateau in N uptake in plant tissue is seen in the data. Even at 22.69 mg-N applied at the Fresh urine 10% equivalent volume application rate, 12.18 mg of N is found in the plant tissue. From the slope of the regression line, we can infer that about 30% of N applied in urine-only treatments is taken up in the plant tissue. Since we do not have isotope tracer or dilution data, we cannot be certain that the N in the plant tissue is the same as that applied to the pot. However, some urine-N is likely present in the plant tissue, as urine-N is excreted in plant available forms, regardless of urine type (Jönsson et al., 2004). It is likely that some of the urine-N applied was lost as gaseous-N such as NH_3 or nitrous oxide or leached as nitrate after soil microbial transformations. Similarly problematic N losses are found in synthetic N fertilizer application (Zhang et al., 2015), and gaseous and leaching losses have been previously demonstrated with urine applied as fertilizer (Kirchmann and Pettersson, 1994; Wachendorf, Taube and Wachendorf, 2005). It is also likely that some urine-N is immobilized in soil microbial tissue. Similar trends were observed in N application and uptake for both above-and belowground biomass (Figures S2-4 and S2-

5). Linear regressions do not fit the UEBC N uptake data as well as for urine-only. Walnut Shell UEBCs exhibit a positive linear trend (R^2 = 0.44). Our results show that urine-N in Stored and CBS urine adsorbed more readily to Walnut Shell than Wood Waste or Sewage Sludge (Figure S2-3). Our results also imply that urine-N desorbed from Walnut Shell biochar more easily, regardless of urine type (Figure 2-3). This trend may be explained by the sorption/desorption behavior of surface functional groups on Walnut Shell biochar. This biochar was produced at 350°C, a relatively low pyrolysis temperature that is known to maintain oxygen-containing surface group functionality (Rasse et al., 2022). Negatively charged functional groups on biochar have shown optimal adsorption capacity for NH_4^+ and urea (Masrura et al., 2020). This may explain the unique behavior of Walnut Shell biochar compared to Wood Waste or Sewage Sludge, as these were produced at ~593°C and 550°C, respectively.

Similar plant N uptake is seen across all application rates of Sewage Sludge-CBS and Stored UEBC, and Wood Waste-CBS and Stored UEBC. It is notable that significantly less N was applied with these UEBCs compared to Fresh UEBC in the same biochar groups. Urine-N applied ranges from 0.02 to 0.88 mg urine-N pot^{-1} for Sewage Sludge and Wood Waste CBS and Stored UEBCs across application rates. However, plant N uptake ranges from 4.40 to 7.27 mg-N uptake $plant^{-1}$. This suggests a soil N priming effect, or native soil N turnover in response to N addition (Kuzyakov, Friedel and Stahr, 2000). It is clear the N in the plant tissue cannot only derive from Wood Waste CBS/Stored UEBCs, as the total N (biochar N + urine N) is lower than the plant uptake for application rates 1% and 2% (Table 2-2). It is also unlikely that much biochar-N is mineralizable. Fiorentino et al. (2019) observed low mineralization of biochar-N and a native soil N priming effect with the co-addition of urea and wheat straw biochar. While Sewage Sludge-CBS and Stored UEBCs had a higher total N content due to the high N content of the raw Sewage Sludge biochar (Table 2-1), it is unlikely that much of this biochar-N is mineralizable in the short term as shown by Wang et al. (2012).

Though Sewage Sludge and Wood Waste Fresh UEBCs at 6% and 10% application rates supplied significantly more urine-N than Sewage Sludge and Wood Waste CBS or Stored UEBCs, their application did not lead to higher N uptake in plant tissue. It is possible that urea molecules in Fresh urine got trapped in small pores in Sewage Sludge and Wood Waste biochars and were slower to diffuse into soil water along a plant root gradient (Rasse et al., 2022). The comparatively higher temperature of these biochars points to a potentially higher porosity, particularly through the formation of micropores (Tomczyk, Sokołowska and Boguta, 2020). If urea-biochar did desorb from UEBCs in our study, it would likely be microbially available. Urea hydrolysis in soils is well documented as a rapid, first order reaction mediated by urea concentration and facilitated by common soil microorganisms utilizing the enzyme urease (Chin and Kroontje, 1963). Additionally, biochar application to soils is found to increase the ureolytic microbial abundance and urea hydrolysis rate (Liu et al., 2021). The linear relationship between Fresh urine-N applied and N uptake in plant tissue in urine-only treatments also supports this theory. Even at the high urine-N application rates of 6% and 10% for Wood Waste Fresh and 6% Sewage Sludge Fresh UEBCs, a potential soil priming effect is still evident. However, for Sewage Sludge-Fresh 10%, 4.02 mg urine-N were applied pot^{-1}, but 3.65 mg-N was present in the plant tissue. This implies a deleterious effect, potentially due to the high N content and/or high ash content of the Sewage Sludge biochar (Table 2-1). Future work should explore high application rates of UEBC prepared with feces-derived biochar *in situ* to determine if it is a suitable biochar for UEBC fertilization.

2.3.3 Plant biomass response

Figure 2-4 shows the total biomass response across treatments. Above-and-belowground biomass response was similar within treatments (Figures S2-4 and S2-5). Thus, all ANOVAs used total biomass as a response variable. Results from the three-way ANOVA show a significant difference between means ($p < 0.001$, three-way ANOVA, total biomass ~ urine*biochar*application rate, Table 2-3). However, few treatments differed significantly when looking at Tukey HSD pairwise comparisons (Table 2-3). Total biomass in Walnut Shell-Stored 1%, Walnut Shell-Stored 10%, Walnut Shell-Fresh 6%, Sewage Sludge-CBS 1%, and Sewage Sludge-Fresh 10% were significantly lower than Stored 10% ($p < 0.05$, Tukey HSD, Table 2-3). Plant biomass in Walnut Shell-Stored 1% and Sewage Sludge-Fresh 10% were also significantly lower than Wood Waste-CBS 10% ($p < 0.05$, Tukey HSD, Table 2-3). Total biomass did not differ significantly between fertilized and unfertilized controls (Table 2-3). However, the unfertilized control was lower (0.251 ± 0.0327 g_{dry}) than the NPK fertilized control (0.275 ± 0.0245 g_{dry}). The organic fertilized control averaged 0.233 ± 0.0253 g_{dry}, likely due to a slow release of organically bound nutrients. The lack of a robust total biomass response overall may be due hyphal nutrient foraging by the mycorrhizal inoculant in the soilless substrate (Cavagnaro et al., 2006). Future work should investigate the effect of UEBC on plant growth with and without mycorrhizal inoculant. Figures for leaf area of largest leaf, leaf number > 1 cm, and height are available in the supplemental material as these growth indicators generally followed the same trend as total biomass (Figures S2-6-8). No significant differences were found across treatments for final percentage of plants germinated (Figure S2-9).

However, some trends in the plant biomass response are evident when grouping predictor variables (biochar, urine, and application rate) in one-way and two-way ANOVAs (Tables S2-1-7). No significant differences were found for total biomass between urine types across biochar types and application rates ($p = 0.494$, one-way ANOVA, total biomass ~ urine, Table S2-1). This may be because the urine types are similarly plant available in the initial phases of plant growth when not adsorbed to biochar. While these data do not indicate the plant availability of the urine types themselves, we can conclude that one type is not significantly detrimental to plant growth over others. We did see a loss of more than 1000 mg-N L^{-1} from CBS urine compared to Stored urine. CBS urine averaged 3211 ± 616 mg-N L^{-1}, while Stored urine averaged 4241 ± 340 mg-N L^{-1}. Though we hypothesized that reduced CBS N content would lead to lower yield, this is still a substantial reduction in the potential fertilization quality of CBS urine and should be addressed in CBS systems. Closing individual urine containers between each use is unrealistic, though urine might be collated in larger, closed containers for decentralized urine-nutrient recovery to mitigate N losses. Another solution could be prevention of urease activity in the urine prior to nutrient recovery through acidification or alkalinization (Senecal and Vinnerås, 2017). Fresh urine averaged 3619 ± 312 mg-N/L, with 3355 ± 312 mg-urea L^{-1}, and 263 ± 28.7 mg- NH_4^+ L^{-1}. The discrepancy between Fresh and Stored total N content may be due to incomplete activity of urease or experimental error.

A significant difference was found for total biomass between biochar types across urine types and application rates ($p = 0.00445$, one-way ANOVA, total biomass ~ biochar, Table S2-2). Post hoc pairwise Tukey HSD tests showed that Walnut Shell differed significantly from urine-only treatments across biochar types and application rates ($p < 0.001$, Tukey HSD, Table S2-2). Average total biomass for Walnut Shell UEBCs was

0.248 ± 0.05 g_{dry}, while the average for urine-only treatments was higher at 0.278 ± 0.05 g_{dry} This is likely due to more immediately available nutrients from urine-only treatments. No other significant differences were found between other biochar types or fertilized or unfertilized controls. There was no significant effect of application rate on total biomass response across urine and biochar types (one-way ANOVA, total biomass~ application rate, $p = 0.205$, Table S2-3). Regardless, optimal application rates likely depend on biochar and urine type *in situ* and should be optimized for different CBS settings based on urine conditions and treatment options, as well as biochar feedstock availability.

A significant difference between biochar/no biochar groups was found ($p = 0.004$, Wilcoxon Rank Sum test, total biomass ~ biochar/no biochar, Table S2-4), with total biomass higher in urine-only treatments. This suggests that in the early growth stages, urine nutrients adsorbed to biochar may be less bioavailable than urine-only nutrients applied alone. These data did not support our hypothesis that UEBC treatments would outperform urine-only treatments. However, there are tradeoffs to applying urine as-is. As previously mentioned, application of urine alone can lead to leaching or gaseous losses of N, like synthetic N fertilizers (Wachendorf, Taube and Wachendorf, 2005). Future research should investigate the best way to use urine as a fertilizer over the course of plant growth. It is possible some urine should be applied as a pre-plant application or sidedress application when using UEBC as a slow-release fertilizer.

2.3.4 Potential for urine-enriched biochar in CBS systems

Our research explores different urine-enriched biochar preparations that could be potentially applied in CBS or other urine-diverting systems. We show that urine storage conditions have consequences for N retention in urine and subsequent adsorption to biochar, that biochar particle size is significant for urine-N sorption, and that urine-N bound to biochar likely releases more slowly than urine-N applied alone. However, future research should address additional questions on the actual application of UEBC in CBS and other urine-diverting systems. We chose a UEBC mixing ratio of 200g:1L based on prior experimentation that found this ratio favorable for urine-N adsorption. However, we recognize that this may be an unrealistic quantity of biochar to supply at the toilet or neighborhood scale. Future work should focus on creating replicable adsorption isotherms using real, urea-hydrolyzed, urease-inhibited, or CBS-style urine and various types of biochar. This is critical to create UEBCs with a predictable N content, which is ultimately necessary for their application and/or sale in real agronomic contexts. The economic feasibility of UEBC production in CBS systems should also be explored.

Additionally, more in situ data is necessary to investigate the potential of UEBC. Longer, field-scale studies should be implemented with different UEBC preparations and application rates. To fully understand the environmental impact of urine-based fertilizers, GHG emissions, NH_3 emissions, and N leaching from both UEBC production and application, as well as urine-only application, should be investigated across agronomic contexts. UEBC application studies that explore plant stress, such as water limitation, should also be undertaken, as the effect of UEBC on soil water dynamics after application is unclear.

2.4 Conclusion

Urine is an abundant source of plant available nutrients that is underutilized in agricultural systems. This research shows that application of Fresh, Stored, or CBS urine alone was positively linearly correlated with plant-N uptake, implying the immediate plant availability of urine-N. Across biochars, the < 500-µm biochar size fraction retained significantly more N than the > 500-µm fraction. Fresh urine-N adsorption to biochar was significantly greater than Stored or CBS urine in the > 500-µm size fraction of Wood Waste and Sewage Sludge biochars. This could be explained by a difference in adsorption mechanisms for urea and NH_3/NH_4^+ in urine to high pyrolysis temperature biochars, or differential adsorption mechanisms of NH_3/NH_4^+ and urea in the > 500-µm and < 500-µm biochar size fractions. Plant-N was not positively linearly correlated with urine-N application for Sewage Sludge and Wood Waste UEBCs and was loosely linearly correlated for Walnut Shell UEBCs. This implies that urine-N adsorbed to biochar is not bio-available in the early stages of plant growth. Future research should investigate the adsorption mechanisms of N species in real urine to biochar. Isotope dilution or tracer studies to understand urea-biochar and NH_4^+-biochar sorption, desorption, and plant-N uptake would also help better explain the phenomena found in this study. This research may help CBS organizations to optimize urine-nutrient recovery and reuse with biochar, as different biochar and urine combinations lead to different agronomic outcomes.

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2.6 Figures

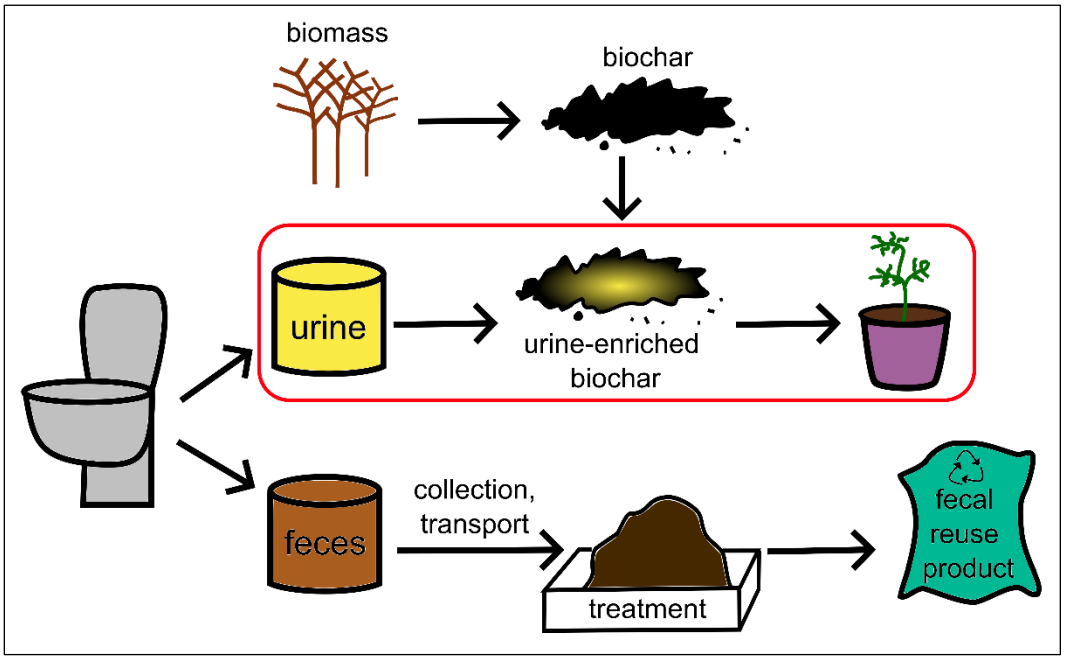

Figure 2-1: CBS service paradigm with proposed UEBC production. The red box highlights how our research approach in this study fits into a CBS system, namely the investigation of CBS urine storage conditions, UEBC production, and UEBC application as a fertilizer.

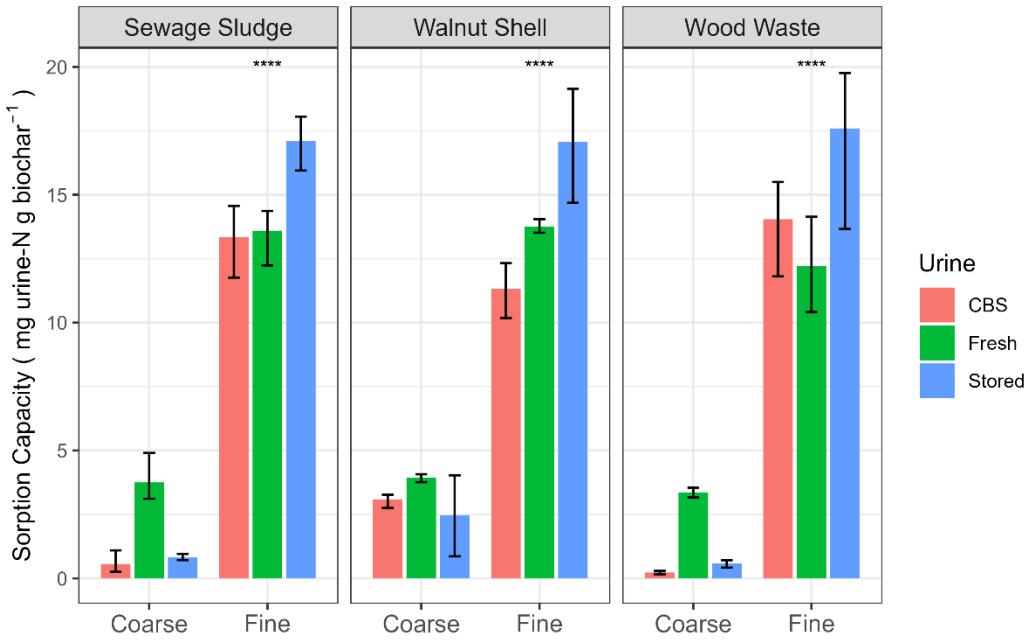

Figure 2-2: Sorption capacities of > 500-µm size fraction (Q_{coarse}), and < 500-µm size fraction (Q_{fine}). Stars indicate significance from a Wilcoxon Rank Sum test between Q_{coarse} and Q_{fine} within biochar type, across urine types ($p < 0.001$).

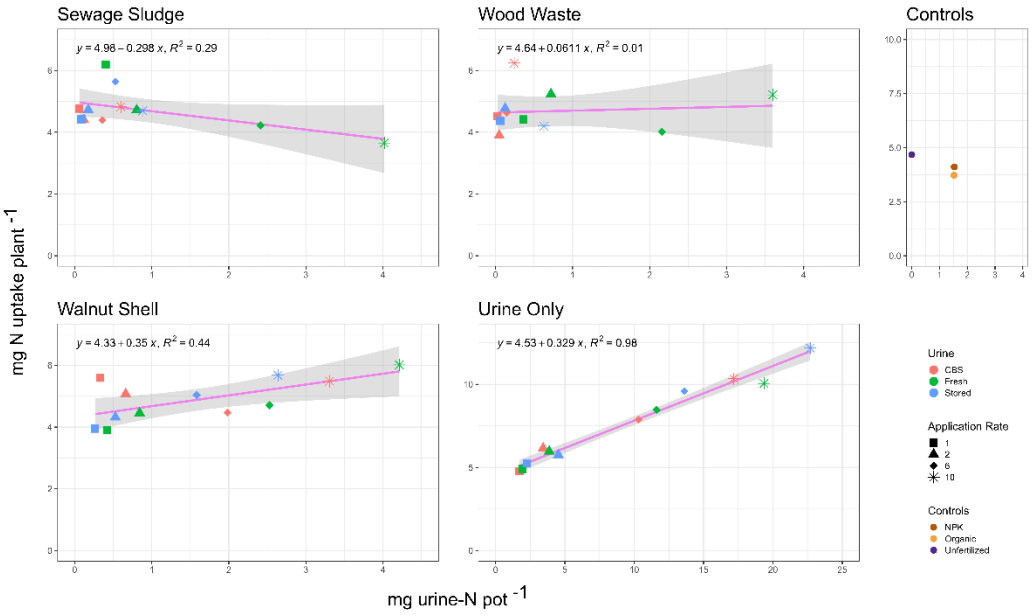

Figure 2-3: Nitrogen uptake in plant tissue compared to urine-N applied with UEBCs or urine-only. Linear regression models were fit to the data within each biochar type (or no biochar, referred to as "urine-only"), across urine types. The equation and fit for each model are displayed on each panel. Urine-N applied per pot (mg) was the predictor variable, and N uptake per plant (mg) was the response variable. Fertilized and unfertilized controls are included in a separate panel.

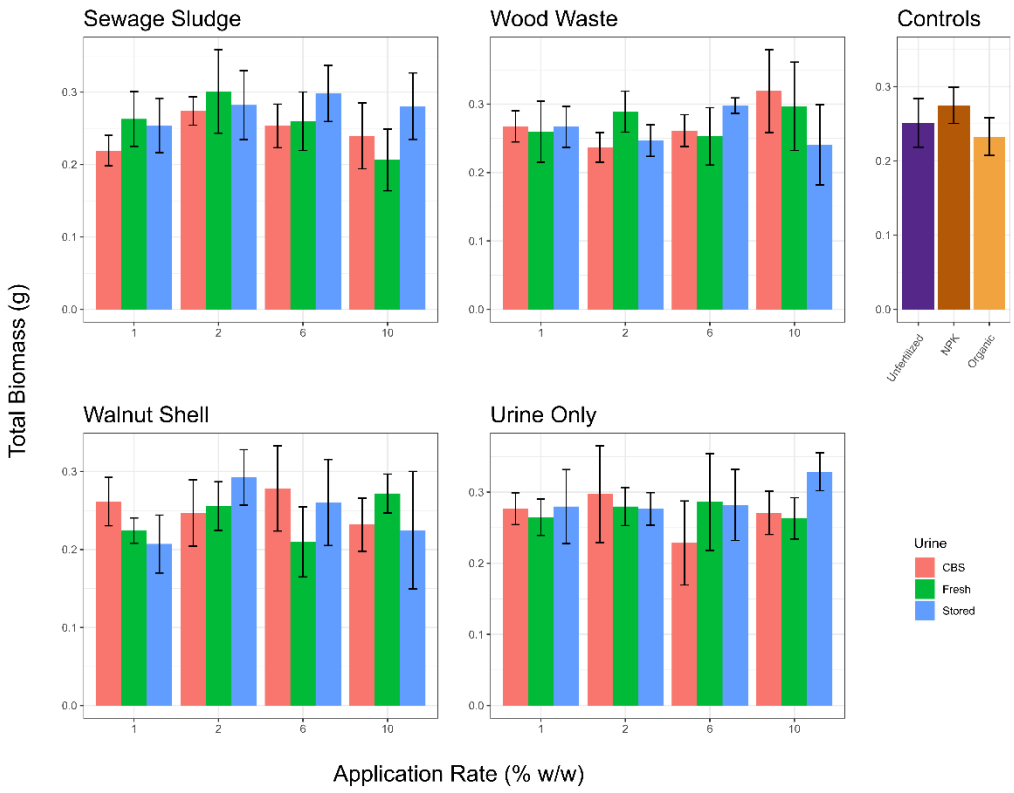

Figure 2-4: Total biomass across treatments. Each biochar type, "urine-only" treatments, and fertilized and unfertilized controls are shown in separate panels. Significant pairwise Tukey HSD post hoc comparisons between treatments are shown in Table 2-3.

2.7 Tables

Table 2-1: Biochar physiochemical properties. All data provided by the supplier (except C, N, pH, and EC, which were updated by researchers).

Biochar	**Aries GREEN™ All Natural Soil Conditioner**	**UKBRC, SS550**	**Walnut Shell biochar**
Feedstock	Recycled wood pallet waste	Sewage Sludge	Walnut Shell
Production method	gasification	pyrolysis	pyrolysis
Temperature (°C)	593	550	350
Moisture (%)	<10	2.48	3.47
Volatile matter (%)	-	21.4	69.3
Ash (%)	<5	59.0	2.21
pH	10.5	7.26	9.99
EC (µs/cm)	4390	563	1930
Total C (%)	83.8	29.9	81.7
Total N (%)	0.677	3.67	0.61
C:N	126	8.16	135
Reference	Aries GREEN™ LLC Franklin, TN, USA	UK Biochar Research Centre, Edinbugh, UK	M. Gonzales *pers. Comm*

Table 2-2: Amount of total N (mg) applied per pot at each unique application rate and amendment combination. The column header is the application rate, and the biochar-urine combination, urine-only treatment, or fertilized control is the row name. The right-most column shows the C:N ratio of UEBCs. Total N is also expressed as *kg-N ha^{-1}* for each treatment. UEBCs were applied on a dry mass basis. (n = 4 for elemental analysis of UEBC samples, n = 5 for raw biochar samples).

	Application Rate				**C:N**
	1%	**2%**	**6%**	**10%**	
Biochar-urine combination or fertilizer					
Sewage Sludge-Fresh	4.33	8.66	26.0	43.3	7.40
kg-N ha^{-1}	811	1620	4860	8110	-
Sewage Sludge-Stored	4.01	8.03	24.1	40.1	7.67
kg-N ha^{-1}	752	1500	4510	7520	-
Sewage Sludge-CBS	4.00	7.97	23.9	39.9	7.74
kg-N ha^{-1}	747	1490	4480	7470	-
Wood Waste-Fresh	1.07	2.15	6.43	10.7	86.1
kg-N ha^{-1}	201	402	1200	2010	-
Wood Waste-Stored	0.775	1.55	4.65	7.75	116
kg-N ha^{-1}	145	291	871	1450	-
Wood Waste-CBS	0.737	1.47	4.42	7.37	124
kg-N ha^{-1}	138	276	828	1380	-
Walnut Shell-Fresh	1.07	2.15	6.44	10.7	82.6
kg-N ha^{-1}	201	402	1210	2010	-
Walnut Shell-Stored	0.915	1.83	5.49	9.15	97.8
kg-N ha^{-1}	171	343	1030	1710	-
Walnut Shell-CBS	0.982	1.96	5.89	9.82	89.8
kg-N ha^{-1}	184	368	1100	1840	-
Fresh	1.94	3.87	11.6	19.4	-

kg-N ha^{-1}	363	725	2170	3634	-
Stored	2.27	4.54	13.6	22.7	-
kg-N ha^{-1}	425	850	2550	4250	-
CBS	1.72	3.44	10.3	17.2	-
kg-N ha^{-1}	322	644	1930	3220	-
NPK	1.53	-	-	-	-
kg-N ha^{-1}	150				
Organic	1.53	-	-	-	-
kg-N ha^{-1}	150				

Table 2-3:Three-way ANOVA results with total biomass as the response variable and urine, biochar, and application rate as predictor variables. Tukey's honestly significant difference (Tukey HSD) pairwise post hoc test results are included. Different letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly**.** *ns* indicates the treatment does not differ significantly from any other treatment.

Total biomass ~ urine*biochar*application rate F = 2.439 p = 6.28E-06					
Treatment	**Total Biomass (g)**	**Tukey HSD**	**Treatment**	**Total Biomass (g)**	**Tukey HSD**
Sewage Sludge Fresh 1 %	0.263 ± 0.0377	*ns*	Walnut Shell Fresh 6 %	0.21 ± 0.0451	**c**
Sewage Sludge Fresh 2 %	0.301 ± 0.0577	*ns*	Walnut Shell Fresh 10 %	0.272 ± 0.0249	*ns*
Sewage Sludge Fresh 6 %	0.26 ± 0.0403	*ns*	Walnut Shell Stored 1 %	0.207 ± 0.0371	**c**
Sewage Sludge Fresh 10 %	0.206 ± 0.0426	**c**	Walnut Shell Stored 2 %	0.292 ± 0.0356	*ns*
Sewage Sludge Stored 1 %	0.254 ± 0.0376	*ns*	Walnut Shell Stored 6 %	0.26 ± 0.0552	*ns*

Sewage Sludge Stored 2 %	0.282 ± 0.0476	*ns*	Walnut Shell Stored 10 %	0.225 ± 0.0753	**bc**
Sewage Sludge Stored 6 %	0.298 ± 0.0387	*ns*	Walnut Shell CBS 1 %	0.261 ± 0.0309	*ns*
Sewage Sludge Stored 10 %	0.281 ± 0.0458	*ns*	Walnut Shell CBS 2 %	0.247 ± 0.0425	*ns*
Sewage Sludge CBS 1 %	0.219 ± 0.0209	**bc**	Walnut Shell CBS 6 %	0.278 ± 0.0547	*ns*
Sewage Sludge CBS 2 %	0.274 ± 0.0197	*ns*	Walnut Shell CBS 10 %	0.232 ± 0.0341	*ns*
Sewage Sludge CBS 6 %	0.253 ± 0.0301	*ns*	Fresh 1 %	0.265 ± 0.0255	*ns*
Sewage Sludge CBS 10 %	0.24 ± 0.0456	*ns*	Fresh 2 %	0.28 ± 0.0268	*ns*
Wood Waste Fresh 1 %	0.26 ± 0.0448	*ns*	Fresh 6 %	0.286 ± 0.068	*ns*
Wood Waste Fresh 2 %	0.289 ± 0.0299	*ns*	Fresh 10 %	0.263 ± 0.0289	*ns*
Wood Waste Fresh 6 %	0.253 ± 0.042	*ns*	Stored 1 %	0.28 ± 0.0519	*ns*
Wood Waste Fresh 10 %	0.297 ± 0.0645	*ns*	Stored 2 %	0.276 ± 0.023	*ns*
Wood Waste Stored 1 %	0.267 ± 0.03	*ns*	Stored 6 %	0.282 ± 0.05	*ns*
Wood Waste Stored 2 %	0.247 ± 0.023	*ns*	Stored 10 %	0.329 ± 0.0267	**a**
Wood Waste Stored 6 %	0.298 ± 0.0114	*ns*	CBS 1 %	0.277 ± 0.0224	*ns*
Wood Waste Stored 10 %	0.241 ± 0.0586	*ns*	CBS 2 %	0.297 ± 0.068	*ns*
Wood Waste CBS 1 %	0.267 ± 0.0227	*ns*	CBS 6 %	0.229 ± 0.059	*ns*
Wood Waste CBS 2 %	0.237 ± 0.0216	*ns*	CBS 10 %	0.271 ± 0.0305	*ns*

Wood Waste CBS 6 %	0.261 ± 0.0233	*ns*	NPK	0.275 ± 0.0245	*ns*
Wood Waste CBS 10 %	0.319 ± 0.0607	**ab**	Organic	0.233 ± 0.0253	*ns*
Walnut Shell Fresh 1 %	0.224 ± 0.0162	**bc**	Unfertilized	0.251 ± 0.0327	*ns*
Walnut Shell Fresh 2 %	0.256 ± 0.0311	*ns*			

Chapter 3.
Nitrogen and phosphorus mineralization dynamics in human excreta-derived fertilizers

Abstract

Growing interest in human excreta-derived fertilizers requires more information on their agronomic relevance. In this study, we measured the nitrogen (N) and phosphorus (P) mineralization from urine, urine-enriched biochar, and feces-derived compost application in a 90-day aerobic soil incubation. Soils were extracted for available N at days 0, 5, 10, 20, 30, 60, and 90, while soils were extracted for four biologically relevant P pools at days 0, 30, 60, and 90. We found that N in urine applied alone was immediately bioavailable, supplying nearly all the 200 kg-N ha^{-1} applied, while urine-enriched biochar supplied approximately half of the N applied. Feces-derived compost application led to a slow release of mineral N. Feces-derived compost application stimulated substantial native soil P mining, while urine-P was likely rapidly immobilized. These results are relevant to container-based sanitation and other source-separated sanitation endeavors.

3.1 Introduction

The growing human population coupled with the exacerbating effects of agricultural practices on climate change point to the need for a paradigm shift towards more sustainable global nutrient management (Rockström et al., 2020). Additionally, the increasing scarcity, climate change impact, and price volatility of mineral fertilizers has increased interest in alternative approaches to fertilization (Crespi et al., 2022; Krein et al., 2023). Human excreta have long been considered as primarily a waste product, with treatment focused on reducing public and environmental health risks (Trimmer, Miller and Guest, 2019). However, we excrete most of the plant-essential nutrients that we consume once we reach adulthood (Jönsson et al., 2004). This means that large quantities of nitrogen (N), phosphorus (P), and potassium (K) are emitted with our waste, with a lack of widespread emphasis on resource recovery (Harder et al., 2020). Most of the N and approximately half of the P and K we excrete is present in urine. The remaining N, P, and K is present in feces, which is also rich in organic matter (Harder et al., 2019). Reuse of human excreta in agriculture was a practice common to most ancient cultures, which was largely lost with the Industrial and subsequent Green Revolution (Ashley, Cordell and Mavinic, 2011). However, recent research and policy are shifting back towards circular approaches to sanitation and agriculture, often termed Ecological Sanitation (or EcoSan) (Langergraber and Muellegger, 2005). Many EcoSan technologies are concerned with the source separation of urine and feces, as a way to increase nutrient recovery efficiencies and reduce environmental impacts (Larsen, Udert and Lienert, 2013).

An emerging application of EcoSan, largely practiced in low-income urban settlements in the Global South, is container-based sanitation. Container-based sanitation employs the

separate collection of urine and feces in sealable containers, which are transported offsite for treatment and resource recovery (Russel et al., 2019). Thermophilic co-composting is a common approach to fecal waste management in container-based sanitation systems, as a way to create a nutrient-rich organic fertilizer that reduces pathogens and greenhouse gas emissions (Preneta et al., 2013; Ryals et al., 2019; McNicol et al., 2020). Source-separated urine can be applied alone as a fertilizer, with demonstrated equivalences to N-rich synthetic fertilizers (Martin et al., 2021). Urine nutrients can also be recovered through adsorption to biochar, the carbon-rich product of pyrolyzed biomass (Masrura and Khan, 2022). Soil application of biochar has the added benefit of soil carbon sequestration, a key climate change mitigation tool (Lehmann, Gaunt and Rondon, 2006). While there is growing interest in the use of human excreta-derived fertilizers, there is a need for more information on their agronomic relevance. Specifically, understanding the mineralization of N and P from such fertilizers is crucial. Comprehensive insights into mineralization dynamics of any fertilizer are essential for recommending optimal application rates that maximize plant uptake and minimize environmental losses (Stanford, 1973). Recent research on human excreta-derived fertilizer mineralization has shed light on certain knowledge gaps (Kelova, Eich-Greatorex and Krogstad, 2021; Martin et al., 2021; Rumeau et al., 2023). However, it is important to study such fertilizers across various agroecological contexts to provide suitable recommendations for farmers.

In this study, we examined six human excreta-derived fertilizers relevant to container-based sanitation or other source-separated sanitation systems: fresh urine, stored urine (urea hydrolyzed), urine-enriched biochar (UEBC) prepared with either fresh or stored urine, and feces-derived composts (FDC) from two prominent container-based sanitation organizations. Our objective was to quantify the N and P mineralization of human excreta-derived fertilizers over the course of a typical 90 day cropping cycle length in an aerobic incubation method. We hypothesized that urine N and P would be immediately plant available from urine applied alone, and that that UEBC N and P would release slowly over the course of the experiment. We also hypothesized that N and P in FDCs would release slowly, with higher availability of P than N.

3.2 Methods

3.2.1 Amendment preparation

A 90-day aerobic amended soil incubation was conducted to assess the N and P mineralization of six human excreta-derived amendments. Fresh and stored urine, two UEBC preparations, and two FDCs were tested. Each FDC was applied on a total N and potentially available N basis. An unfertilized control was also incubated, for a total of nine treatments. Each treatment was replicated three times.

Urine was collected from willing participants over age 18 (IRB number UCM2020-171) and refrigerated at -4°C until use. Fresh urine had an electrical conductivity of ~11.4± 1.47 mS cm^{-1} and pH of ~6.77 ± 0.05, typical of fresh urine values (Ray, Saetta and Boyer, 2018, Bischak et al., 2023). A sample of fresh urine was analyzed for ammonium (NH_4^+). Urease (CAS 9002-13-5, Fisher Scientific) was applied to a subsample of fresh urine at 0.533 g L^{-1} to determine urea content by analysis as NH_4^+. Fresh urine-N content was considered as the combined urea and NH_4^+ content. To prepare the stored urine, 0.533 g L^{-1} of urease was added based on methods used by (Ray, Saetta and Boyer,

2018), shaken for 30 minutes at 180 RPM, and left at 25°C in a sealed container until all urea was hydrolyzed. Urea hydrolysis was considered complete when the electrical conductivity stabilized at ~22.0 ± 1.67 mS cm^{-1} and pH at 9.37 ± 0.09, approximately one day later. Stored urine-N content was considered as NH_4^+ content, assuming all N was present as NH_4^+ after complete urea hydrolysis. Urine-N, in both stored and fresh urine, was determined by NH_4^+ analysis using the microplate colorimetric salicylate-nitroprusside method (Agilent BioTek Gen5 Microplate Reader, Agilent Technologies, Santa Clara, CA, USA).

UEBCs were prepared with a walnut shell biochar pyrolyzed at 350°C (NextChar, Amherst, MA) mixed with fresh urine (hereafter Fresh UEBC), and stored urine (hereafter Stored UEBC). Urine and biochar were mixed at a 200 g biochar: 1 L urine ratio, allowed to saturate in a sealed container at room temperature for 48 hours, and drained freely over a 53 μm sieve. An effluent sample from each UEBC was filtered through a 0.45 μm polypropylene syringe filter for later analysis to determine the N adsorbed to biochar (Qe) using the following equation:

$$Q_e = \frac{(C_o - C_e)*V}{M}$$

[Eq. 1]

Where Q_e is the mass of urine-N adsorbed to biochar (mg urine-N g-$biochar^{-1}$), C_o is the urine-N concentration in solution before adsorption (mg L^{-1}) and C_e is the urine-N concentration in solution after adsorption (mg L^{-1}), V is the volume of urine (L), and M is the mass of biochar (g). Urease was applied to a subsample of the Fresh-UEBC effluent to correct for urea content, which was negligible. The same equation was used to calculate P sorption to UEBCs.

FDCs were sourced from two prominent container-based sanitation organizations, Sustainable Organic Integrated Livelihoods (SOIL) in Cap-Haïtien, Haiti, and Sanergy in Nairobi, Kenya. Both SOIL and Sanergy use thermophilic co-composting to transform fecal matter into nutrient-rich compost while disinfecting fecal pathogens, a process in which compost piles are maintained at greater than 55°C for approximately ten weeks (Berendes et al., 2015; Tarpeh et al., 2023). The SOIL compost, Konpos Lakay, is hereafter referred to as SOIL Konpos Lakay. The Sanergy compost, Evergrow, is hereafter referred to as Sanergy Evergrow. Amended soils are hereafter referred to by the name of their treatment. For example, soil amended with fresh urine is referred to as Fresh Urine.

3.2.2 Amendment application and incubation

The amendments were mixed with a gravelly loam soil mapped in the Redding series (Fine, mixed, active, thermic Abruptic Durixeralfs). Relevant soil properties are shown in Table 3-1. Soil was collected from four sampling locations from 0-15 cm in Merced, CA, and stored at 4°C until use. Six 10 g soil subsamples were oven dried at 105°C to determine the initial moisture content. Soils were sieved to 4 mm to remove gravel prior to mixing with amendments. Fresh soil equivalent to 200 g oven dry soil was added to each 473 mL polypropylene incubation jar.

All amendments were applied to soil at a rate of 200 kg-N ha^{-1}. Each FDC was also applied at a rate of 2860 kg-N ha^{-1}. This rate assumes that 7% of compost-N is

bioavailable (Ryals et al., 2021), and is referred to hereafter as potentially available nitrogen (PAN). FDC was otherwise applied on a total N basis. Approximately 14 times as much N and P were applied with FDC PAN treatments as with FDC total N treatments. The amount of N and P applied for each amendment are shown in Table 3-2. Since amendments were applied on an N basis, the P applied varies across amendments. UEBCs were applied on a urine-N basis (Q_e from Eq. 1), assuming that biochar-N mineralization was negligible over the course of the study. Urine-only treatments were applied on a total N basis assuming all urine-N is available. Amendment application corrected for the moisture content of the given amendment. All soil + amendment mixtures were homogenized at the beginning of the experiment. Jars were adjusted to 60% of field capacity (approximately 22% moisture) with deionized water and maintained between 19% and 26% moisture throughout the study. Field capacity was determined by preparing three jars of approximately 200 g of soil. The initial soil moisture content was analyzed and the initial jar weight was noted. Jars were saturated with water until they began to leach. They were allowed to drain freely and weighed three days later to ensure leaching was finished. The pertentage soil moisture at field capacity was considered as the difference between the initial and final weight divided by the final weight, multipled by 100. Incubation jars had perforated lids to ensure adequate aeration. All jars were kept in the dark to prevent plant growth throughout the study. Each treatment was incubated in triplicate at 24°C.

3.2.3 Soil N and P extractions

Amended soils were extracted for available N (NO_3^- and NH_4^+) seven times throughout the study: on days 0, 5, 10, 20, 30, 60, and 90. Five grams of amended soil was shaken with 25 mL of 2M KCl for 1 hour at 200 RPM. Three KCl-only samples were prepared per extraction time point**.** Extracts were filtered using Whatman #1 filter paper. Samples were stored at -20°C until analysis for available $NH_4^+$and NO_3^- using the sodium nitroprusside and vanadium chloride spectrophotometric method, respectively (Mulvaney, 1996) (Agilent BioTek Gen5 Microplate Reader, Agilent Technologies, Santa Clara, CA, USA). The average absorbance from KCl-only samples was subtracted from analysis data to correct for background absorptivity, per time point.

Amended soils were also extracted for four biologically relevant pools of P using the biologically based-P (BBP) method (DeLuca et al., 2015) at four times during the study: on days 0, 30, 60, and 90. One gram of amended soil was shaken with 10 mL of each extractant. 0.01M Calcium chloride was used to extract soluble and weakly adsorbed inorganic P, 0.01M citric acid was used to extract active inorganic P pool sorbed to clay particles or weakly bound in inorganic precipitates, 0.02 EU ml-1 phosphatase enzyme solution in 50 mM sodium acetate buffer was used to extract organic P readily attacked by acid phosphatase enzymes, and 1M hydrochloric acid was used to extract soluble, active, and moderately stable inorganic P adsorbed to mineral surfaces or present in inorganic precipitates. These extractants are hereafter referred to as $CaCl_2$, Citric Acid, Enzyme, and HCl, respectively. Three extractant-only samples were prepared per extractant and extraction time point. Extracts were shaken for 3 hours at 200 RPM and allowed to settle in a refrigerator at -4°C for 30 minutes. Extracts were filtered using Whatman #1 filter paper. Samples were stored at -20°C until orthophosphate analysis using the Malachite green method (Ohno and Zibilske, 1991). The average absorbance from extractant-only samples was subtracted from analysis data to correct for background absorptivity, per extractant and time point.

3.2.4 Statistical Analysis and Modeling

The percent of N mineralized was calculated as follows for each time point ($N_{min}t$), adapted from methods used by Lazicki et al. (2020):

$$N_{min}t = \frac{(NH_4^+\text{-}N + NO_3^-\text{-}N)_{\text{ treatment}} - (NH_4^+\text{-}N + NO_3^-\text{-}N)_{\text{ control}}}{N_{total}} * 100$$

[Eq. 2]

The total mineral N extracted from the unfertilized control was subtracted from the total mineral N for a treatment at day t, divided by the total N applied for said treatment (N_{total}), and multiplied by 100. $N_{min}t$ at day 0 represents the percent of N initially in mineral form. The same approach was used for P mineralized ($P_{min}t$) for each BBP reagent. For example, $P_{min}t_{HCl}$ was calculated as follows:

$$P_{min}t_{HCl} = \frac{(\text{HCl-extracted } PO_4\text{-}P)_{\text{ treatment}} - (\text{HCl-extracted } PO_4\text{-}P)_{\text{ control}}}{P_{total}} * 100$$

[Eq. 3]

All statistical analyses were performed in R Statistical Software (v 4.1.3; R Core Team 2022). Linear models were used to compare the amount of each extracted analyte at each extraction date using the *lm* function. Pairwise Tukey's Honestly Significant Difference (Tukey HSD) post hoc comparisons between treatments at each extraction date were performed using the *emmeans* function in the *emmeans* package. The same statistical analyses were performed on $N_{min}t$ and $P_{min}t$ data. Standard error of $N_{min}t$ and $P_{min}t$ by time point was propagated using the *crossing* function in R to calculate the uncertainty space for all combinations of N or P $\text{mineralized}_{\text{treatment}}$ - N or P $\text{mineralized}_{\text{control}}$. All references to statistical significance consider $p < 0.05$. As most discussion of statistical significance is of multiple pairwise comparisons, exact p values are not reported in the main text.

3.3 Results

3.3.1 Nitrogen

Net N mineralization varied over time and between treatments (Figure 3-1, Table S3-1). Most N mineralization occurred within the first five days for all treatments. Stored Urine and Fresh Urine treatments had the highest amounts of extracted mineral N in this period, followed by UEBCs, and composts applied on a PAN basis. For much of the 90 days, extractable N from compost amended soils did not differ significantly from unfertilized controls, aside from initially available mineral N from PAN composts and a significant N release from total N composts on day 60. Soil mineral N accumulation was not observed during the experiment, implying N volatilization, N immobilization in microbial biomass after mineralization, and/or N sorption to organic matter. The bulk of the extracted mineral N from all amendments was NH_4^+ rather than NO_3^- (Figure S3-1 & S3-2). The statistics for $N_{min}t$ remain largely the same as for the extracted mineral N data (Table S3-2, Figure S3-3). The exception is $N_{min}0$ for composts applied on a PAN basis, which do not differ significantly from the control due to the large amount of N applied. We chose

not to express our results as cumulative N mineralized, since we cannot be certain what mineral N was newly mineralized from one extraction day to the next, what N was volatilized, and what N remained in the system immobilized in microbial biomass.

On day 0, 93.8 ± 6.61 mg mineral-N kg dry soil $^{-1}$ was extracted from Stored Urine, significantly more than any other treatment. This accounts for approximately 88% of the N applied (Figure S3-3). The second-most was extracted from Fresh Urine, at 74.3 ± 7.39 mg mineral-N kg dry soil $^{-1}$, or approximately 69% of the N applied, significantly higher than all other treatments. Fresh UEBC and Stored UEBC treatments were significantly lower on day 0 than urine-only treatments and significantly higher than all FDC treatments and the unfertilized control, with 37.7 ± 6.64 mg mineral-N kg dry soil $^{-1}$ (approximately 32% of N applied) and 34.9 ± 8.74 mg mineral-N kg dry soil $^{-1}$ extracted (approximately 29% of N applied), respectively. 16.1 ± 0.21 mg mineral-N kg dry soil $^{-1}$ (approximately 0.7% of N applied) and 17.4 ± 1.52 mg mineral-N kg dry soil $^{-1}$ (approximately 0.8% of N applied) were extracted from SOIL Konpos Lakay PAN and Sanergy Evergrow PAN, respectively. SOIL Konpos Lakay total N and Sanergy Evergrow total N did not differ significantly from the unfertilized control at day 0. Significantly more NO_3^- was extracted from the unfertilized control than all treatments except Fresh and Stored Urine on day 0, indicative of an initial NO_3^- depression period (Figure S3-2).

On day 5, Fresh Urine mineralized significantly more N than all other treatments, with 41.4 ± 5.33 mg mineral-N kg dry soil $^{-1}$ extracted, approximately 40% of N applied. Store Urine had the second most, with 35.8 ± 3.65 mg mineral-N kg dry soil $^{-1}$ extracted (approximately 35% of N applied), significantly higher than all other treatments. This is followed by UEBCs, significantly higher than all FDCs and the unfertilized control, with 15.2 ± 1.51 mg mineral-N kg dry soil $^{-1}$ extracted (approximately 14% of N applied) for Fresh UEBC and 19.8 ± 7.98 mg mineral-N kg dry soil $^{-1}$ (approximately 19% of N applied) for Stored UEBC.

From days 10-30, extractable mineral N declined across treatments. No treatments differed significantly from each other or the unfertilized control on days 10, 20, or 30. A significant mineral N release was observed for SOIL Konpos Lakay and Sanergy Evergrow total N on day 60 compared to all other treatments. 18.3 ± 10.66 mg mineral-N kg dry soil $^{-1}$ (approximately 17% of N applied) was extracted from Sanergy Evergrow total N, and 5.9 ± 4.44 mg mineral-N kg dry soil $^{-1}$ (approximately 5% of N applied) from SOIL Konpos Lakay total N. No treatments differed significantly on day 90.

3.3.2 Phosphorus

Phosphorus mineralization also varied over time and between treatments (Figure 3-2, Table S3-3). The most P was generally extracted with HCl for all treatments and time points, followed by Citric Acid, Enzyme, and $CaCl_2$. HCl extracted-P increased, and Enzyme extracted-P decreased over time for SOIL Konpos Lakay PAN and Sanergy Evergrow PAN. There is a spike of $CaCl_2$ extractable-P for FDCs applied on a PAN basis at day 60. Significantly more P was extracted from PAN FDCs compared to all other treatments at nearly every time point for each P extractant (Figure 3-2, Table S3-3).

The most significant differences between treatments were observed for HCl-extracted soils. On day 0, HCl Sanergy Evergrow PAN was significantly higher than other treatments, with 164 ± 15 mg PO_4-P kg dry soil $^{-1}$. HCl SOIL Konpos Lakay PAN was

significantly higher than Stored Urine, Fresh UEBC, and Stored UEBC, and the control, with 135 ± 5 mg PO_4-P kg dry soil $^{-1}$. On day 30, significantly more P was extracted from HCl SOIL Konpos Lakay PAN than any other treatment, with 252 ± 158 mg PO_4-P kg dry soil $^{-1}$. HCl Sanergy Evergrow PAN, saw 110 ± 40 mg PO_4-P kg dry soil $^{-1}$ extracted, which was significantly higher than Stored Urine, Fresh UEBC, and the control. On day 60 the HCl PAN FDCs were significantly higher than all others, with an average of 468 mg PO_4-P kg dry soil $^{-1}$ extracted for both. The same trend held for day 90, with an average of 501 mg PO_4-P kg dry soil $^{-1}$ extracted for both. For the $CaCl_2$, Citric Acid, and Enzyme extractions, the PAN FDCs treatments were the only treatments that differed significantly from the control, which was true at each extraction date.

The statistics for $P_{min}t$ remain largely the same when compared to the extracted mineral P data (Table S3-4 , Figure S3-4). $P_{min}t_{HCl}$ Fresh urine is not statistically significant at any date, due to the relatively large amount of P applied compared to P extracted. However, all FDCs treatments are significantly higher than other treatments at all dates for all BBP extractants, due to the relatively small amount of P applied compared to large amount of P extracted (Table S3-4). Substantial mining of native soil P is evident for these treatments. This was most evident for total N composts, as a small amount of P was applied (0.34 mg PO_4-P kg dry soil $^{-1}$ for SOIL Konpos Lakay and 0.19 mg PO_4-P kg dry soil $^{-1}$ for Sanergy Evergrow), and a comparatively large amount of P was extracted. As for the N data, we chose not to express our results as cumulative P mineralized, since we cannot be certain what mineral P was newly mineralized from one extraction day to the next, and what P was immobilized in microbial biomass as the BBP method does not account for microbial biomass P (DeLuca et al., 2015). As there is also overlap between the P extraction pools (DeLuca et al., 2015), we cannot sum them to indicate total P availability of each amendment.

3.4 Discussion

3.4.1 Nitrogen availability

3.4.1.1 Urine only

Our results show that N is more available from urine-derived fertilizers compared to FDCs. Extracted mineral N was much higher for urine-derived fertilizers, with the highest from Stored Urine in the first five days of the experiment (Figure 3-1). The lack of soil mineral N accumulation over the course of the study for urine-only treatments implies that N was either volatilized, sorbed, mineralized and subsequently immobilized, or leached. Because soils were maintained at field capacity and not allowed to drain, the possibility of leaching can be excluded.

Nitrogen volatilization as NH_3 has been shown to be high from urine application as evidenced by Martin et al. (2023), who found that stored urine applied at a rate of 145 kg-N ha $^{-1}$ to the surface of a loamy haplic Luvisol resulted in 34% N volatilization of the total urine-N applied under field conditions. Rumeau et al. (2023) modeled NH_3 volatilization from stored urine applied at 170 kg-N ha^{-1} to a calcareous loamy clay soil and found that between 57% and 67% urine-N applied would be lost to NH_3 volatilization. The lack of substantial NO_3^- accumulation from urine-only treatments (Figure S3-2) implies potentially high volatilization of NH_3 from Fresh Urine and Stored Urine in our study. Effort should be made when applying urine alone as a fertilizer to prevent volatilization. The Rich Earth Institute, a prominent urine nutrient recovery

research institute, recommends applying urine in a furrow and covering it with soil, tilling after application, irrigating after application, or diluting urine before application (Rich Earth Institute, 2019). Urea hydrolysis appeared to happen within the first five days of the experiment for Fresh Urine. Stored Urine had the highest extracted mineral N at day 0, and Fresh Urine the highest at day 5. These results imply a brief period of urea hydrolysis mediated by soil microbes, by which Fresh Urine urea-N is hydrolyzed to NH_4^+-N. This is consistent with literature on urea hydrolysis, which is shown to be a rapid, first-order reaction in soils (Chin and Kroontje, 1963).

It is possible that mineral urine-N that was not volatilized was immobilized in microbial biomass for urine-only treatments. Ma et al. (2021) found preferential immobilization of fertilizer NH_4^+-N in microbial biomass compared to fertilizer NO_3^--N, particularly for treatments with low carbon availability, in an incubated agricultural Andosol with a pH of 6.55. As most urine-N in our study was in ammoniacal form, and negligible carbon was added with urine-only treatments, some urine-N may have been similarly immobilized. Christie and Wasson (2001) found similarly low immobilization rates of NH_4^+-N without carbon addition in an incubated clay loam grassland soil with a pH of 6.0, which they attribute in part to non-microbial fixation of NH_4^+-N as nonexchangeable NH_4^+-N. Thus, NH_4^+-N fixation is another possible explanation for the lack of mineral N accrual over the course of our study. It is likely that NH_4^+-N from urine-derived fertilizers in our study may have sorbed to clay minerals, rendering it non-extractable with a KCl solution (Mulvaney, 1996). Urine-NH_3 may have also chemisorbed to soil organic matter in either of the urine-only treatments, following urea hydrolysis in the Fresh Urine treatment (Nommik and Vahtras, 1982; Johansson, 1998).

3.4.1.2 Urine-enriched biochar

UEBC-N was most available within the first five days of our aerobic incubation, though the activity of plant roots would likely stimulate further desorption and thus bioavailability in planted soils. A slight slow release effect was observed for Stored UEBC, with a small, significant amount of extractable NO_3^- on day 30 (Figure S3-2). While some of the aforementioned N fates may be the same for the UEBCs as for the urine-only treatments, there are also likely some differences. The negligible urea content in Fresh-UEBC effluent suggests urea hydrolysis happened during the 48-hour UEBC saturation period. This implies that urine-N in Fresh UEBC likely sorbed as NH_4^+ (after hydrolysis) and urea (before hydrolysis). Previous research demonstrates the entrapment of urea molecules in biochar pores in N-enriched biochars (Bakshi et al., 2021; Castejón-del Pino et al., 2023). This urine-urea-N may have been desorbed, hydrolyzed by microbes, and microbially assimilated or adsorbed to clay or organic surfaces over the course of the incubation. It also could have remained entrapped in the biochar pores, and thus microbially inaccessible. If urea-urine-N remained entrapped in biochar pores, it could explain the lack of any significant slow release behavior in Fresh UEBC compared to Stored UEBC.

As urea hydrolysis was catalyzed with urease for the stored urine used to prepare the Stored UEBC, all urine-N in this treatment can be assumed as NH_4^+. Cai et al. (2016) demonstrated NH_4^+ desorption ratios lower than 10% for biochar pyrolyzed at 200°C and 300°C, and a desorption ratio of around 30% for biochar pyrolyzed at 400°C, for biochars produced from corn cob, pomelo peel, and banana stalk. Our walnut shell biochar was pyrolyzed at 350°C, suggesting a NH_4^+ desorption ratio potentially similarly low. This would explain the nonsignificant extractable mineral N for both Stored and Fresh UEBCs

after day 5. Regardless, this dynamic would likely differ in the presence of plant roots, as roots may stimulate desorption from N-enriched biochar fertilizers by building ion gradients (Rasse et al., 2022). This explains the previously observed slow release effect of walnut shell UEBCs when used as fertilizer for tomatoes (*Bischak* et al., 2023).

It is also possible that mineral N extracted from UEBC treatments at days 0 and 5 was from urine absorbed in large biochar pores or floating in the biochar slurry. The UEBC was drained over a 0.53-µm sieve to retain small biochar particles, based on prior research that demonstrated the importance of small biochar particles to retain urine-N (*Bischak* et al., 2023). This small sieve size created a wet UEBC product that was more difficult to handle than our prior preparations which used a 500-µm sieve (*Bischak* et al., 2023). The wetness of the UEBCs may have contributed to some NH_3 volatilization from UEBC treatments as well. Further research on UEBC should seek to optimize the retention of urine-N in UEBC, while making it practical and safe to handle. It is important to note that while we did not see a large slow release effect with UEBC, its application may lead to soil N accrual and subsequent release with repeated applications, or release N when the biochar weathers (Haider et al., 2020). As noted with urine-only treatments, the lack of mineral N accrual in UEBC treatments could be due to microbial immobilization or binding to clay or organic surfaces.

3.4.1.3 Feces-derived composts

Nitrogen was less available from FDCs compared to urine-derived fertilizers. On day 0, the third-most mineral N was extracted from PAN FDCs, while total N FDCs did not differ significantly from the control. From days 5 – 30, mineral N extracted from all compost treatments did not differ significantly from the control (Figure 3-1). This is likely due to either the N in FDC being relatively stable, and thus not readily decomposed, or N mineralization and subsequent volatilization, sorption to organic matter, and/or sorption to clay minerals as discussed in previous sections. Kelova et al. (2021) found low N mineralization rates after 90 days of soil incubation for a mixture of fecal matter, sanitary bark, urine, and water composted at 38°C, with a 2.6% increase in extractable mineral N. The C:N ratio of the FDCs also likely explains the lower N mineralization when compared to urine-derived fertilizers. Sanergy Evergrow had a C:N of 20, while SOIL Konpos Lakay had a C:N of 6.7 (Table 3-2). Though these are quite different ratios, they both supplied more labile organic matter than the urine-derived fertilizers. The C content of urine alone is negligible, and while the C content of the UEBCs was quite high, biochar C is generally assumed to be marginally labile (Mašek et al., 2013). The significant release of NH_4^+ from both total N FDCs on day 60 is likely due to the well-documented slow release nature of compost as a nitrogen fertilizer (Amlinger et al., 2003). These results are also consistent with Kelova et al. (2021), who saw a notable N release from FDCs after 60 days. Though the extractable mineral N was low from FDC treatments in our study, a single compost application is shown to increase soil N years after addition (Ryals et al., 2014), and increase soil N availability (Sullivan et al., 2003).

Over the course of the study, the amount of extracted mineral N from PAN FDCs was not proportional to the amount of N applied (Table S3-5). Since we assumed 7% of the N in compost was bioavailable, PAN FDCs supplied more than 14 times the amount of N as total N FDCs. However, on Day 0, approximately 2.4 times more N was extracted from the PAN FDCs compared to the total N FDCs (Table S3-5). At no point throughout the incubation did the N release from PAN FDCs become proportional to the amount of N

applied. This may be due to N mineralization and subsequent sorption to organic matter, as the PAN FDCs supplied a large amount of organic matter. Our results suggest that less than 7% of FDC-N is initially bioavailable for total N FDCs, as $N_{min}t$ did not exceed 2% until day 60 when $N_{min}t$ was 17.2% for Sanergy Evergrow, and $N_{min}t$ was 4.8% for SOIL Konpos Lakay. $N_{min}t$ did not exceed 1% for PAN FDCs at any time point (Table S3-2). Negative $N_{min}t$ values at various time points for most FDCs treatments also suggest some immobilization of FDC-N throughout the growing season (Table S3-2).

3.4.2 Phosphorus availability

3.4.2.1 Urine only and urine-enriched biochar

Our results show that FDCs stimulate more P mineralization than urine-derived fertilizers, even though more P was applied with urine-derived fertilizers. The most P was applied with UEBCs, with 9.11 mg PO_4-P kg dry soil $^{-1}$ for Fresh UEBC and 6.64 mg PO_4-P kg dry soil $^{-1}$ for Stored UEBC. However, only Stored UEBC was significantly higher than the control at day 30 for HCl extracted soils. Fresh UEBC never differed significantly from the control for any date or extractant. This implies that though urine-P was concentrated in UEBCs compared to urine applied alone, urine-P desorption was negligible. The Qe values for P were low compared to Qe for N (Table S3-6). Low P sorption to biochar is consistent with other studies, likely due to negatively charged biochar surface functional groups (Hale et al., 2013; Takaya et al., 2016).

A relatively large amount of P was also applied with Fresh and Stored Urine alone, with 5.57 mg PO_4-P kg dry soil $^{-1}$ and 3.19 mg PO_4-P kg dry soil $^{-1}$, respectively. However, extracted P for either treatment never differed significantly from the control for any extractant or extraction time point. This implies that urine-P may be rapidly assimilated into microbial biomass. The BBP method notably does not extract for microbial biomass P (DeLuca et al., 2015), meaning that this P pool is not accounted for in our study. Inorganic phosphate comprises nearly all of the P in urine (Bonvin et al., 2015). Rapid microbial inorganic P uptake (within the first 24 hours of an isotope dilution experiment) in permanent grassland soils has been demonstrated (Bünemann et al., 2012). All our day 0 extractions were performed within 24 hours of amendment application, which may account for the apparent rapid immobilization of urine-P. The soil used in our study was collected from a naturalized grassland managed for cattle grazing in Merced, CA.

While applied urine-P may have been rapidly immobilized, a potential explanation for low levels of extractable P from Stored Urine is that while high P concentrations were analyzed from small, well-mixed urine samples, only a small amount of urine-P may have actually been applied. Phosphorus precipitation as struvite is well documented as being triggered by urea hydrolysis in stored urine, and can reach maximum concentrations in a few hours (Udert et al., 2003). These precipitates settle to the bottom of a container (Tilley, Atwater and Mavinic, 2008), and thus may have not been present in representative quantities in the pipette tip when urine was applied (though the container was also well-mixed before application). From a practical standpoint, this makes recovery of P-rich precipitates difficult when applying stored urine alone as a fertilizer. Different recovery pathways should be prioritized to recover P from stored urine, such as struvite precipitation with magnesium addition, sorption, or membrane separation (Harder et al., 2019).

3.4.2.2 Feces-derived composts

The high $P_{min}t$ values (Table S3-4 , Figure S3-4) indicate FDC application led to large stores of native soil P becoming available during the incubation. These data indicate that even small applications of FDC simulate mineralization of soil P reserves. While the Olsen-P and Bray-P tests used to evaluate the soil P status indicated moderate native soil P availability (Table 3-1), these tests may underestimate soil P status, particularly in previously fertilized soils (Barrow, Roy and Debnath, 2022). Both Citric Acid and HCl extracted more P from unfertilized soils at day 0 than the Olsen-P or Bray-P values (Figure 3-2, Table S3-3). The pool isolated with these extractants is P adsorbed to clay minerals or present in inorganic precipitates (Crain et al., 2018). Our soil was acidic, with an initial pH of 5.8 (Table 3-1). Acidic soil conditions tend to favor P precipitation or adsorption to iron or aluminum (Doydora et al., 2020). It is also possible that P was precipitated as hydroxyapatite, due to the large amount of calcium native to the soil (Table 3-1) (Doydora et al., 2020). The organic matter content of FDCs, in combination with the relatively high organic matter content of 4.9% of our soil (Table 3-1) (Hiederer and Köchy, 2011), may have stimulated soil P mining. There is a demonstrated positive correlation between soil organic matter content and P availability, suggesting that organic matter molecules may prevent P diffusion into micropores and thus increase P availability (Hawkins et al., 2022; Vermeiren et al., 2022). This may explain the high $P_{min}t$ values for all FDCs, and the significant amount of extracted available P for PAN FDCs across extractants and time points. We also observed a decline in Enzyme-extractable P over the course of the incubation, and an increase in HCl-extractable P for compost treatments. As Enzyme-extractable P represents the organic P fraction (DeLuca et al., 2015), this suggests that some organic P was mineralized over the course of the incubation and adsorbed to mineral surfaces or incorporated in inorganic precipitates. This is consistent with literature on the decomposition of organic P (Taylor et al., 1978; Jalali and Ranjbar, 2009; Gagnon et al., 2012).

3.4.3 Implications for human excreta-derived fertilizer application

Our results have implications for the application of human excreta-derived fertilizers. We demonstrate that urine and UEBC are a significant source of plant available N, while FDCs are a significant source of plant available P. Urine-N is a relatively fast cycling pool of N, while FDC-P is a fast cycling pool of P. FDC also provides a moderate amount of slow cycling N, and urine-P appears to be a much slower cycling pool that was unavailable over the course of our study.

Stored Urine applied alone results in nearly the entire crop N demand being met immediately, with 187.6 kg-N ha^{-1} available on day 0 (Table S3-1). Similarly, 148.6 kg-N ha^{-1} was available for Fresh Urine on day 0. On day 5, slightly more of the crop N demand was met with Fresh Urine, with 82.8 kg-N ha^{-1} available compared to 71.6 kg-N ha^{-1} for Stored Urine. This implies that Stored or Fresh Urine applied alone is an excellent source of plant available N at the start of the growing season, with a slight preference for immediate availability from Stored Urine. This is consistent with high mineral fertilizer equivalencies observed from urine fertilizers (Martin et al., 2021). As noted previously, N volatilization was likely high from Fresh and Stored Urine, potentially depleting their fertilization values. We recommend that urine should be well incorporated into the soil when applied alone to reduce volatilization risk. Urine could be used similarly to synthetic fertilizer as a preplant or sidedress to meet specific crop N demand throughout the growing season. UEBC prepared with a low pyrolysis temperature, lignocellulosic biochar supplied approximately half of the available N as

Fresh or Stored Urine applied alone, with 75.4 kg-N ha^{-1} from Fresh UEBC and 69.8 kg-N ha^{-1} from Stored UEBC on day 0, and 30.4 kg-N ha^{-1} from Fresh UEBC and kg-N ha^{-1} and 39.6 kg-N ha^{-1} from Stored UEBC at day 5. Like urine-only treatments, N was mainly available from UEBCs within the first five days. For this reason, we recommend applying UEBC to crops with a high initial N demand, with adequate soil incorporation to prevent volatilization. Based on our results, we recommend applying similar UEBC preparations at a rate of 400 kg-N ha^{-1} to provide approximately 200 kg-N ha^{-1} However, as noted previously, UEBC-N desorption dynamics likely differ with plant root gradients and biochar weathering. We observed a slow release of N for FDCs applied on a total N basis in our study, with 36.6 kg-N ha^{-1} from Sanergy Evergrow total N and 11.8 kg-N ha^{-1} from SOIL Konpos Lakay total N on day 60. Applying FDCs on a PAN basis did not result in proportional N release, with approximately 34 kg-N ha^{-1} available from each at the start of the growing season, but declining availability thereafter. For this reason, we recommend applying smaller amounts of FDC as an N fertilizer, though applying larger quantities may result in long-term soil health benefits (Courtney and Mullen, 2008). Additionally, the carry-over effect of PAN FDC application on crop yield and soil carbon accrual has been demonstrated (Ryals et al., 2021).

Though we did not normalize amendment application on a P basis, we demonstrated that even low rates of FDC-P application stimulate significant native P mining, likely from inorganic precipitates or P adsorbed to clay minerals, in an acidic soil with a relatively high organic matter content of 4.9% (Hiederer and Köchy, 2011). Indeed, FDCs applied on a total N basis supplied an average of 0.53 kg-P ha^{-1} (Table S3-3) but stimulated an average of 174 kg-PO_4-P ha^{-1} extracted with 1M HCl at day 0. Practically, this means that while FDC supplies low rates of P when applied on an N basis, considerable soil P mining may be expected for soils with legacy P. Available P was high across BBP extractants for PAN FDCs, particularly for Citric Acid and HCl extractions. As a P fertilizer, applying FDC on a PAN basis may be expected to stimulate approximately 1000 kg-PO_4-P ha^{-1} at the end of a growing season, compared to the approximately 7.5 kg-PO_4-P ha^{-1} applied (Table S3-3). We also demonstrate that urine-P is potentially rapidly immobilized after application and not liberated over the course of the growing season, implying that urine alone is not a reliable source of P fertilizer. Our research points to the need for BBP extraction from soils amended with human excreta-derived fertilizers across soil types with various site histories to better elucidate the results of this study. This work provides valuable insight into the potential of human excreta-derived amendments to meet specific crop N and P demand over the course of a typical growing season, which may be applied to a diversity of agroecosystems with different fertilization strategies and cropping systems.

Future work should address key knowledge gaps in this research area. The mineralization of K from human excreta has been understudied and should be prioritized. Isotopic studies of N and P uptake from human excreta-derived fertilizers should also be undertaken. We used a 0.53-μm sieve to prepare the UEBC in this study, which resulted in a wet urine-biochar slurry that was difficult to handle. Future UEBC research should focus on optimizing N and P recovery and plant availability while creating a drier product that is easy and safe to handle and reduces the mass of the product. Additionally, the co-application of P-rich FDC and N-rich urine or UEBC should be studied, to determine if mineralization dynamics differ and if total crop N and P demand can be met through human excreta alone.

3.5 Conclusion

This research demonstrates that human excreta-derived fertilizers are a good source of plant available N and P. Our results suggest that comparatively more available N is present in urine-derived fertilizers, and more available P is present in FDCs in a simulated 90-day cropping cycle. Urine-N was available during the first five days of a simulated cropping cycle, with nearly the entire N demand met from Stored Urine and slightly less from Fresh Urine. Approximately half of the urine-N in UEBC was plant available, also within the first five days. Urine-P was rapidly immobilized. FDC applied on a total N and PAN basis stimulated substantial mining of legacy P incorporated in inorganic precipitates or adsorbed to clay minerals. Feces-derived-N stimulates a slow release of mineral N. These results are relevant to container-based sanitation and other source-separation based sanitation organizations.

3.6 References

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3.7 Figures

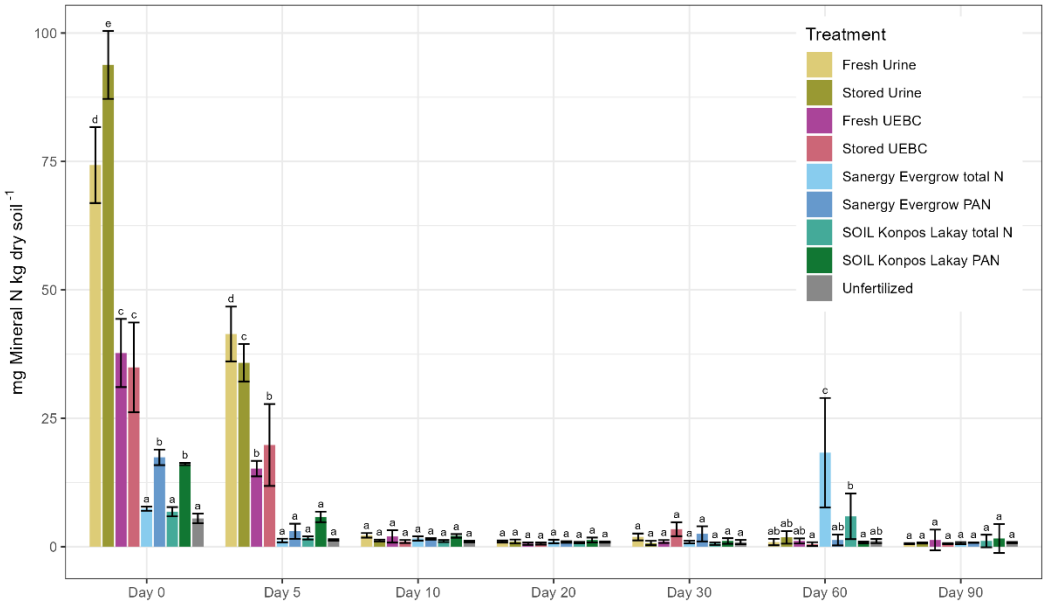

Figure 3-1: Extracted mineral N of human excreta-derived fertilizer amended soils over the course of a 90 day aerobic incubation. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly Error bars represent ± one standard deviation (n = 3).

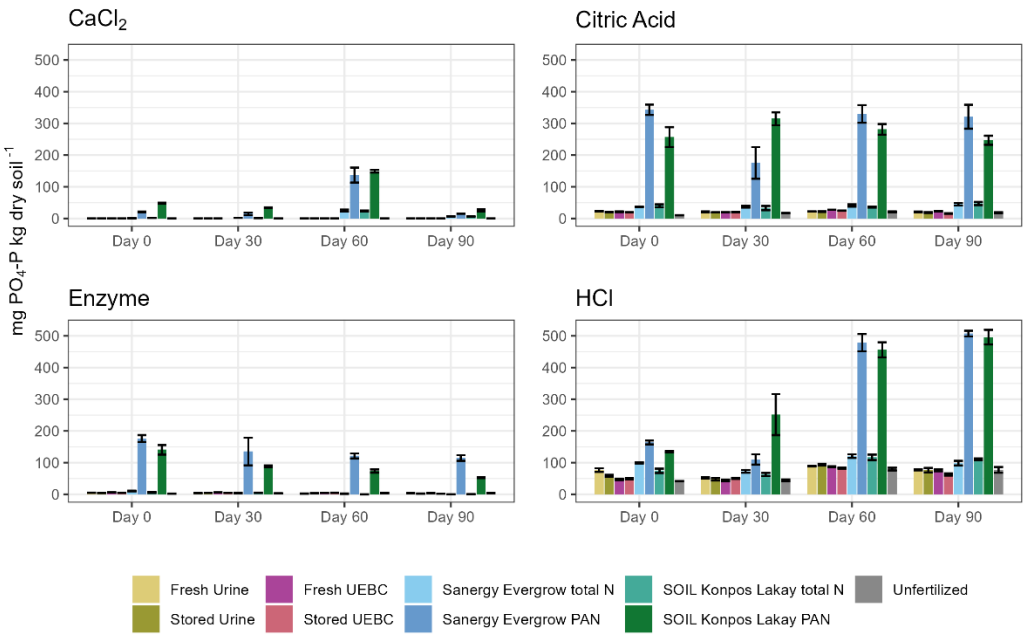

Figure 3-2: Extracted PO4-P of human excreta-derived fertilizer amended soils over the course of a 90 day aerobic incubation. Error bars represent ± one standard deviation (n = 3). Significant differences between treatments by extraction date are shown in Table S3-3.

3.8 Tables

Table 3-1: Relevant soil properties. Average values are shown.

Soil property	**Unit**	**Value**
Location	NA	Merced, CA
Soil series	NA	Redding gravelly loam
pH	NA	5.80
EC	mS cm^{-1}	0.38
total C	%	2.64
total N	kg ha^{-1}	5000
K	kg ha^{-1}	666
Mg	kg ha^{-1}	1030
Ca	kg ha^{-1}	4800
Na	kg ha^{-1}	150
CEC	m_{eq} 100 g^{-1}	15.6
Organic matter	%	4.90
Bray-P	kg ha^{-1}	36.5
Olsen-P	kg ha^{-1}	29.4

Table 3-2: Ntotal and Ptotal applied per amendment. Ntotal and Ptotal do not include the native soil N and P, which can be found in Table 3-1. All amendments were applied at a rate of 200 kg ha-1, aside from compost applied on a PAN basis assuming 7% of the N was available. UEBC values express only urine N or P adsorbed to the biochar, assuming biochar N or P mineralization was negligible. We assume the C content of urine to be negligible. Variation between Fresh and Stored Urine N content can be attributed to experimental error, while the difference between Fresh and Stored UEBC N content can be attributed to differences in urea-N and NH_4^+-N adsorption affinities to biochar (Table S3-6).

	N				P		
	N content (mg N g amend.$^{-1}$)	C:N	mg N kg dry soil^{-1}	kg N ha^{-1}	P content (mg P g amend.$^{-1}$)	mg P kg dry soil^{-1}	kg P ha^{-1}
Treatments							
Fresh UEBC	6.22	59.0	100.0	200.0	0.39	9.11	18.2
Stored UEBC	5.40	69.7	100.0	200.0	0.25	6.64	13.3
Fresh Urine	2.76	NA	100.0	200.0	0.15	5.57	11.2
Stored Urine	3.10	NA	100.0	200.0	0.10	3.19	6.38
SOIL Konpos Lakay total N	39.6	6.7	100.0	200.0	0.13	0.34	0.68
SOIL Konpos Lakay PAN	39.6	6.7	1430	2860	0.13	4.89	9.78
Sanergy Evergrow total N	23.5	20	100.0	200.0	0.04	0.19	0.37
Sanergy Evergrow PAN	23.5	20	1430.6	2860	0.04	2.66	5.32

Chapter 4.
Enhancing soil health through feces-derived compost application: a case study in Northern Haiti

Abstract

Ecological Sanitation (EcoSan) may help achieve multiple United Nations Sustainable Development Goals. However, more information on the impact of the land application of human waste-derived fertilizers such as thermophilically co-composted human feces on soil health, soil carbon stocks, and crop yield is necessary in the areas where EcoSan products are applied. We conducted a sorghum growth trial comparing feces-derived compost application to synthetic fertilization over two consecutive cropping cycles in northern Haiti. We found that feces-derived compost, particularly when overapplied on the basis of total nitrogen, led to significant increases in bioavailable soil macro- and micronutrients. Feces-derived compost application resulted in a decrease in bulk density at a depth of 0-10 cm after a single application and caused modest increases in carbon (C) and nitrogen (N) concentration in the same 0-10 cm soil layer by the end of the second sorghum crop cycle. No experimental treatments differed from unfertilized soil in either cropping cycle. This research is relevant to EcoSan systems, particularly container-based sanitation services that utilize thermophilic co-composting.

4.1 Introduction

Achieving the United Nations Sustainable Development Goals (SDGs) by 2030 requires innovative approaches to resource management (Bleischwitz et al., 2018). Ecological Sanitation (EcoSan) is a circular approach to human excreta nutrient management, by which resources embedded in human excreta are safely recovered for reuse in agriculture (Langergraber and Muellegger, 2005). EcoSan offers a multi-benefit opportunity towards achieving SDG 6 (water and sanitation for all), SDG 2, (end hunger and achieve food security), and SDG 13 (take climate action). However, more research on EcoSan reuse products is necessary to gain a comprehensive understanding of the whole-system benefits of EcoSan, particularly as they pertain to achieving SDG 2 and 13.

As of 2020, 4.5 billion people lack access to safely managed sanitation, 2 billion of which have no access to basic sanitation (United Nations, 2020). SDG 6 will likely not be met by 2030 if rates of implementation do not increase (United Nations, 2020). However, sewered sanitation systems are unlikely to meet the sanitation needs of the Global South. Sewerage uses large amounts of fresh water, requires extensive infrastructure and upfront investment, and does not easily serve the often-informal pattern of urban development (Öberg et al., 2020). Container-based sanitation (CBS) is an approach to EcoSan that is helping to bridge the SDG 6 implementation gap. In CBS systems, feces and urine are source-separated in containers and transported to a local facility for waste treatment, processing, and reuse (Russel et al., 2019). CBS provides a resilient, low-to-no water,

decentralized approach to sanitation with the added benefit of nutrient recovery and reuse. Sustainable Organic Integrated Livelihoods (SOIL) is a Haitian NGO that provides a CBS EcoSan service, EkoLakay. Founded in 2006, SOIL EkoLakay now serves over 2300 households with safe and dignified access to sanitation (*SOIL Haiti*, no date). SOIL utilizes thermophilic co-composting to transform source-separated fecal matter into a nutrient-rich, organic soil amendment, Konpòs Lakay (Preneta et al., 2013). SOIL's work as a sanitation service is particularly salient, as sanitation access in Haiti is extremely underserved. As of 2010, access to improved sanitation in urban areas is estimated at 24%, and as low as 10% in rural areas (Gelting et al., 2013).

There is high potential for EcoSan reuse products to address SDG 2. Current "linear" approaches to human excreta management ultimately release most of the nutrients embedded in human waste back to the environment. This leads to watershed pollution, including eutrophication and groundwater contamination, and atmospheric pollution, including greenhouse gas emissions that contribute to global climate change and stratospheric ozone depletion (Fowler et al., 2013; Trimmer and Guest, 2018). Improper waste treatment also leads to enteric disease (Orner and Mihelcic, 2018). However, the nutrients we excrete are the same plant-essential nitrogen (N), phosphorus (P), and potassium (K) that we apply as fertilizers (Jönsson et al., 2004). If safely recovered and treated, human feces generated in Haiti could meet 13, 22, and 11% of the N, P, and K demand for major Haitian crop production (Ryals et al., 2021). However, there is a separate global economy dedicated to the extraction and production of inorganic fertilizers to feed the global population. Phosphorus and K fertilizers are mined from spatially heterogeneous and nonrenewable mineral deposits (Cordell and Neset, 2014; Cañedo-Argüelles et al., 2017). Inert atmospheric N is converted into reactive N through the Haber-Bosch process, the main source of N fertilizers globally (Fowler et al., 2013). While the overapplication of these inorganic fertilizers has various negative environmental impacts, their provision is also unequal (FAO, 2017). Smallholder farmers, a foundational part of the global food system, often lack access to synthetic fertilizer (Rapsomanikis, 2015). The circular approach to nutrient management in EcoSan not only has the potential to restore disrupted biogeochemical cycles but can also provide organic, nutrient-rich fertilizers to support underserved agroecological systems at a local level. The application of organic amendments, such as Konpòs Lakay, are also known to improve soil health (Urra, Alkorta and Garbisu, 2019). Improvement in soil health can lead to improved crop nutrition, and thusly, better human health outcomes (Lehmann et al., 2020).

The climate change mitigation potential of SOIL's thermophilic fecal co-composting process has been rigorously studied. Research has shown SOIL's co-composting process has the potential to mitigate 8.6 kg CO_2e kg^{-1} BOD, accounting for the full sanitation cycle including avoided emissions (McNicol et al., 2020). This mitigation potential is mainly due to a large reduction in methane emissions due to compost pile aeration. This contrasts with high methane-emitting alterative waste fates in Haiti, such as waste stabilization ponds and unmanaged disposal sites (Ryals et al., 2019). This is further relevant as methane emissions from pit latrines, a wastewater management strategy that serves approximately one-quarter of the global population, are estimated to account for as much as 1% of global methane emissions (Van Eekert et al., 2019; Reid et al., 2014), though this figure may be overstated (Bourgault et al., 2019).

While the climate change mitigation potential of thermophilic fecal co-composting has been quantified, the land application of feces-derived compost is understudied in local

agroecosystems. Ryals et al. (2021) demonstrated the potential of one application of Konpòs Lakay to elevate crop yield over six consecutive cropping cycles and increase soil carbon (C) content in a greenhouse growth experiment. However, a land application study on the impact of Konpòs Lakay application in Haitian agroecosystems is lacking in the literature. Specifically, it is important to quantify the impact of Konpòs Lakay application on soil C, which has implications for soil C sequestration, a scalable and powerful climate change mitigation strategy (Trimmer, Miller and Guest, 2019). Additionally, the impact of Konpòs Lakay application on other soil health indicators, and crop production compared to other locally available fertilization regimes, is lacking (Ryals et al., 2021). To address this gap, we conducted a sorghum growth study using Konpòs Lakay. The objective of this study was to assess the effect of feces-derived compost application on sorghum production, soil health indicators, and soil C stocks on a farm in Cap-Haïtien, Haiti over two consecutive growing cycles. We were interested in whether successive Konpòs Lakay applications had an accrued effect on soil C stock, soil health indicators, and crop growth. We hypothesized that Konpòs Lakay application would lead to improved soil health outcomes due to the positive impact of C additions on soil biological, physical, and chemical processes (Larney and Angers, 2012). We expected Konpòs Lakay application to perform similarly to synthetic fertilization in Haitian agroecosystems, due to the restoration of C and macro- and micronutrients to degraded soil (Bargout and Raizada, 2013). We expected all fertilized treatments to outperform the unfertilized control.

4.2 Methods

4.2.1 Study site

In January 2022, a sorghum growth experiment was established at a farm under the management of Université Anterior Fermin (UNAF) in Cap-Haïtien, Haiti (19°43'21.9"N 72°09'51.9"W). The regional climate is characterized as tropical, with an average annual temperature of approximately 25.7 °C and an average annual precipitation of 1256 mm (*Cap-Haitien climate: Average Temperature, weather by month, Cap-Haitien water temperature*, accessed September 2023). The site had not received any recent fertilization. Management in the previous year included rotational crops, a fallow period, and cassava cultivation. The soil texture was characterized as a loam using the hydrometer method (Gee and Bauder, 1986) using a composite soil sample collected from 0-30 cm. The soil was classified as 23.99% clay, 36.42% sand, and 39.60% silt, which is within the loam soil texture class. A lack of local soil survey data made further classification difficult.

4.2.2 Experimental design and management

The study was organized in a randomized block design with five treatments, each replicated five times. Treatments included compost applied at 100% of the N demand (hereafter Compost), compost applied at 150% of the N demand (hereafter Compost 150), 50% of the N demand from synthetic fertilizer and 50% from compost (hereafter NPK-Compost), 100% of the N demand from synthetic fertilizer (hereafter NPK), and an unfertilized control. Relative physiochemical properties of the compost are found in Table 4-1. All amendments were applied at 76 kg-N ha^{-1}, except for Compost 150, on a dry mass basis. Amendments were applied based on total N content. Compost application supplied 510 kg-C ha^{-1} while Compost 150 application supplied 765 kg-C ha^{-1}, on a dry mass basis. 2.21 Mg ha^{-1} of compost was applied for the Compost treatment, while 3.31

Mg ha^{-1} of compost was applied for the Compost 150 treatment, on a dry mass basis. Synthetic fertilization consisted of a 20-20-10 NPK fertilizer applied at 30 kg-N ha^{-1} at planting, with a urea sidedress applied at 46 kg-N ha^{-1} five weeks later, based on local synthetic fertilization recommendations. Plots were weeded and hand-tilled prior to planting the first cycle. Amendments were surface applied and then spread evenly with a rake.

The study involved two consecutive sorghum cropping cycles, each of which lasted approximately 4 months. The first cycle lasted from February to June 2022, and the second cycle lasted from June to October 2022. A semi-dwarf sorghum cultivar, *Tinen-2*, was grown in this study. Seeds were provided by La Brasserie Nationale d'Haiti. The cultivar was bred for yield intensification with increased planting density and fertilization, and for aphid resistance. The line was developed specifically for Haiti (Pressoir, accessed September 2023). Each plot was 2.5 m by 4 m. A 140 cm buffer was allocated between plots, which was planted with one row of sorghum in the first cycle, but not planted for the second cycle to allow easier movement between the plots. An unplanted aisle was left between each block. Each plot consisted of 4 rows with approximately 16 plants per row. Each row was 70 cm apart, and plants within rows were approximately 25 cm apart. This planting density was chosen based on past local research (Aristil, 2019). Two seeds were planted manually per hole, and thinned a week after emergence, for a total of 64 plants per plot. Chlorpyrifos pesticide was applied at the recommended rate as needed in locations with aphid infestations during both cycles. The crop was watered with a bucket three times per week unless it rained heavily. Heavy bird grazing was problematic in both cycles. A scarecrow was used in both cycles, in addition to paper covers on the panicles, and reflective tape in the second cycle. Amendments were applied prior to planting in the same plots for the first and second cycle.

4.2.3 Plant growth indicators and productivity

Plant growth indicators were measured throughout the study. Germination, plant height, stem circumference, number of leaves, length of the longest leaf and width of the widest leaf were monitored for 10 plants per plot in 3 out of 5 blocks. During the first cycle, height and number of leaves were monitored 4 times per week from emergence to the 3-leaf stage (the first two weeks of the growing season.) Growth indicator monitoring was reduced to four times over the course of the first two weeks of the second cycle due to logistical constraints. After the 3-leaf stage, all indicators were monitored weekly until harvest. Final plant survival was surveyed approximately two months before harvest.

At harvest, five plants were chosen randomly from each plot in three of the five blocks. Selected plants were at least three plants into the plot and within the middle two rows. Plants were harvested above the adventitious root with a machete. The panicle was cut off and bagged. All biomass parts were dried in the sun and later weighed. Between cycles, the residue was removed, and the field was hand tilled carefully to remove the roots and any weeds. Plants were harvested based on seed maturity, approximately 110 days after germination.

4.2.4 Soil health indicators

Soils were sampled for analysis prior to planting, immediately prior to the first harvest, and immediately prior to the second harvest. Three random sample locations per plot were chosen based on the same criteria as for the monthly soil sampling locations and flagged at the start of the study. For the baseline and first cycle sampling, soils were

sampled with a 5.5 cm diameter PVC corer from 0-10 cm and with a soil knife from 10-30 cm. For the second cycle, soils were sampled with a soil knife from 0-10 cm. Soils were air dried and stored for later analysis. Most soil health analyses were adapted from the Soil Health Evaluation Manual from the Soils Cross Cutting Project (Vanek, Fonte and Magonziwa, 2018). Wet aggregate stability was measured with the method outlined in the Soil Health Evaluation Manual. Briefly, a 70 g composite soil sample from each plot from the 0-10 cm fraction was allowed to completely soak for 5 minutes in a 2 mm sieve with aggregates larger than 8 mm removed. The sample was sieved at 50 beats per minute for 2 minutes using a smartphone metronome app. The aggregates remaining on the sieve were dried and weighed. The soil that washed through the sieve was transferred to a 250 μm sieve and processed following the same procedure. Soil bulk density was measured by weighing the 0-10 cm sample and taking the soil moisture content in triplicate with a moisture analyzer. Bioavailable nutrients were measured using Plant Root Simulator (PRS®) ion exchange membrane probes (Western Ag Innovations, Saskatoon, Saskatchewan, Canada). Three pairs of anion and cation probes were inserted in a randomized zigzag design in each plot immediately after planting. The probes were collected two weeks after installation, cleaned, and shipped to the manufacturer for total ion analysis. The three pairs per plot were analyzed as one average sample by the manufacturer. A control probe sample was also analyzed. Soil C and N were measured via elemental analysis using a Costech 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA) on a composite sample for each depth increment.

Soils were also sampled monthly for additional soil health indicators, including pH, basal respiration, soil moisture, EC, and erosion/deposition. Three random sampling locations were flagged within each plot at the beginning of the study based on the intended planting scheme. Sampling locations were adjacent to plants, at least three plants into the plot lengthwise and within the two innermost rows. Samples were at least three plants apart from one another. Monthly soil samples were taken systemically on each occasion, approximately 30 cm from the center of the sampling area. At the next sampling event, the flag was moved approximately 10 cm down clockwise, and soil was sampled from that area. The three random samples were composited in-field and bagged. Soil pH was measured with a subsample of each monthly soil sample using the 1:2 soil:DI water method (Thermo Scientific™ Expert pH Pocket Tester, Thermo Fisher Scientific Inc., USA). Soil basal respiration was measured with a separate subsample of each monthly soil sample using the Solvita CO_2 Burst Test and the Solvita Digital Color Reader (Woods End Laboratories, Inc., Mount Vernon, ME, USA). Soil moisture was measured in-field during monthly sampling events, at one random location per plot (Extech Instruments Soil Moisture Meter Model MO750, Teledyne FLIR LLC, USA). Soil electrical conductivity (EC) and temperature was also measured in-field during monthly sampling events, at one random location per plot (Hanna Instruments GroLine HI98331, Hanna Instruments USA, Smithfield, RI, USA).

4.2.5 Methods constraints

Due to political and civil unrest in Haiti in late 2022, our data collection methods were modified during the second cropping cycle. To reduce time spent in the field for the sake of worker safety, we used modified protocols. Only the 0-10 cm soil samples were collected, with a soil knife rather than bulk density corer. Soil aggregate stability was not measured, and the final basal respiration measurement was also not taken. Plant growth indicators were monitored less frequently in the second cycle. Due to the lack of 10-30 cm bulk density data and 0-10 cm bulk density data for the second cycle, we represent

soil C data as C concentration rather than C stocks. Due to heavy bird grazing in both cycles, we were unable to accurately measure yield. Thus, total biomass, stalk biomass, and seed head biomass with seeds removed are reported.

4.2.6 Statistical analyses

All statistical analyses were performed in R Statistical Software (v 4.1.3; R Core Team 2022). All data were tested for normality prior to statistical analysis with Shapiro Wilk tests and QQ plots. Analysis of variance (ANOVA) tests with post hoc Tukey's HSD tests were used to test for treatment differences by cycle on bioavailable nutrients, harvest data, plant indicator data, and all soil physiochemical data. T-tests were used to test for differences within treatment between the baseline and first cycle. Linear mixed effect models were used to compare time series data between treatments within each cycle (plant growth, soil pH, EC, moisture, and temperature), with treatment and date as fixed effects and block as a random effect. Pairwise Tukey's Honestly Significant Difference (Tukey's HSD) post hoc comparisons between treatments by date were performed using the *emmeans* function in the *emmeans* package. Due to irregular data collection time points for plant growth indicators and soil physiochemical data between the two cycles, treatments are not compared statistically between cycles. Trends comparing the two cycles are discussed when relevant. The difference in the lagged value for plant growth and survival data was used as the predictor variable to address temporal autocorrelation. For example, the difference between plant height on a date and the previous date was calculated to perform a linear mixed effect model on plant height data. Relevant statistical tables are found in the supplemental material. All other data is represented graphically in the supplemental material, with a note of significance in the figure caption and/or main text. ANOVA or t-test p values are reported in the main text and the reader is referred to post hoc test p values in the supplemental material when relevant. All reference to statistical significance considers $p < 0.05$.

4.3 Results and discussion

4.3.1 Compost application and bioavailable nutrients

Compost application significantly increased bioavailable soil nutrients, particularly after the second application. No significant differences between treatments were observed for bioavailable nutrients during the first cycle. In the second cycle, both compost application rates led to significant increases in bioavailable soil nitrate (NO_3^-), P, K, and sulfur (S) compared to other treatments (Figure 4-1, Figure 4-2). Our results demonstrate the potential for feces-derived compost to restore Haitian soil fertility by supplying plant-essential nutrients with repeated application better than synthetic fertilizer.

4.3.1.1 NO_3^- and NH_4^+ bioavailability

Second cycle Compost 150 soil NO_3^- was significantly higher than the Control, with 80.8 ± 51.37 μg-NO_3^- 10 cm^{-2} 14 day^{-1} observed (Figure 4-1). For Compost, 79 ± 38.87 μg-NO_3^- 10 cm^{-2} 14 day^{-1} was observed in the second cycle, which was also significantly higher than the Control. NPK and NPK-Compost did not differ significantly from the Control, with 30 ± 12.88 and 58.2 ± 39.1 μg-NO_3^- 10 cm^{-2} 14 day^{-1} observed, respectively. Repeated compost application is documented to increase soil N concentrations, primarily as NO_3^- (Hartl, Putz and Erhart, 2003; Habteselassie et al., 2006). Additionally, high NO_3^- to NH_4^+ ratios in compost are considered an indicator of

compost stability (Bernal et al., 1998). This likely explains the significant increase in available soil NO_3^- for both compost application rates compared to other treatments in the second cycle, and comparatively low available soil NH_4^+. When comparing second cycle NO_3^- to the first cycle within each treatment, none of the differences were significant (Table S4-1), though the average NO_3^- concentration increased for each treatment besides the control (Figure 4-1). However, a statistically significant increase in bioavailable NH_4^+ was observed across treatments in the second cycle compared to the first (Table S4-1). This may be due to the accumulation of NH_4^+ in the topsoil, a noted dynamic in moderate or no tillage systems (Ollivier et al., 2011). While the adventitious root balls were hand tilled out prior to planting in the second cycle, seeds were planted in undisturbed soil between the tilled areas for the second cycle. This contrasts with the entirely hand tilled field at the start of the first cycle. Additionally, watering throughout the first cycle likely stimulated soil N mineralization, accounting for the NH_4^+ accumulation seen at the beginning of the second cycle (López-Bellido et al., 2014). These results show that two successive applications of feces-derived compost led to more bioavailable soil NO_3^- than synthetic N fertilizer, in a moderate tillage system with loamy soil.

4.3.1.2 P and K bioavailability

Second cycle Compost 150 bioavailable P was significantly higher than the Control and NPK, with 8.04 ± 4.48 μg-P 10 cm^{-2} 14 day^{-1} observed (Figure 4-1). Compost and NPK-Compost did not differ significantly from any other treatment, with 4.54 ± 1.61 μg-P 10 cm^{-2} 14 day^{-1} and 4.82 ± 2.53 μg-P 10 cm^{-2} 14 day^{-1} observed, respectively. The increase in soil P from the first to second cycle was statistically significant for Compost 150, Compost, and NPK-Compost (Table S4-1). Increases in surface soil P after multiple years of repeated cattle manure applications are well documented (Qian et al., 2004; Weyers et al., 2016). Similar soil P accumulation is seen with repeated sewage sludge application (Kidd et al., 2007; Antoniadis, Koutroubas and Fotiadis, 2015). These trends are consistent with our findings of the highest soil available P in the second cycle for Compost and Compost 150, and significant increases in soil P status for Compost, Compost 150, and NPK-Compost between cycles. While the compost itself was 1.25% P on a dry mass basis (Table 4-1) and thus likely supplied some available P, the high organic matter content (46% dry mass basis, Table 4-1) of the compost also likely stimulated native soil P mineralization. Organic matter may prevent diffusion of P into soil micropores, increasing soil P availability (Hawkins et al., 2022). The potential of SOIL Konpos Lakay to increase soil P availability in local Haitian agricultural soils is salient, as a survey of 1500 soils from farms in five major Haitian watersheds found 62% of them to be P deficient (Hylkema, 2011). SOIL Konpos Lakay application could build soil fertility by increasing soil P status, ultimately leading to increased crop quality and thus crop nutrition. However, soil P budgets should be considered with repeated feces-derived compost application to meet crop P demand and reduce P losses to the environment, as repeated cattle manure application over 16 years led to significant soil P accumulation and P losses attributed to leaching (Whalen and Chang, 2001).

A similar trend was observed for bioavailable K. In the second cycle, Compost 150 was significantly higher than the Control, NPK, and NPK-Compost, with 44.2 ± 18.07 μg-K 10 cm^{-2} 14 day^{-1} observed (Figure 4-1). Compost did not differ significantly from any other treatment, 27.6 ± 9.66 μg-K 10 cm^{-2} 14 day^{-1} observed. Our results are supported by the literature, as repeated sewage sludge application is also shown to increase soil available K (Kidd et al., 2007; Antoniadis, Koutroubas and Fotiadis, 2015). While K is one of the most important major plant nutrients, K fertilizer usage is lower in the Global

South due to high import costs and reduced availability (Sardans and Peñuelas, 2015; Yakovleva et al., 2021). Our results demonstrate that successive feces-derived compost applications supplied more bioavailable soil K than synthetic fertilizer and could potentially supply a critically overlooked nutrient to smallholder farmers in the Global South.

4.3.1.3 S and micronutrient bioavailability

Second cycle Compost 150 bioavailable S was also significantly higher than the Control and NPK, with 19.62 ± 10.01 μg-S 10 cm^{-2} 14 day^{-1} observed (Figure 4-2). Compost and NPK-Compost did not differ significantly from any other treatments, with 14.56 ± 4.66 μg-S 10 cm^{-2} 14 day^{-1} and 18.69 ± 9.71 μg-S 10 cm^{-2} 14 day^{-1} observed, respectively. Soil available S increased significantly for Compost between cycles (Table S4-1). This is important as soil S deficiency is widespread, particularly in low income countries (Behera et al., 2021), though S fertilization is important for increasing sorghum yield and crop quality (Sahrawat et al., 2008). Further, the need to recycle S-rich organic materials such as feces as fertilizers to reduce reliance on mineral fertilization has been identified, particularly as the supply of sulfuric acid decreases as the global economy moves towards decarbonization (Lisowska et al., 2022; Maslin, Van Heerde and Day, 2022). Nonsignificant accumulation of other important crop nutrients such as Fe, Mg, Ca, Mn, and Cu were observed in the second cycle in compost treatments, while Zn availability increased in the second cycle across treatments (Table S4-2). These results further demonstrate the potential of feces-derived compost to supply critical crop nutrients in smallholder systems.

4.3.1.4 Heavy metal bioavailability

No treatments differed significantly for either cycle in bioavailability of Pb or Cd. However, nonsignificant accumulation was seen in compost treatments in the second cycle for Pb, while Cd was largely nondetectable in either cycle (Figure 4-3). As noted, Cu, Fe, and Mn bioavailability increased for compost treatments in the second cycle, and Zn bioavailability increased between cycles across treatments. While Cu, Fe, Mn, and Zn are important plant micronutrients, they can cause plant toxicity when present in excess quantities in the soil (Arif et al., 2016). Heavy metal accumulation is a topic of concern with fecal recovery and reuse, and is well-explored in the literature (Nunes et al., 2021). While our results did not show a significant accumulation of heavy metals of interest with two successive feces-derived compost applications, the risks of heavy metal soil and crop accumulation with long term application should be noted. Additionally, the effect of feces-derived compost application on other heavy metal bioavailability, such as Hg, Ni, and As, should be further researched.

4.3.2 Harvest biomass

No statistically significant differences were observed between treatments for either cycle for total biomass, stalk biomass, or seed head biomass (Figure 4-4, Figures S4-1-2, Tables S4-3-8). There was also no statistical significance when comparing treatments across cycles (Tables S4-9-11). This is likely due to the high native C and N status of the soil (Figure 4-5), indicating good baseline soil fertility. If the field was cropped for subsequent cycles, we might expect treatment differences in the longer term. However, the results of this short term study indicate that feces-derived compost treatments perform as well as synthetically fertilized treatments, but no better than the unfertilized control, in a Haitian agricultural loam.

4.3.3 Plant survival and growth monitoring indicators

In the first cycle, no treatments differed significantly in plant survival rate ($p = 0.87$, Table S4-12). This trend was the same for the second cycle ($p = 0.96$, Table S4-13). Plant survival increased across treatments between cycles (Figure S4-3). This increase was significant for NPK ($p = 0.028$) and NPK-Compost ($p = 0.035$) (Table S4-14). However, both compost treatments had significantly lower initial germination than other treatments during some dates in the first month of the cropping cycle (Figure S4-3). This was true for both cycles, though more pronounced in the first cycle. This suggests that applying feces-derived compost may cause an initial lag in or inhibition of germination upon first application. There is a demonstrated negative correlation between the phenolic acid content in composts derived from animal waste and the germination of sorghum seeds (Marambe and Ando, 1992). As the phenolic acid content of human fecal water fraction is high (Jenner, Rafter and Halliwell, 2005), phenolic acids may partially explain the lag in germination with compost treatments though further research is necessary. The pH of the compost itself was 4.9, characteristic of the acidic nature of thermophilically composted human feces (Shi et al., 2016). A study on *Sorghum almum* germination found a decline in germination rates at pH below 5 (Eberlein, 1987). We did see lower initial soil pH values for both cycles in Compost and Compost 150 treatments (Figure S4-12). If some seeds were planted in acidic compost-soil microenvironments, this may explain the lag in germination and nonsignificant but lower plant survival for compost treatments. Farmers might consider NPK-Compost as an alternative for faster germination and better seedling survival. However, as plant survival did not differ significantly between treatments at the end of either cycle, this may not be of concern. The impact of feces-derived compost application on germination and plant survival should be further studied with other crops and soil types.

Stem circumference, leaf number, length of the longest leaf, and width of the widest leaf, did not differ significantly between treatments for either cycle. P values are shown in Tables S4-15-22, and data is visualized in Figures S4-4-7. Plant height did not differ significantly between treatments for the first cycle ($p = 0.18$, Table S4-23). In the second cycle, treatments did differ significantly for plant height ($p = 2.42E-03$, Table S4-24). Compost 150 and Compost were significantly lower than the Control across the cycle (Table S4-25).

4.3.4 Soil bulk density and aggregate stability

No significant differences in soil bulk density were observed between treatments for the baseline or first cycle for the 0-10 cm depth (Figure S4-9). However, when comparing bulk density for each treatment between the two cycles, a significant reduction in bulk density in the second cycle was observed for Compost, Compost 150, and NPK (Figure S4-9, Table S4-26). This amounted to a 12.9 ± 8.64 % reduction for Compost, 15.8 ± 12.4 % reduction for Compost 150, and 16.0 ± 6.96 % reduction for NPK. Reduced bulk density in compost amended soils is well established (Brown and Cotton, 2011). Reduced bulk density is an indicator of increased soil pore space and thus better soil structure. Reduced bulk density in compost amended soil may also be attributed to enhanced soil aggregation (Brown and Cotton, 2011). However, we did not see significant treatment differences for aggregate stability at 0-10 cm for both aggregates less than 250 μm and those larger than 2 mm (Figures S4-10 &11). However, aggregates less than 250 μm

generally decreased across treatments between cycles, while those larger than 2 mm generally increased, implying enhanced aggregation between cycles.

4.3.5 Soil pH, EC, moisture, temperature, and basal respiration

Soil pH did not differ significantly between treatments during the first cycle ($p = 0.06$, Table S4-27), but differences were significant in the second cycle ($p = 0.02$, Table S4-28). Second cycle Compost 150 soil pH averaged 6.8 ± 0.3, which was significantly lower than NPK-Compost at 6.9 ± 0.3 ($p = 0.011$, Table S4-29). Though both pH ranges are within the optimal range for sorghum production (Butchee et al., 2012), farmers might consider NPK-Compost for crops that require more alkaline soil conditions. Further research on changes in soil pH with repeated application of feces-derived compost should be pursued, as the impact of repeated organic amendment application on soil pH varies across studies (Diacono and Montemurro, 2011). This is important as the availability of heavy metals is inversely correlated with soil pH (Diacono and Montemurro, 2011), which may be an important factor that determines best practices for feces-derived compost application, as seen with the nonsignificant increase of Cu and Pb bioavailability with repeated Compost 150 application in this study.

Soil basal respiration fluxes were marginally lower for Compost and Compost 150 compared to NPK and NPK-Compost the third month after planting during the first cycle (Figure S4-13, Table S4-30, Table S4-31). Otherwise, there were no statistical differences in basal respiration flux. Fluxes were significantly lower across treatments in the second cycle compared to the first (Table S4-33). Soil EC, moisture, and temperature did not differ significantly between treatments for either cycle. P values are shown in Appendix C SI Tables S4-34-39, and data is visualized in Appendix C SI Figures S4-14-16.

4.3.6 Soil C and the climate change mitigation potential of feces-derived compost application

No significant differences between treatments were observed for soil C at the 0-10 cm depth or 10-30 cm depth for any cycle (Table S4-40). A reduction in average soil C for all treatments at the 10-30 cm depth was observed between the baseline and first cycle sampling (Table S4-41). This may be due to soil C loss from initial tillage prior to planting the first cycle (Haddaway et al., 2017). This loss was significant for NPK, amounting to an -12.8 ± 4.75 % ($p = 0.016$). This is likely due to the documented depletion of soil organic C in the subsoil with synthetic fertilization across agroecosystems (Khan et al., 2007). This may have longer term implications for continual synthetic fertilization of Haitian soils for soil C stocks. A positive, nonsignificant trend in soil C concentration in 0-10 cm soil was observed for Compost 150 and NPK-Compost over the course of the study, while NPK saw a decline in 0-10 cm soil C over the course of the study (Figure 4-5). Compost 150 0-10 cm C concentration increased by an average of 4.01 ± 4.4 % by the first cycle, and 2.92 ± 7.95 % by the second cycle. Similarly, NPK-Compost increased by 1.27 ± 10.37 % by the first cycle and 3.68 ± 10.11 % by the second cycle. This contrasts with NPK increasing by 0.18 ± 7.96 % by the first cycle and decreasing by -10.1 ± 2.99 % by the second cycle. Compost increased by 12.23 ± 7.48 % by the first cycle and decreased by -9.9 ± 3.75 % by the second cycle, and the Control increased by 9.66 ± 23.39 % by the first cycle and decreased by -7.34 ± 20.18 % by the second cycle. While the variability in these data are high, these results may indicate the potential of Compost 150 or NPK-Compost to increase soil C stocks in the topsoil in the long term. While soil C concentration has implications for soil C sequestration, more data

is necessary to scale the climate change mitigation potential of feces-derived compost application. Studies estimating the soil C sequestration potential of organic amendment application on agricultural land often utilize long term field study data or modeling to estimate the net climate change mitigation potential at the decadal or centennial time scale (Jarecki and Lal, 2003; Ryals et al., 2015). Additional spatially and temporally robust GHG flux measurements after feces-derived compost application, deep soil C measurements over multiple years of a given compost application practice, and complete bulk density and/or equivalent soil mass data sets are necessary to determine the net climate change impact of feces-derived compost application on cropland. Feces-derived compost application on different crop types, soil degradation statuses, land management scenarios, and regions should also be explored.

4.3.7 Soil N

No significant differences between treatments for soil N at the 0-10 cm depth or 10-30 cm depth were observed for any cycle (Table S4-42). Soil N at the 10-30 cm depth declined across treatments between the baseline and first cycle (Table S4-43). This is also likely due to microbial activity being stimulated by hand tillage used to prepare the field for the first cycle (Song et al., 2022). This loss was significant for NPK at -14.2 ± 6.90% ($p = 0.011$). This is consistent with findings on the long term loss of soil N in the subsoil after synthetic fertilizations across agroecosystems, attributed to fertilizer-N stimulation of microbial C usage and native soil N mineralization (Mulvaney, Khan and Ellsworth, 2009). This may have longer term impacts on native soil N depletion and ultimately crop quality with continual synthetic fertilization in Haitian agroecosystems. Like soil C, a positive trend in soil N concentration in 0-10 cm soil was observed for Compost 150 and NPK-Compost, while NPK saw a decline in N in 0-10 cm over the course of the study (Figure 4-5). Compost 150 0-10 cm N concentration decreased by -1.67 ± 5.01 % by the first cycle and increased by 8.50 ± 9.54 % by the second cycle, while NPK-Compost declined by -9.06 ± 6.61 % by the first cycle and increased by 10.52 ± 13.53 % by the second cycle. Compost 0-10 cm N increased by 7.69 ± 7.97 % in the first cycle and declined by -5.59 ± 4.37 % in the second cycle, while NPK declined by -5.50 ± 7.48 % in the first cycle and -2.64 ± 5.19 % in the second cycle. While the variability in soil N is also high, these results imply that overapplying feces-derived compost on a total N basis or coapplying feces-derived compost and synthetic fertilizer may increase soil N concentration in the topsoil. This may have longer term implications for building soil organic N, as compost application is demonstrated to increase soil N availability (Sullivan et al., 2003), and a single compost application is shown to increase soil N years after application (Ryals et al., 2014).

4.3.8 Challenging dominant narratives about Haitian agroecosystems

Though agriculture is the most important source of income in Haiti and soil fertility, erosion, and deforestation issues are among the most commonly cited in discussion of Haitian agroecosystems, there is a lack of peer-reviewed data on Haitian agroecosystems (Bargout and Raizada, 2013). Indeed, Churches et al. (2014) performed a supervised classification of Haitian land use data using 2010–2011 Landsat imagery and found approximately 29% was forested, and a total of 32% of Haitian land area was tree covered. This contrasted with the oft-cited FAO statistic of 4% forest cover and 0% other wooded land in Haiti at the time of publishing. More recent research estimates Haitian forest cover at 21% as of 2015 (Pauleus and Aide, 2020), while another study estimated an increase in Haitian forest cover from 2002 to 2010 (Rodrigues-Eklund et al., 2021). This is to say that predominant narratives have misrepresented Haitian natural and

agroecological systems, though recent research challenges that. This study lends an important contrast to the dominant narrative of massive soil depletion and fertility issues in Haiti. As harvest biomass data did not differ significantly between treatments for either cycle, we can conclude that the unfertilized soil had adequate natural fertility to sustain biomass production comparable to both feces-derived compost and synthetic fertilizer over two consecutive cropping cycles. This contrasts with our hypothesis. While we expected feces-derived compost application to perform similarly to synthetic fertilizer, we expected they would both outperform the unfertilized control. Further, the baseline soil C stock in our study was 30.41 ± 6.64 Mg C ha^{-1} in the 0-10 cm depth, equivalent to 1.92 ± 0.36 % C. This falls within the median range for baseline agricultural SOC stocks for dry sub-humid and humid climatic zones (Emde et al., 2021). These insights point to the need for rigorous agricultural research, particularly supported financially by the Global North, to better quantify Haitian soil C status and overall fertility. Insight and support from local agronomists and farmers are particularly important to better elucidate the ecological status of various Haitian agroecosystems.

4.4 Conclusion

CBS EcoSan systems have the potential to help achieve multiple SDGs, including SDGs 6, 2, and 13. This research demonstrates the potential of a CBS EcoSan reuse product, feces-derived compost, to enhance key soil health indicators in a Haitian agroecosystem over two consecutive sorghum cropping cycles. Feces-derived compost, particularly applied at 150% of sorghum N demand, significantly increased bioavailable soil NO_3^-, P, K, and S after the second application. Feces-derived compost application reduced 0-10 cm bulk density after one application and led to moderate increases in 0-10 cm C and N content at the end of the second sorghum crop cycle. This has implications for long term positive soil health impacts with repeated feces-derived compost application in Haitian agroecosystems. However, further research is necessary on the tradeoffs between improved soil health indicators, soil C stocks, and ultimately climate change mitigation potential, and soil heavy metal concentration or P leaching with long term feces-derived compost application. Feces-derived compost application should be studied longer term in multiple Haitian agroecosystems.

4.5 References

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4.6 Figures

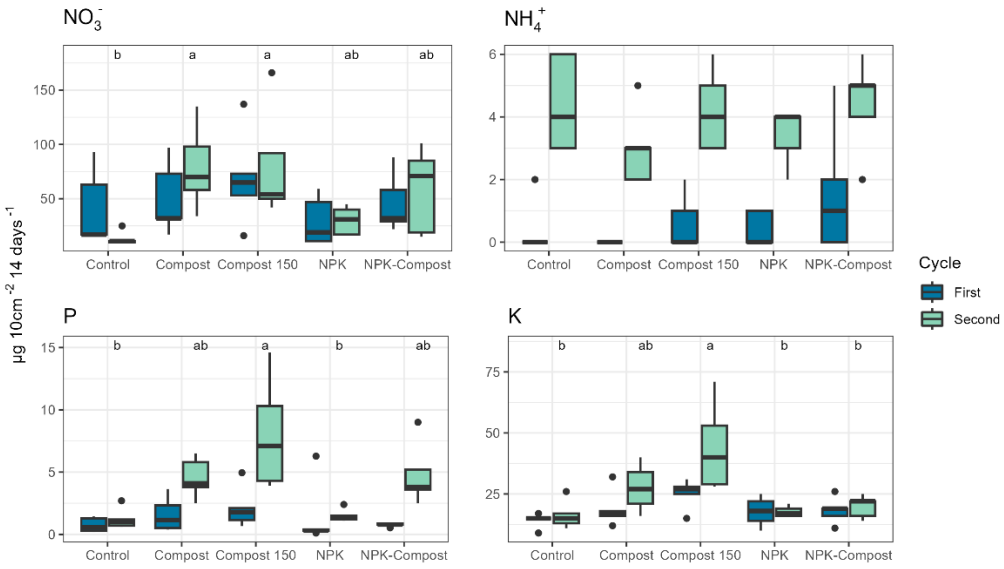

Figure 4-1: Bioavailable soil N, P, and K in the first and second cropping cycles. As no significant differences were observed between treatments in the first cycle, letters represent significant differences between treatments in the second cycle. If treatments share a letter do not differ significantly at $p < 0.05$. Data is expressed as the average ± one standard deviation (n = 5).

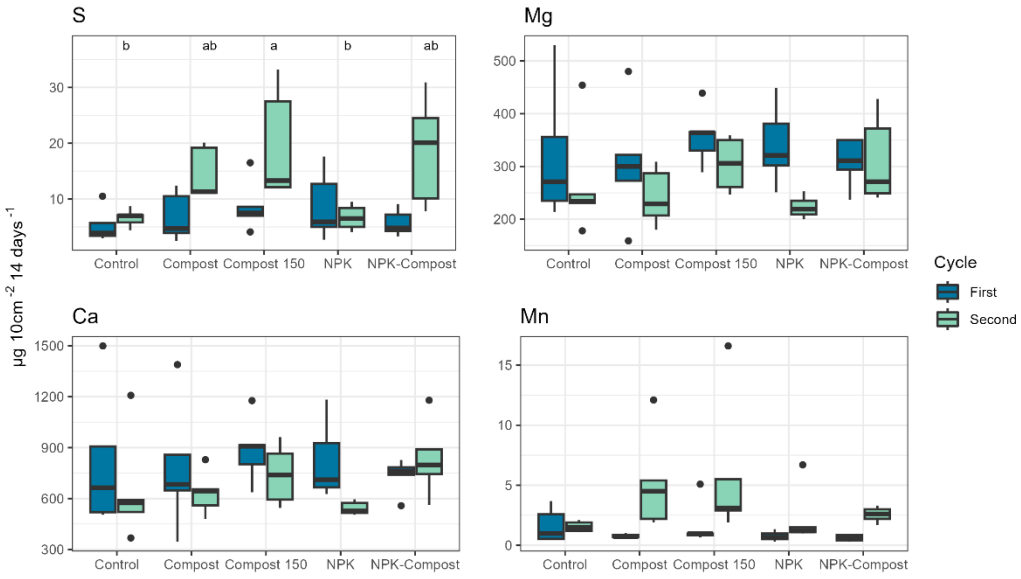

Figure 4-2: Bioavailable soil S and micronutrients in the first and second cropping cycles. As no significant differences were observed between treatments in the first cycle, letters represent significant differences between treatments in the second cycle. If treatments share a letter do not differ significantly at $p < 0.05$. Data is expressed as the average ± one standard deviation (n = 5).

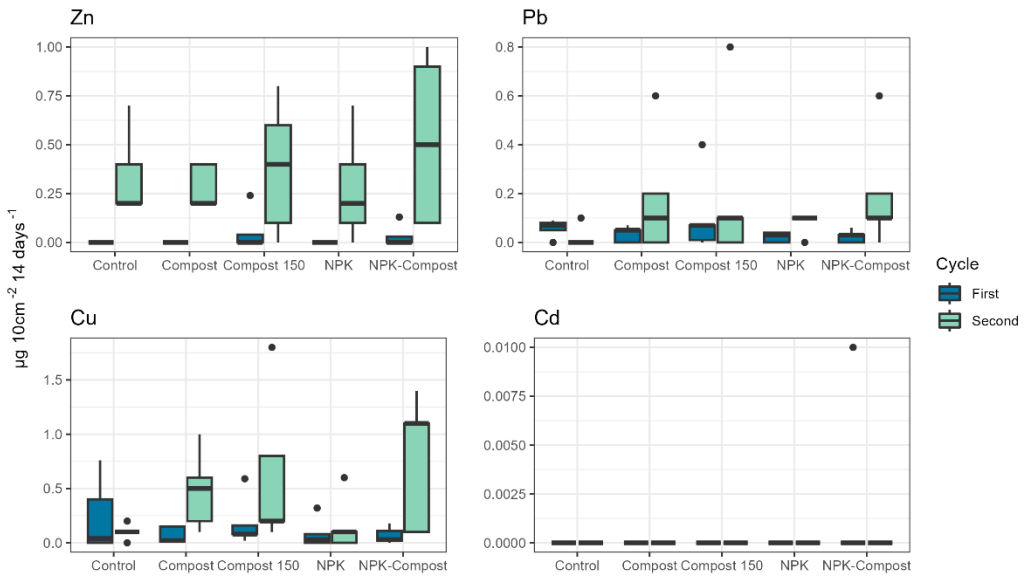

Figure 4-3: Bioavailable soil heavy metals in the first and second cropping cycles. Data is expressed as the average ± one standard deviation (n = 5). No significant differences were detected between treatments for either cycle at $p < 0.05$.

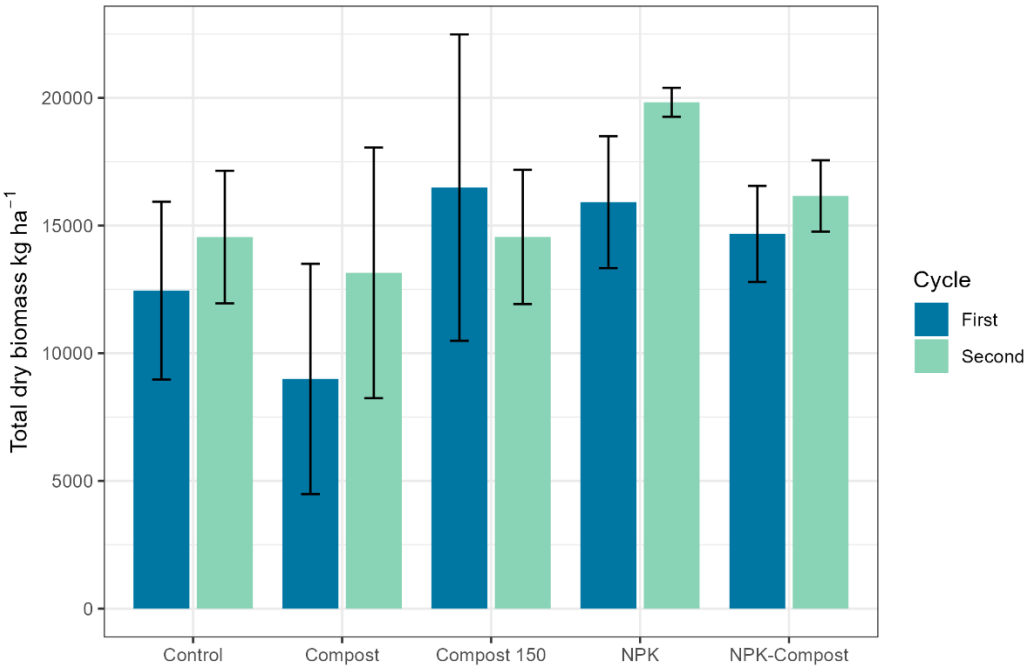

Figure 4-4: Total dry biomass for the first and second cycle. Data is expressed as the average ± one standard deviation (n = 5). No significant differences were detected between treatments for either cycle at $p < 0.05$.

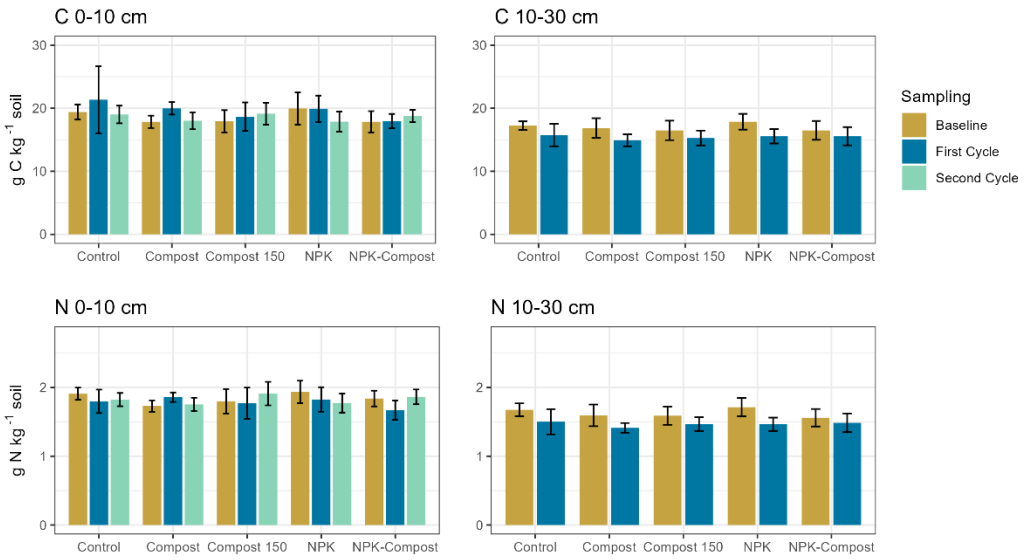

Figure 4-5: Soil C and N content in the 0-10 cm depth increment for the baseline, first cycle, and second cycle soil sampling. The 10-30 cm depth was not sampled in the second cycle due to threats to worker safety. Data is expressed as the average ± one standard deviation (n = 5).

4.7 Tables

Table 4-1: Compost physicochemical analysis. All results are reported on a dry mass basis except moisture and solids content.

Analysis	**Unit**	**Result**	**Method**
Moisture (70 °C)	%	40.4	TMECC 03.09-A
Solids	%	59.7	TMECC 03.09-A
Total Nitrogen (N)	%	3.44	TMECC 04.02-D
Phosphorus (P)	%	1.25	TMECC 04.03-A
Phosphate (P_2O_5)	%	2.86	TMECC 04.03-A
Potassium (K)	%	1.43	TMECC 04.04-A
Potash (K_2O)	%	1.72	TMECC 04.04-A
Sulfur (S)	%	0.62	TMECC 04.05-S
Magnesium (Mg)	%	1.2	TMECC 04.05-MG
Calcium (Ca)	%	1.98	TMECC 04.05-CA
Sodium (Na)	%	0.56	TMECC 04.05-NA
Iron (Fe)	%	1.35	TMECC 04.05-FE
Aluminum (Al)	%	0.92	TMECC 04.07-AL
Copper (Cu)	mg kg^{-1}	78	TMECC 04.05-CU
Manganese (Mn)	mg kg^{-1}	447	TMECC 04.05-MN
Zinc (Zn)	mg kg^{-1}	313	TMECC 04.05-ZN
pH	-	4.9	TMECC 04.11-A
Soluble salts	dS m^{-1}	11.1	TMECC 04.10-A
Ash (550 C)	%	53.8	TMECC 03.02-B
Organic matter	%	46.2	TMECC 05.07-A
TOC	%	23.1	TMECC 04.01-A
C:N	-	6.7:1	TMECC 05.02-A

Respiration - CO2-C/g TS	mg CO_2-C g TS $^{-1}$ day $^{-1}$	0.4	TMECC 05.08-B
Respiration - CO2-C/g OM	mg CO_2-C g OM $^{-1}$ day $^{-1}$	0.4	TMECC 05.08-B
Compost Stability Index	-	Very Stable	TMECC 05.08
Maturity by CO_2 Respiration	-		TMECC 05.08-E Solvita

Chapter 5.
Reflecting on Ecological Sanitation research methods in the lab and field

Abstract

Conducting Ecological Sanitation (EcoSan) research is a unique pursuit with various inherent barriers. Collecting and safely handling human excreta for research is itself a complex process. Further, the complex chemical composition of human excreta makes its analysis difficult. In this chapter, I reflect on EcoSan research challenges and triumphs I faced in both the field and lab. I present the perspectives of the research team involved in my third chapter, which coincided with substantial civil unrest in Haiti in late 2022. I also present my perspective on the unique barriers in urine-as-fertilizer research. This work can be of use to other EcoSan researchers, as the barriers I faced are likely common in this field and should be considered with future work.

5.1 EcoSan research challenges in the field

5.1.1 Background and framing

From September to December 2022, Haiti experienced cascading challenges resulting from increased unrest due to gang violence, protests against fuel shortages and rapid inflation, discontent with the interim government put in place after the assassination of President Jovenel Moise in 2021, and the reemergence of cholera (Danticat, 2022). My research partner, Sustainable Organic Integrated Livelihoods (SOIL), operates a container-based sanitation service for low-income households in Cap-Haïtien, Haiti (Preneta et al., 2013). With a mission to create a replicable model, the organization pursues research and innovation with the objectives of operational improvements, increased service inclusivity, and participation in the global conversation on safe sanitation and climate change mitigation. SOIL has a long standing research partnership with the University of California, Merced (UCM) focused on the climate change mitigation potential of SOIL's flagship sanitation service, EcoLakay.

Starting in September 2022, SOIL began to operate under *ijans nivo oranj* (level orange emergency), the third of four emergency levels internal to the organization. Subsequently, they prioritized employee safety and essential waste treatment services, and reduced time spent on research and innovation. This had a significant impact on research outcomes for the third chapter of my dissertation. Each member of the research partnership – UCM leading the research, SOIL managing data collection on the ground, and l'Université Anténor Firmin (UNAF) that provided the interns to execute the project and provide technical agronomic expertise – experienced the *ijans* differently. Some obstacles included inflation rendering transportation prices unsustainable, the inability of the in-

country team to collect data or even leave their homes, and the evacuation of the NGO's non-Haitian project coordinator, Patrick Ward.

Here, I share perspectives from the research team in-country and evacuated NGO staff during the crisis, as well as my own perspective. Together, we sought to answer the question: "What is the value of pursuing research to each stakeholder amidst crises?". This answer is relevant to researchers and implementers involved in Global North-Global South research partnerships in which civil unrest may need to be navigated.

5.1.1.1 Stakeholder study participation

This study was a collaboration between myself and my advisor, Dr. Rebecca Ryals at UCM, Patrick Ward and other staff at SOIL, and the interns from UNAF. Patrick Ward of SOIL managed the team of interns and served as a liaison between the different stakeholders. UNAF provided seven interns in total, four interns for each of the two growing cycles and one intern from the first cycle continuing into the second in a leadership role. The interns for the second growing cycle, who experienced the impact of the ijans on the research project, were Eldine Dacéus, Limane Joachim, Yamouch Bazile, and Frantz Philias (incumbent intern). The UNAF interns managed the in-cycle soil physiochemical and plant growth data collection and analysis. This included soil pH, moisture, temperature, electrical conductivity, soil basal respiration, erosion pin monitoring, and plant growth monitoring. Each experimental plot was watered three times a week in a normal week by the interns. When gas was not available to pump water to the reservoir, the interns had to pull water from the well, increasing the time in the field. Baseline soil sampling was conducted with UNAF interns and help from one SOIL staff member. Soil sampling before the first harvest was led by myself, Patrick Ward from SOIL, and the UNAF interns. Soil sampling before the second harvest was conducted with modified protocols by the UNAF interns only due to the *ijans*.

5.1.1.2 Ijans protocol adaptation

In September 2022, the ability to conduct work was hindered due to the protests and fuel supply chain disruptions. Safety was prioritized above all, and the interns collected data when agreed safe by SOIL staff and themselves. To accomplish this, fixed dates for data collection had to become flexible and the actual data collected was stripped to its most essential components to reduce time in the field. This was particularly true for the time intensive second harvest soil sampling. The *gadyen* (caretaker) of the UNAF plots who lives on the premises was paid to take care of the watering responsibilities in lieu of the interns, decreasing the time the interns would need to spend in the field.

5.1.2 Reflecting on Chapter 3 research in the field

After the completion of intern-led data collection and once the *ijans nivo oranj* was lifted, we wanted to reflect as a team on this varied research process. To do so, we all answered questions related to the project, objectives, research outcomes, and our personal feelings on the matter. The questions were developed by the SOIL staff with my revision. Respondents included myself, Patrick, three others from SOIL staff, and the four interns that were a part of the research team in the second cropping cycle during the ijans. The questions can be found in Appendix D. Bridj Ozeris, a SOIL Research Assistant unaffiliated with this project, coordinated and interviewed each of the interns. Bridj was chosen to conduct the interviews because of his background in agronomy, native proficiency in Haitian Creole, and his lack of prior involvement in the project.

Translations in Haitian Creole, as asked to the interns, can be found in the Supplemental Material. Once answered, the interview questions were qualitatively analyzed for common themes by me and Patrick Ward. Neither of us coded our own answers. As this work was simply a reflection on the research process translated to Haitian Creole for the interns and Haitian SOIL staff, UCM determined that IRB approval was unnecessary.

Each member of the research partnership experienced the ijans differently. Several themes emerged from our collective reflection on the research process, including challenges with communication, transportation, inflation, stress, safety, completing objectives, and increased workload. All team members responded that they felt the research project, and particularly the ijans, taught them something new about working on a research team.

5.1.3 Conducting research on the ground

Communication was a theme in all team members' interview responses. Some interns felt the transition between the two groups of interns could have happened more smoothly. Occasional miscommunication led to some data not being collected. Prior to the ijans, Patrick saw the interns in person at least weekly when they did soil tests and planned to go to the field. Once he was evacuated, he was limited to communication through Whatsapp. Increasing irregularity of cell service in Haiti due to energy shortages caused by the gas crisis, as well as communicating through a language barrier and in different time zones, made communication more difficult. However, the interns felt Patrick did a good job communicating, especially effectively explaining revised tasks, during the ijans.

SOIL operational staff were concerned with securing and implementing transportation for the interns during the ijans. During normal operations, the interns would sometimes benefit from free transportation on SOIL vehicles if their destination was on the normal route. However, during the ijans, SOIL preferred that the interns use motorcycle taxis instead because of their ability to navigate through or away from unforeseen blockades and protests. Although the interns were provided with a stipend accounting for the cost of transportation, they would have preferred to travel by SOIL vehicles at no cost because of the rapid inflation. However, the second cycle harvest required the use of a SOIL vehicle in which extra care and planning had to be implemented. The ijans caused inflation, and thus transportation costs were higher and made intern transportation on their monthly stipend impossible. Their monthly stipend was increased on two separate occasions to accommodate this rapid inflation. This had to be approved by UCM, but also by the SOIL staff so as not to create unsustainable precedents in intern payment schemes, while ensuring their needs were being met.

A theme of stress related to completing the work emerged from the interviews. Interns mentioned the protests affecting them psychologically. There was a sense of fear of not being safe in the field when completing the work, and stress of not being able to go on days when field work was deemed unsafe. Multiple interns mentioned their families' fear at them leaving the house for work. SOIL staff emphasized the stress the ijans caused in project management. Additional time had to be spent organizing new protocols and prioritizing certain tasks. The evacuation of international SOIL staff in mid-September 2022 added an additional level of stress to the project. Around the time the sorghum grain began to emerge during the second cycle, the ijans period began and life in Cap-Haïtien was increasingly unsafe. The was a source of strife, as the grain was eaten by birds in the

first cycle, and thus we were unable to collect yield data during the first cycle. We ordered sorghum head bags to protect the grain from birds but were unable to deliver them to the field in time during the second cycle. This yield loss was a source of stress for all involved, and the interns were intent on preventing further yield loss, though ultimately yield data was not collected for the second cycle either.

The ijans caused us to amend protocols to prioritize the safety of the interns working in the field. Patrick and I worked to amend the protocols before he was evacuated in September 2022. The interns were generally supportive of the new protocols, with two saying they would have done the same if they were in charge. Another said if he had been in charge, he would have amended the safety protocols slightly, such as choosing to go out on different days or taking different routes to the field. Roadblocks and protests often prevented the interns from traveling on their usual route to the field. There were increased safety concerns around the time of the second harvest, which was a source of stress to all involved. The gayden, who lived on site, was instrumental in operating safely during the ijans. He often watered or weeded when interns were not able to safely travel to the field. Everyone was grateful for the role the gayden played in the project. Many of the interns mentioned the workload surrounding the harvest, and how they felt rushed in the field completing the necessary tasks. From the SOIL and UCM side, workload was increased, because protocols had to be rapidly changed.

Most research team members mentioned that the project taught them about working on a team. A theme of persistence through times of adversity was common in interns' responses, and that working during a crisis is typical in Haiti. SOIL staff mentioned the research project was important for framing SOIL's work in terms of climate change mitigation and adaptation, which in the future can lead to carbon financing, ultimately allowing SOIL to expand their sanitation service provision.

5.1.4 Conducting research from afar

As this project was a part of my dissertation, my focus was on managing the conduct of rigorous scientific research that resulted in publishable data. The start of the project was delayed nearly two years due to the COVID-19 pandemic and security concerns resulting from the assassination of president Jovenel Moise. Thus, the study goals and design were modified many times prior to the study start to accommodate changing plans. My main role in the project was project design, protocol development, soil processing, data analysis and interpretation, and manuscript preparation. The main tension that the *ijans* created for me was the potential for data loss, and the fear that completing my research objectives could create an unsafe environment for others on our research team. Regular updates between UCM, SOIL, and UNAF were necessary to triage new data collection protocols and identify which datasets to prioritize over others. For example, labor intensive soil aggregate stability measurements and soil bulk density were abandoned in the second cycle to allow for the interns to only spend one day in the field during the final harvest. In-cycle monitoring also happened less frequently in the second cropping cycle. Some replication was lost in the second cycle harvest data collection to reduce time spent in the field, as triplicate soil samples from each plot were composited in-field. While these changes in data collection have shaped the quality of the final data, none of this data collection or project monitoring would have been possible without the direction of SOIL and UNAF. From my perspective, this ultimately points to the necessity of strong relationships with local partners to do agronomic research in fragile contexts. Furthermore, flexibility and regular communication led to a stronger partnership overall.

Another consideration about this chapter was how my involvement contributed to neocolonial narratives and power structures. I traveled to Haiti once during the project, though the total sorghum growth time was approximately eight months and total project implementation a year. While I had good communication with SOIL staff and through them, the interns, I was ultimately getting most of my information about Haitian agroecosystems from the sparse peer-reviewed literature on the subject. This means that my ideas about what were meaningful research questions in this context might not have aligned with the reality. For example, in reflecting on the research project, one of the interns from the second cycle said that they "learned sorghum can be grown in northern Haiti." We chose this crop in the first cycle, under the assumption that sorghum is an important staple crop for Haitian agriculture. While this might be true in other parts of the country, it was apparently not the most relevant for the Cap-Haïtien area. Our assumptions ran contrary to local advice in other moments, such as when the interns recommended that we let the soil "rest" between cycles, and technical assistants from the Brasserie Nationale d'Haïti advised against applying the same amendments in the same plots consecutively. This points to a larger conversation about inequalities inherent to North-South academic collaboration (Tilley and Kalina, 2021).

This research also evoked questions about the permanence of climate mitigation research in the Global South. Prior to the *ijans*, we worked with SOIL to plan for the hiring of a full-time agronomist to support further cropping cycles of the project. The plan was to sample soils from the experiment for years to come to assess the long term effect of repeated feces-derived compost application on soil C stocks, which is an important missing piece of the research at SOIL to assess the full climate change mitigation potential of their operations. However, the *ijans* precluded this possibility, and the project was ultimately stopped. This led me to reflect on the potential for soil C sequestration research in fragile sociopolitical contexts. Soil C sequestration is an inherently slow process, and there is significant dispute between scientists and policymakers about the permanence of newly sequestered soil C (Dynarski, Bossio and Scow, 2020). It also requires monitoring in the long term to verify its impact (Smith et al., 2020)—ideally including soil greenhouse gas emissions which were logistically difficult to sample in this project. Building strong and lasting Global North- South partnerships, with consistent funding and engagement from project stakeholders, is ultimately necessary to fully account for soil C sequestration projects in the Global South. However, while this is difficult, effort should be made towards the decolonization of ecological research (Baker, Eichhorn and Griffiths, 2019), which may also bring about improved opportunities for accurately accounting for soil C sequestration.

5.2 EcoSan research challenges in the lab

5.2.1 Collecting and analyzing human urine

Urine nutrient recovery research in the lab required the collection of human urine. Since our lab operates in a building with typical Western-style flush toilets with no source-separation, all urine had to be collected from volunteers in specimen cups. This required Institutional Review Board and Biological Use Authorization approval from UCM, which took a significant amount of time. One of the requisites of IRB approval was that the origin of each urine sample remained anonymous, and that urine samples from multiple donors be collated. However, a urine sample from a dehydrated individual could skew the N content higher of the aggregated urine sample. This made it difficult to conduct studies in the lab based on a predicted initial N content from multiple batches of aggregated

urine, as the N content in the aggregated urine samples could range from 3000 mg L^{-1} to 7000 mg L^{-1}. The chemical composition of urine is based on human diet, hydration, and environmental exposure (Jönsson et al., 2004). The N and P content also varies widely based on whether a diet is plant-based or animal-based (Jönsson et al., 2004; Rose et al., 2015). Ultimately, this points to the need for better on-site analytical techniques for N, P, K, or other plant-essential micronutrients at urine-to-fertilizer operations, to better predict the chemical content if urine use as a fertilizer is to become more widespread.

5.2.2 Producing and analyzing urine-enriched biochar

I initially became interested in urine-enriched biochar as a potentially inexpensive, low-technology solution to urine nutrient recovery in source-separated sanitation systems that could also sequester C when soil applied. After reviewing relevant literature and beginning work in the lab, a few questions emerged: How much urine should be mixed with how much biochar? Did the type of biochar matter? Many studies used fresh urine, completely urea-hydrolyzed urine, or something in between. Did the nitrogenous species in the urine matter? Mixing two naturally occurring substances together quickly became complex. I spent a semester trying to develop urine-nitrogen (N) biochar adsorption isotherms to determine if urine-N sorbed in a predictable manner to biochar. In my work, the adsorption was unpredictable. This is likely due in part to the complex chemical matrix of urine (Tettenborn, Behrendt and Otterpohl, 2007). While urine contains plant-essential N as urea, ammonium, creatine and nitrate, and phosphorus (P) and potassium (K), it also contains various salts and a diversity of metabolites (Bouatra et al., 2013; Rose et al., 2015). Research has demonstrated the competitive sorption of metabolites and pharmaceuticals for biochar surfaces (Sun et al., 2018; Solanki and Boyer, 2019; Masrura et al., 2022). This may in part explain the unpredictable sorption behavior of urea-N in fresh urine to biochar surfaces I observed. It is also likely that NH_4^+ sorption in urea-hydrolyzed urine was reduced by competition with other cations such as Ca^{2+}, Mg^{2+} or K^+ (Fidel, Laird and Spokas, 2018). I ultimately decided to choose a biochar-to-urine mixing ratio based on this preliminary work and performed the urine-enriched biochar greenhouse experiment presented in Chapter 1, to study plant growth with urine-enriched biochar made from multiple biochar types and with urine under various storage conditions. However, adsorption isotherms should be further pursued with urine-enriched biochar research. Ultimately, if urine-enriched biochar or any urine-nutrient adsorbed amendment is to be scaled and marketed as a fertilizer, some prediction of the N, P, and K content is necessary.

5.3 Conclusion

In conclusion, I hope that this insight on my dissertation research provides valuable information to other EcoSan researchers. I shared a reflection on the challenges in conducting EcoSan research in the field due to the civil unrest in Haiti in late 2022. In this reflection, themes of communication challenges, transportation issues, inflation, stress, and workload emerged, but ultimately the resilience of the research team was underscored. Further, the laboratory component of EcoSan research I conducted focused on urine-nutrient recovery, which faced challenges in IRB approval, variability in urine composition, and the complex sorption behavior of urine components to biochar. In reflecting on my research, I emphasize the importance of adapting to crisis situations, and promoting stronger partnerships for Global North-Global South ecological research. I also highlight the need for improved analytical techniques and predictable sorption behavior when using urine as a fertilizer.

5.4 References

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Chapter 6.
Conclusion

This dissertation demonstrates the potential of Ecological Sanitation (EcoSan) fertilizers, or novel fertilizers derived from human excreta, to provide plant essential nutrients to enhance crop production. We also show the capacity of EcoSan fertilizers, particularly feces-derived compost produced from thermophilic co-composting, to enhance key soil health indicators. In Chapter 2, we show that urine-enriched biochar made from three biochar types (wood, walnut shell, and sewage sludge) combined with three urine storage conditions (fresh, stored, and simulated container-based sanitation) exhibit different urine-nitrogen (N) adsorption, desorption, and plant uptake behavior. In this chapter, we also show the preferential sorption of urine-N to biochar particles < 500-μm. In our EcoSan fertilizer-amended soil incubation in Chapter 3, we show that human excreta-derived fertilizers are an effective source of plant available N and phosphorus (P) over a simulated 90-day cropping period. We show that urine-derived fertilizers provide more plant-available N while feces-derived composts provide more plant-available P. In this chapter, we also observed substantial mining of native soil-P with feces-derived compost application. In Chapter 4, we demonstrate that various feces-derived compost application rates increase bioavailable soil nutrients after the second application, which has implications for building soil health in the long term. We recommend longer agronomic studies to fully understand the potential of feces-derived compost application to build both surface and subsurface soil carbon (C). Our reflection on EcoSan research methods in Chapter 5 revealed important insights, including the resilience of a multi-stakeholder research partnership based in northern Haiti, and the logistical and analytical challenges unique to urine nutrient recovery research.

Further EcoSan research should address knowledge gaps revealed by this dissertation. To scale the production of novel fertilizers such as urine-enriched biochar, it is essential to develop adsorption isotherms to model urine-nutrient adsorption onto biochar to predict their nutrient content. Further, N isotope dilution or tracer data could elucidate plant uptake dynamics of urine-N and urine-enriched biochar-N. Urine-enriched biochar preparation methods should also be studied, to optimize the recovery of urine-nutrients, while making the product easy and safe to handle. While the potential of feces-derived compost application to sequester soil C is an important piece of the climate change mitigation potential of container-based sanitation EcoSan services, longer studies are necessary to fully account for change in soil C stocks. Another gap this dissertation work introduced is the lack of a research focus on recycling human excreta-derived potassium.

APPENDICES

Appendix A: Supplemental Information for Chapter 2

Figure S2-1. CBS-style container used in the experiment. The top was cut from a 1-gallon (3.78 L) container based on standard practices at SOIL.

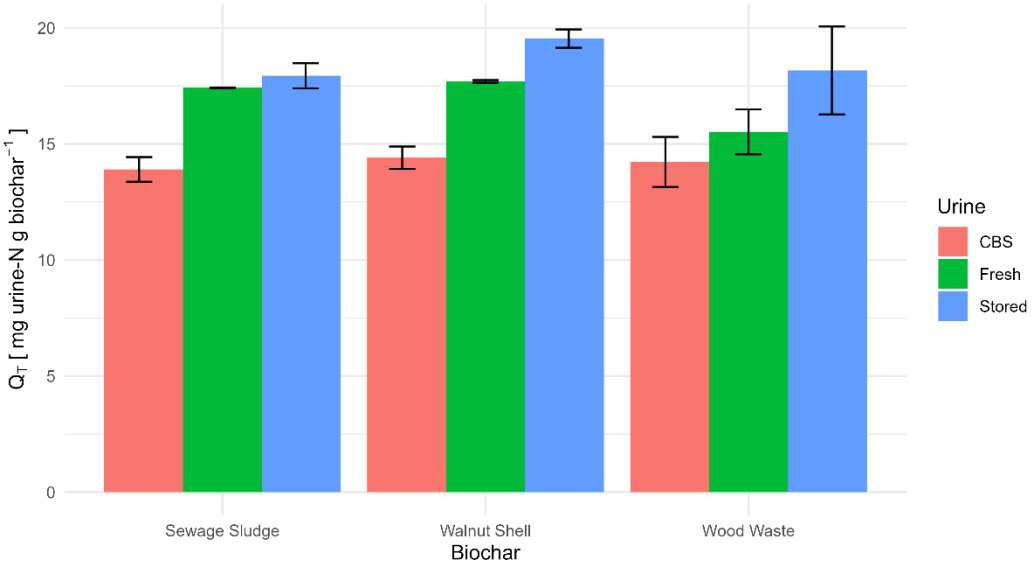

Figure S2-2. Total sorption capacity (Q_T) of each UEBC. Error bars represent the average value ± one standard error.

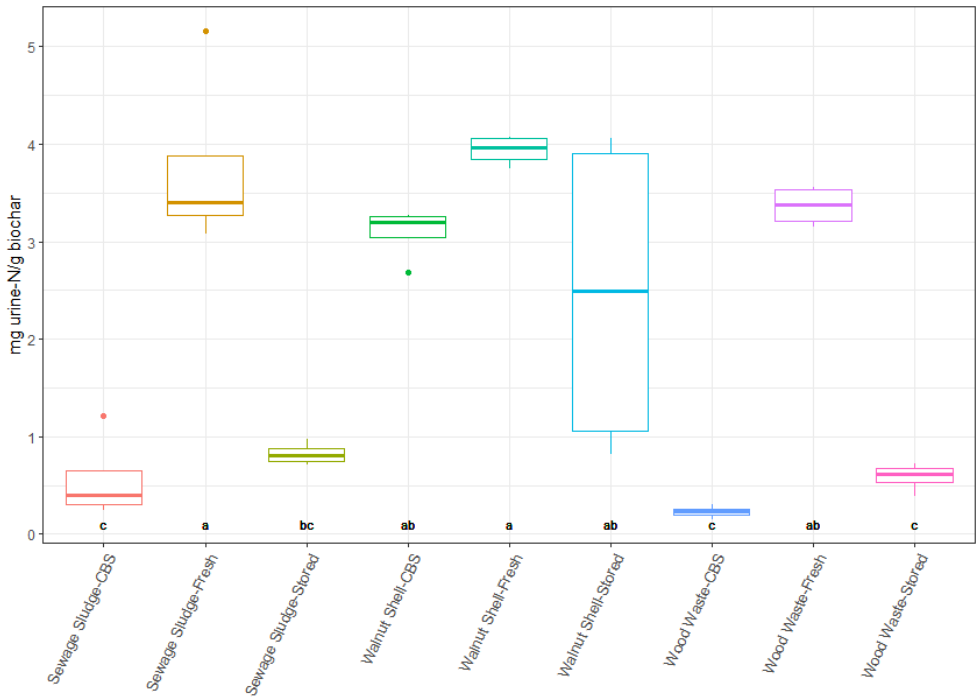

Figure S2-3. Increase in N content of enriched biochar relative to unenriched biochar (n = 4). Letters below boxes indicate significant difference between means at $p < 0.05$ (one-way Kruskall-Wallis test with Bonferroni correction). If treatments share at least one letter they do not differ significantly.

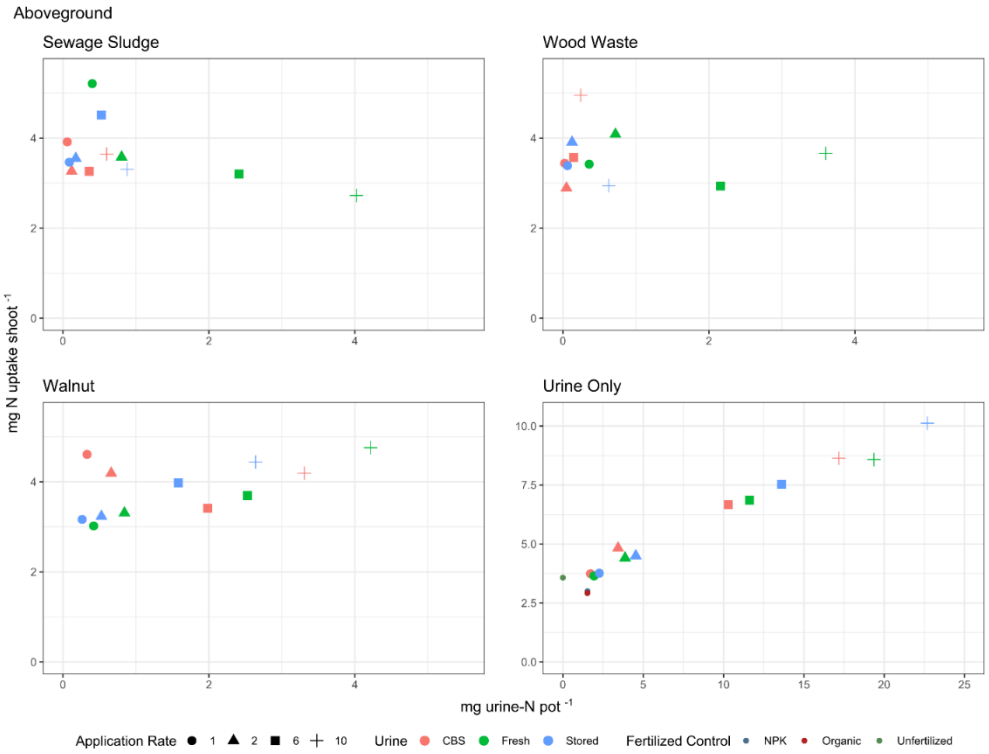

Figure S2-4. Nitrogen uptake in aboveground plant tissue compared to urine-N applied with UEBCs or urine-only. Fertilized and unfertilized controls are included in the "urine-only" panel for purposes of comparison.

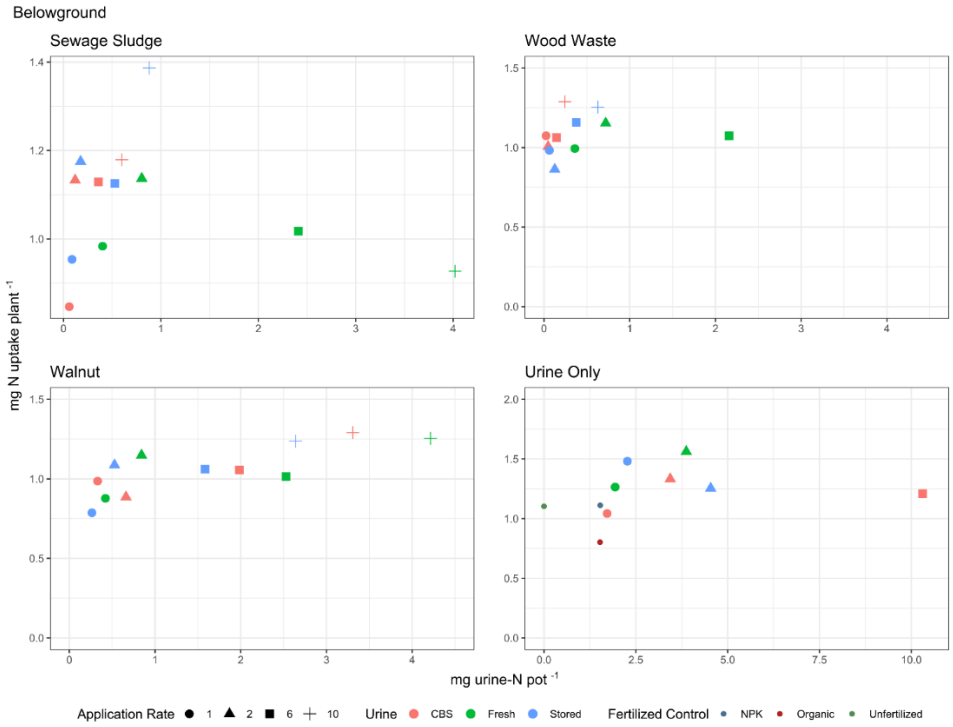

Figure S2-5. Nitrogen uptake in belowground plant tissue compared to urine-N applied with UEBCs or urine-only. Fertilized and unfertilized controls are included in the "urine-only" panel for purposes of comparison.

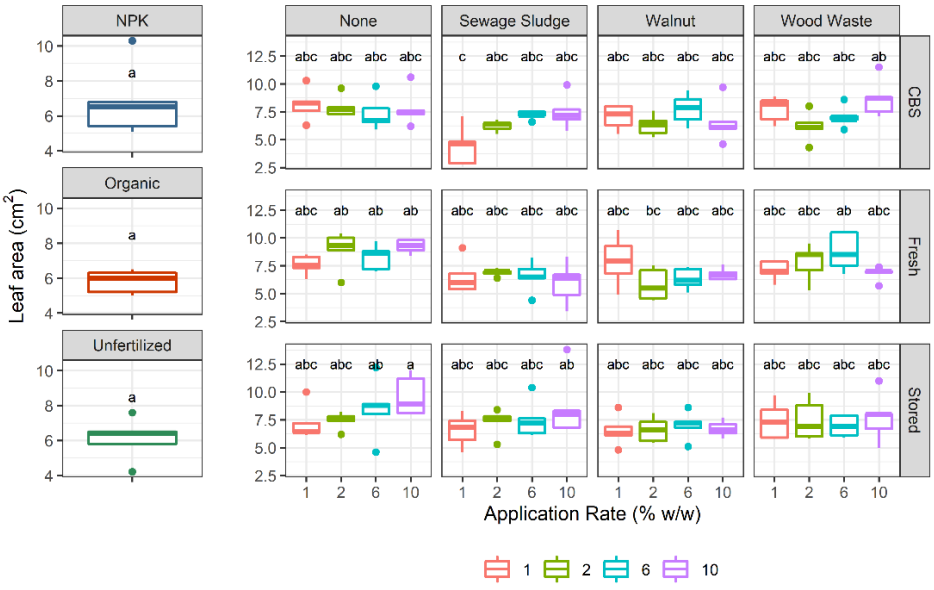

Figure S2-6. Leaf area of leaves > 1 cm. Letters indicate significant differences between means from a three-way ANOVA with leaf area of leaves > 1 cm as a response and biochar type, urine type, and application rate as factors ($p < 0.05$). If treatments share at least one letter they do not differ significantly.

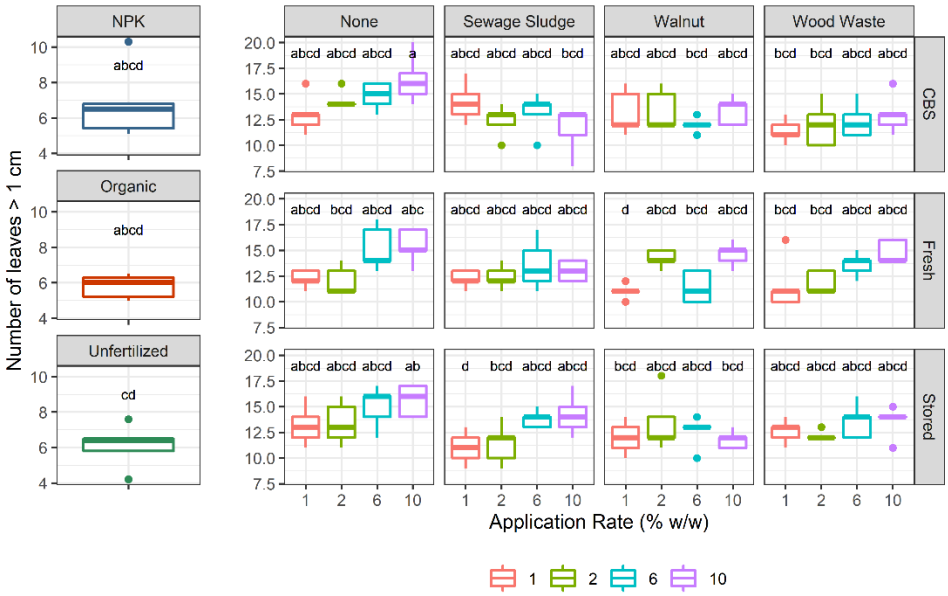

Figure S2-7. Number of leaves > 1 cm. Letters indicate significant differences between means from a three-way ANOVA with number of leaves > 1 cm as a response and biochar type, urine type, and application rate as factors ($p < 0.05$). If treatments share at least one letter they do not differ significantly.

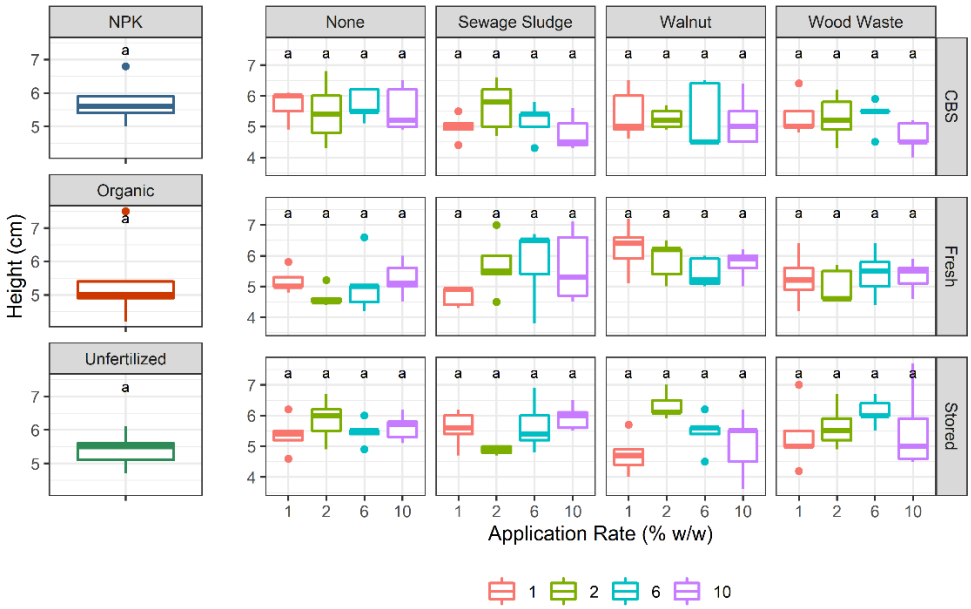

Figure S2-8. Plant height. Letters indicate significant differences between means from a three-way ANOVA with height as a response and biochar type, urine type, and application rate as factors ($p < 0.05$). If treatments share at least one letter they do not differ significantly.

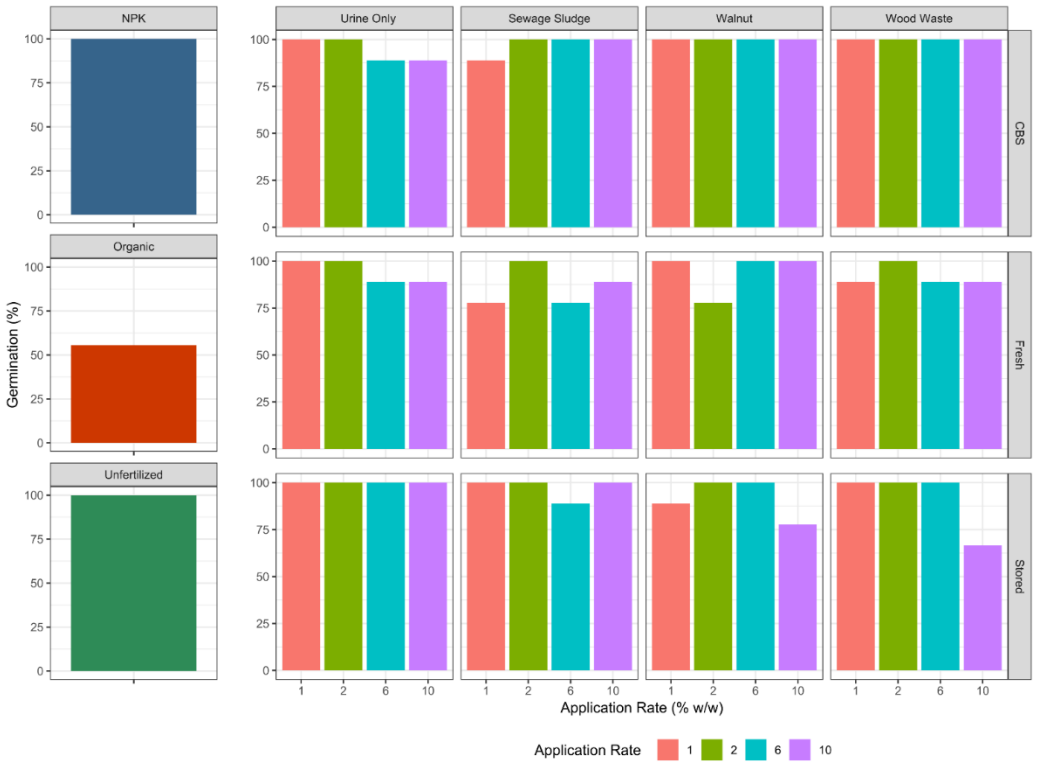

Figure S2-9. Germination percentage. A general linear model found no significance differences for germination percentage between treatments ($p < 0.05$).

Table S2-1: **One-way ANOVA with total biomass as a response variable and urine as a predictor variable.** Fertilized and unfertilized controls were compared to urine type in the model. Tukey's honestly significant difference (Tukey HSD) post hoc test results are included. Letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly. *ns* indicates the treatment does not differ significantly from any other treatment.

Total Biomass ~ Urine F = 0.882 p = 0.494		
Urine	**Total biomass (g)**	**Tukey HSD**
Fresh	0.262 ± 0.0455	*ns*
Stored	0.27 ± 0.0491	*ns*
CBS	0.26 ± 0.0439	*ns*

NPK	0.275 ± 0.0245	*ns*
Organic	0.233 ± 0.0253	*ns*
Unfertilized	0.251 ± 0.0327	*ns*

Table S2-2: **One-way ANOVA with total biomass as a response variable and biochar as a predictor variable.** Fertilized and unfertilized controls and Urine Only were compared to biochar in the model. Tukey's honestly significant difference (Tukey HSD) post hoc test results are included. Letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly. *ns* indicates the treatment does not differ significantly from any other treatment.

Total Biomass ~ Biochar
F = 3.235

p = 0.00445

Biochar	**Total Biomass (g)**	**Tukey HSD**
Sewage Sludge	0.261 ± 0.0455	*ns*
Wood Waste	0.27 ± 0.0434	*ns*
Walnut Shell	0.248 ± 0.0461	**b**
Urine Only (No Biochar)	0.278 ± 0.0454	**a**
NPK	0.275 ± 0.0245	*ns*
Organic	0.233 ± 0.0253	**b**
Unfertilized	0.251 ± 0.0327	*ns*

Table S2-3: **One-way ANOVA with total biomass as a response variable and application rate as a predictor variable.** Fertilized and unfertilized controls were compared to application rate in the model. Tukey's honestly significant difference (Tukey HSD) post hoc test results are included. Letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly. *ns* indicates the treatment does not differ significantly from any other treatment.

Total Biomass ~ Application Rate
F = 1.425

p = 0.205

Application Rate (%)	Total biomass (g)	Tukey HSD
1	0.254 ± 0.0375	*ns*
2	0.273 ± 0.0402	*ns*
6	0.265 ± 0.0476	*ns*
10	0.265 ± 0.0562	*ns*
NPK	0.275 ± 0.0245	*ns*
Organic	0.233 ± 0.0253	*ns*
Unfertilized	0.251 ± 0.0327	*ns*

Table S2-4: Two-sample Wilcoxon Rank Sum test using "biochar/no biochar" as grouping terms. Stars show that the two groups differ significantly at $p < 0.01$.

Total Biomass ~ Biochar/No Biochar p = 0.00397	
Group	**Total Biomass (g)**
UEBC	0.259 0.0447
Urine	0.278 0.0454 **

Table S2-5: **Two-way ANOVA with total biomass as a response variable and biochar and urine as predictor variables.** Fertilized and unfertilized were compared to urine and biochar combinations in the model. Tukey's honestly significant difference (Tukey HSD) post hoc test results are included. Letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly. *ns* indicates the treatment does not differ significantly from any other treatment.

Total Biomass ~ Biochar*Urine F = 2.163 p = 0.00974			
Urine	**Biochar**	**Total biomass (g)**	**Tukey HSD**
Fresh	Wood Waste	0.275 ± 0.0465	*ns*

Fresh	Sewage Sludge	0.258 ± 0.0534	*ns*
Fresh	Walnut Shell	0.241 ± 0.0357	**b**
Stored	Wood Waste	0.263 ± 0.0396	*ns*
Stored	Sewage Sludge	0.279 ± 0.0424	*ns*
Stored	Walnut Shell	0.246 ± 0.0594	**b**
CBS	Wood Waste	0.271 ± 0.0452	*ns*
CBS	Sewage Sludge	0.246 ± 0.035	*ns*
CBS	Walnut Shell	0.256 ± 0.0408	*ns*
Fresh	No Biochar	0.274 ± 0.0392	*ns*
Stored	No Biochar	0.292 ± 0.043	**a**
CBS	No Biochar	0.268 ± 0.0516	*ns*
NPK		0.275 ± 0.0245	*ns*
Organic		0.233 ± 0.0253	**b**
Unfertilized		0.251 ± 0.0327	*ns*

Table S2-6: **Two-way ANOVA with total biomass as a response variable and biochar and application rate as predictor variables.** Fertilized and unfertilized controls were compared to biochar/no biochar and application rate combinations in the model. Tukey's honestly significant difference (Tukey HSD) post hoc test results are included. Letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly. *ns* indicates the treatment does not differ significantly from any other treatment.

Total Biomass ~ Biochar*Application Rate F = 2.336 p = 0.0021			
Biochar	**Application Rate (%)**	**Total Biomass (g)**	**Tukey HSD**
Sewage Sludge	1	0.245 ± 0.0362	*ns*
Sewage Sludge	2	0.287 ± 0.0413	*ns*
Sewage Sludge	6	0.27 ± 0.0396	*ns*

Sewage Sludge	10	0.242 ± 0.0518	*ns*
Wood Waste	1	0.265 ± 0.0315	*ns*
Wood Waste	2	0.257 ± 0.033	*ns*
Wood Waste	6	0.271 ± 0.032	*ns*
Wood Waste	10	0.286 ± 0.0662	*ns*
Walnut Shell	1	0.231 ± 0.036	**b**
Walnut Shell	2	0.265 ± 0.0396	*ns*
Walnut Shell	6	0.251 ± 0.054	*ns*
Walnut Shell	10	0.244 ± 0.0501	*ns*
Urine Only	1	0.274 ± 0.0338	*ns*
Urine Only	2	0.285 ± 0.042	*ns*
Urine Only	6	0.266 ± 0.0614	*ns*
Urine Only	10	0.288 ± 0.0406	**a**
NPK		0.275 ± 0.0245	*ns*
Organic		0.233 ± 0.0253	*ns*
Unfertilized		0.251 ± 0.0327	*ns*

Table S2-7: **Two-way ANOVA with total biomass as a response variable and urine and application rate as predictor variables.** Fertilized and unfertilized controls were compared to urine type and application rate in the model. Tukey's honestly significant difference (Tukey HSD) post hoc test results are included. Letters indicate significant differences between means ($p < 0.05$). If treatments share at least one letter they do not differ significantly**.** *ns* indicates the treatment does not differ significantly from any other treatment.

Total Biomass ~ Urine*Application Rate F = 1.202 p = 0.275			
Urine	**Application Rate (%)**	**Total biomass (g)**	**Tukey HSD**

Fresh	1	0.253 ± 0.0347	*ns*
Fresh	2	0.282 ± 0.0379	*ns*
Fresh	6	0.254 ± 0.0516	*ns*
Fresh	10	0.26 ± 0.052	*ns*
Stored	1	0.252 ± 0.0462	*ns*
Stored	2	0.274 ± 0.0357	*ns*
Stored	6	0.284 ± 0.042	*ns*
Stored	10	0.269 ± 0.0652	*ns*
CBS	1	0.256 ± 0.0319	*ns*
CBS	2	0.264 ± 0.0461	*ns*
CBS	6	0.256 ± 0.0445	*ns*
CBS	10	0.266 ± 0.0529	*ns*
NPK		0.275 ± 0.0245	*ns*
Organic		0.233 ± 0.0253	*ns*
Unfertilized		0.251 ± 0.0327	*ns*

Appendix B: Supplemental Information for Chapter 3

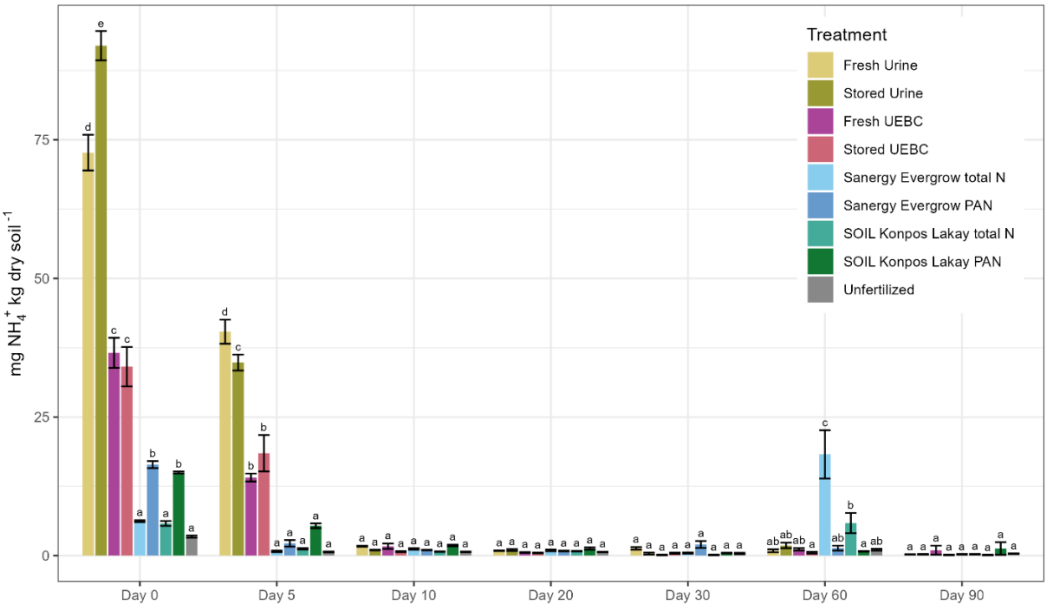

Figure S3-1: Extracted NH_4^+ of human excreta-derived fertilizer amended soils over the course of a 90 day aerobic incubation. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly. Error bars represent ± one standard deviation (n = 3).

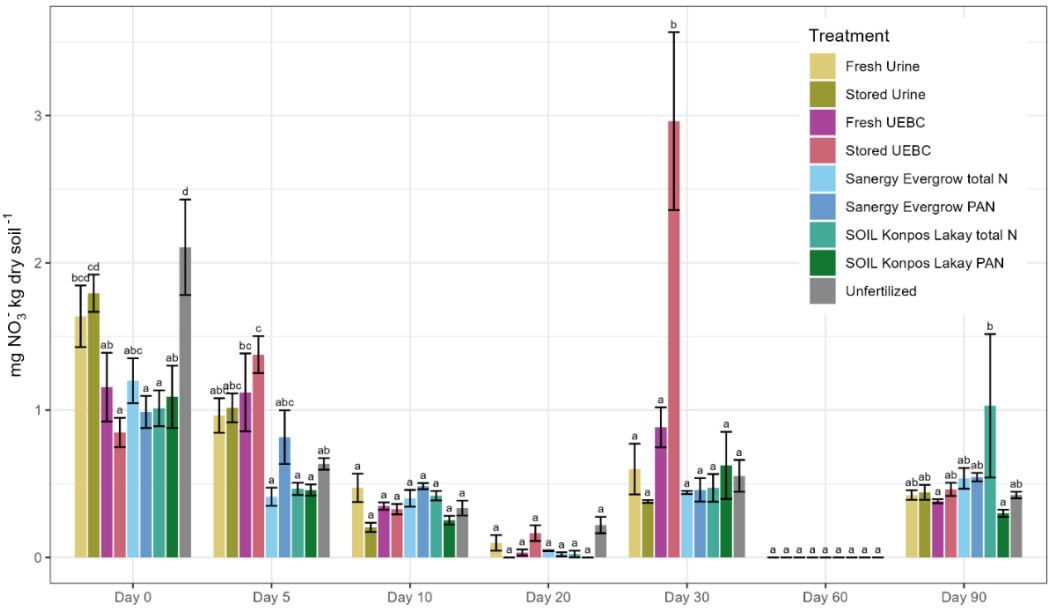

Figure S3-2: Extracted NO_3^- of human excreta-derived fertilizer amended soils over the course of a 90 day aerobic incubation. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If

treatments share a letter, they do not differ significantly. Error bars represent ± one standard deviation (n = 3).

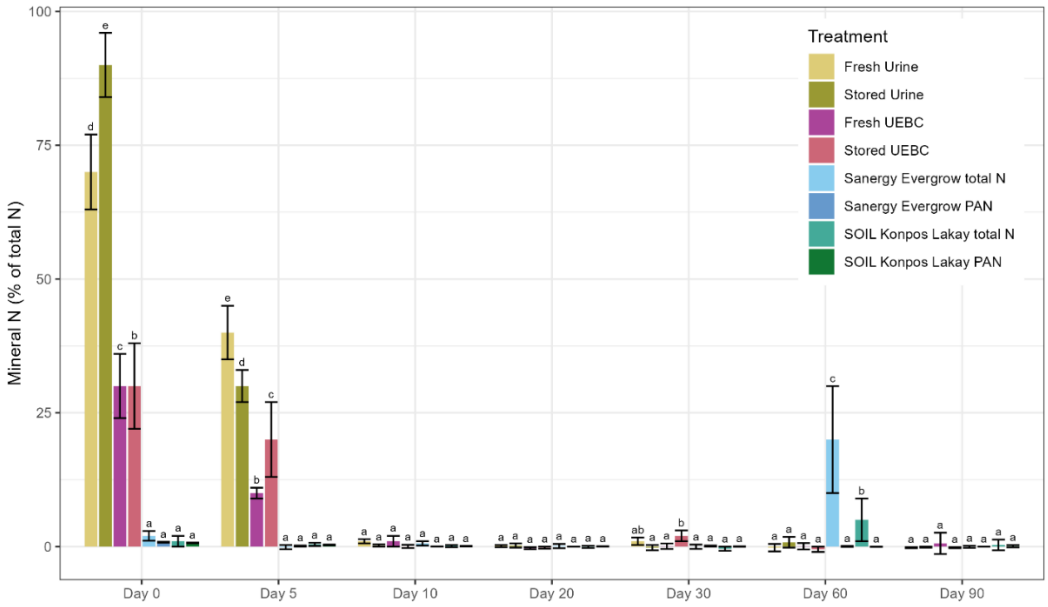

Figure S3-3: $N_{min}t$, or the percentage of N mineralized from the amendment correcting for N mineralization in the unfertilized control, relative to the amount of N applied. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly. These results are expressed in Table S3-2.

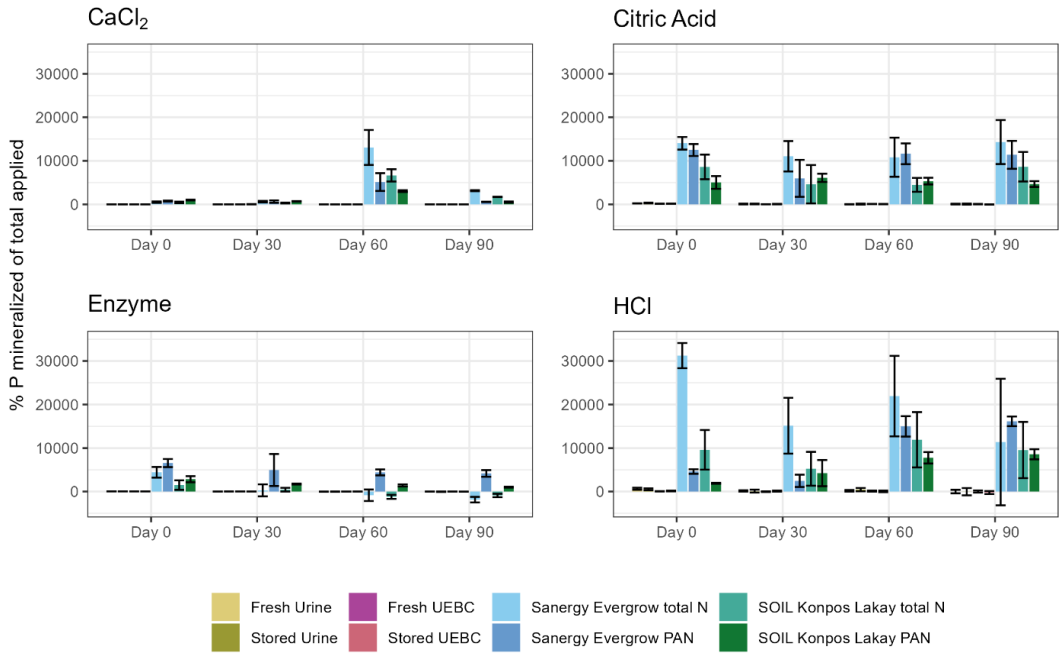

Figure S3-4: $P_{min}t$, or the percentage of P mineralized from the amendment correcting for P mineralization in the unfertilized control, relative to the amount of P applied. These results, with significant differences, are expressed in Table S3-4.

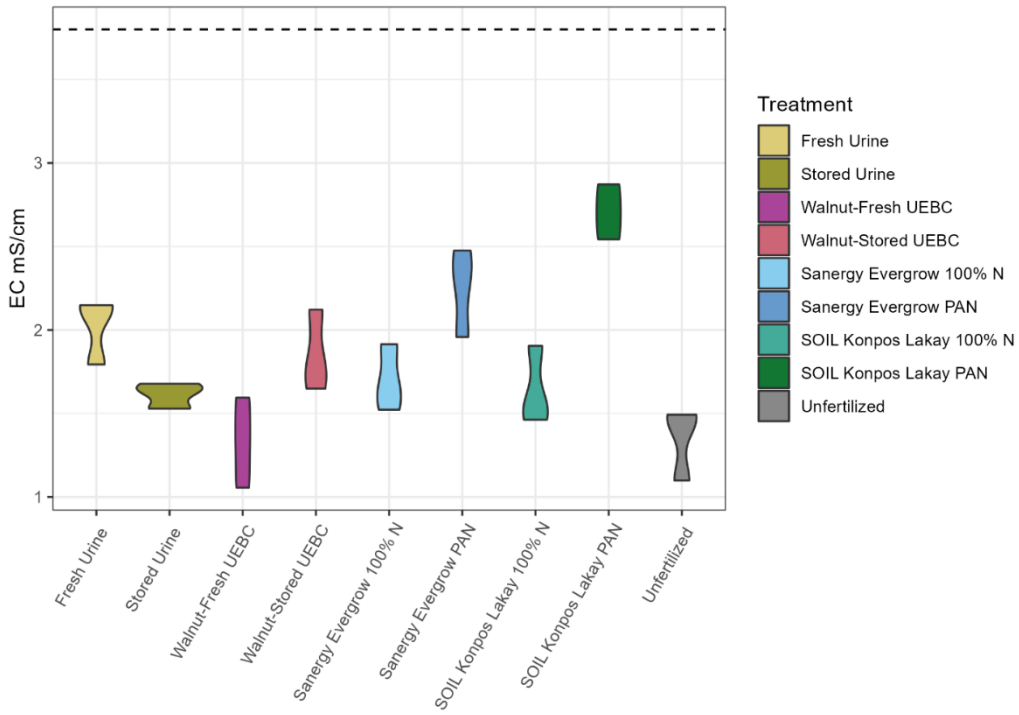

Figure S3-5: Electrical conductivity of soils after incubation (n = 3). The dashed line shows the initial, unamended soil EC (3.8 mS cm^{-1}). Treatments differed significantly after incubation (Kruskal-Wallis test, $p = 0.006$, $\alpha = 0.05$). Post hoc Dunn's test showed that Fresh-UEBC ($p = 0.034$) and the Unfertilized control ($p = 0.025$) were significantly lower than Soil Konpos Lakay PAN at $\alpha = 0.05$.

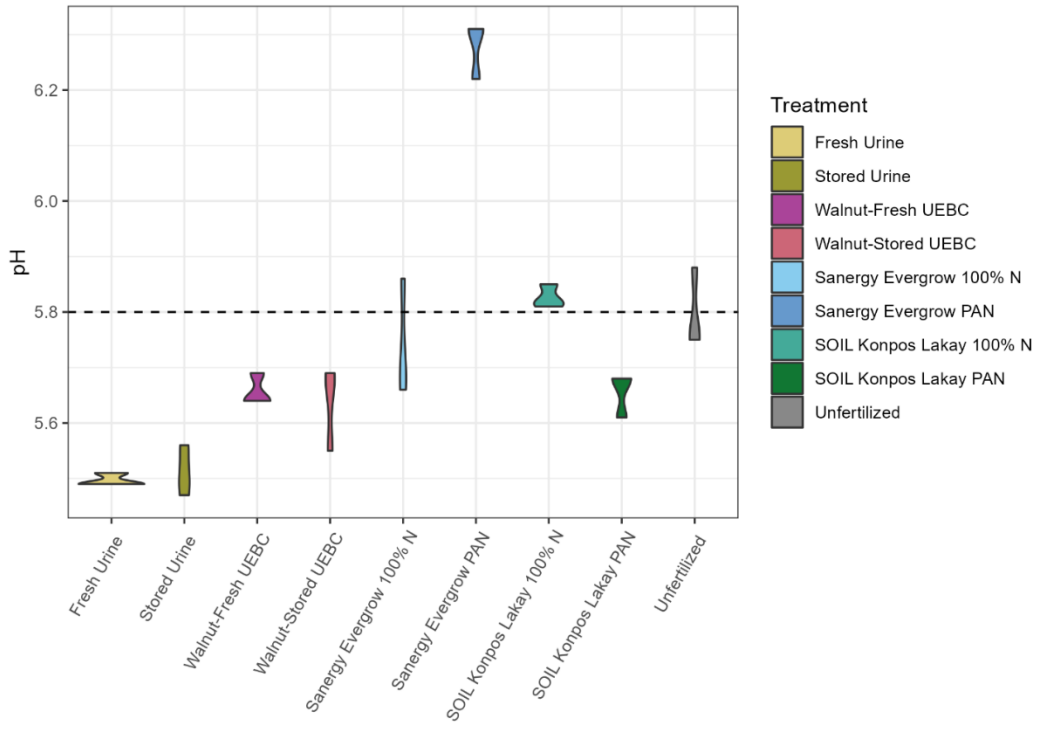

Figure S3-6: pH of soils after incubation (n = 3). The dashed line shows the initial, unamended soil pH (5.8). Treatments differed significantly after incubation (Kruskal-Wallis test, $p = 0.004$, $\alpha = 0.05$). Post hoc Dunn's test showed that Fresh Urine ($p = 0.015$) and Stored Urine ($p = 0.026$) were significantly lower than Sanergy Evergrow PAN at $\alpha = 0.05$.

Table S3-1: Extracted mineral N by treatment and date. Extracted nitrate and ammonium are also shown. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly.

	NO_3^-					**NH_4^+**				**mineral N**			
Treatment	Day	mg NO_3^--N kg dry soil $^{-1}$	sd	kg NO_3^--N ha-1	HSD	mg NH_4^+-N kg dry soil $^{-1}$	sd	kg NH_4^+-N ha $^{-1}$	HSD	mg min-N g dry soil $^{-1}$	Sd	kg min-N ha $^{-1}$	HSD
Fresh Urine	0	1.64	0.512	3.28	**bcd**	72.7	7.89	145.4	**d**	74.3	7.39	148.6	**d**

Stored Urine	0	1.79	0.309	3.58	**cd**	92	6.45	184	**e**	93.8	6.61	187.6	**e**
Fresh UEBC	0	1.16	0.572	2.32	**ab**	36.6	6.63	73.2	**c**	37.7	6.64	75.4	**c**
Stored UEBC	0	0.85	0.244	1.7	a	34.1	8.7	68.2	**c**	34.9	8.74	69.8	**c**
Sanergy Evergrow total N	0	1.2	0.372	2.4	**abc**	6.2	0.45	12.4	a	7.4	0.41	14.8	a
Sanergy Evergrow PAN	0	0.99	0.266	1.98	a	16.4	1.55	32.8	**b**	17.4	1.52	34.8	**b**
SOIL Konpos Lakay total N	0	1.01	0.298	2.02	a	5.8	1.09	11.6	a	6.8	0.91	13.6	a
SOIL Konpos Lakay PAN	0	1.09	0.518	2.18	**ab**	15	0.43	30	**b**	16.1	0.21	32.2	**b**
Unfertilized	0	2.11	0.795	4.22	**d**	3.4	0.5	6.8	a	5.5	0.94	11	a
Fresh Urine	5	0.96	0.287	1.92	**abc**	40.4	5.34	80.8	**d**	41.4	5.33	82.8	**d**
Stored Urine	5	1.02	0.242	2.04	**abc**	34.8	3.52	69.6	**c**	35.8	3.65	71.6	**c**
Fresh UEBC	5	1.12	0.648	2.24	**bc**	14.1	1.76	28.2	**b**	15.2	1.51	30.4	**b**
Stored UEBC	5	1.38	0.307	2.76	**c**	18.5	8.05	37	**b**	19.8	7.98	39.6	**b**

Sanergy Evergrow total N	5	0.41	0.151	0.82	a	0.8	0.35	1.6	a	1.2	0.36	2.4	a
Sanergy Evergrow PAN	5	0.82	0.447	1.64	**abc**	2.2	1.45	4.4	a	3	1.47	6	a
SOIL Konpos Lakay total N	5	0.47	0.101	0.94	a	1.2	0.28	2.4	a	1.7	0.3	3.4	a
SOIL Konpos Lakay PAN	5	0.46	0.093	0.92	a	5.4	1.07	10.8	a	5.8	1.03	11.6	a
Unfertilized	5	0.63	0.096	1.26	**ab**	0.6	0.23	1.2	a	1.3	0.15	2.6	a
Fresh Urine	10	0.47	0.235	0.94	a	1.7	0.25	3.4	a	2.2	0.44	4.4	a
Stored Urine	10	0.2	0.078	0.4	a	1	0.13	2	a	1.2	0.18	2.4	a
Fresh UEBC	10	0.35	0.06	0.7	a	1.7	1.19	3.4	a	2	1.21	4	a
Stored UEBC	10	0.33	0.086	0.66	a	0.7	0.25	1.4	a	1	0.29	2	a
Sanergy Evergrow total N	10	0.4	0.14	0.8	a	1.2	0.29	2.4	a	1.6	0.41	3.2	a
Sanergy Evergrow PAN	10	0.48	0.05	0.96	a	1	0.11	2	a	1.5	0.13	3	a
SOIL Konpos Lakay total N	10	0.42	0.078	0.84	a	0.7	0.13	1.4	a	1.1	0.2	2.2	a

SOIL Konpos Lakay PAN	10	0.25	0.072	0.5	a	1.8	0.37	3.6	a	2.1	0.35	4.2	a
Unfertilized	10	0.34	0.125	0.68	a	0.7	0.18	1.4	a	1	0.16	2	a
Fresh Urine	20	0.1	0.13	0.2	a	0.9	0.14	1.8	a	1	0.21	2	a
Stored Urine	20	0	0	0	a	1	0.4	2	a	1	0.4	2	a
Fresh UEBC	20	0.03	0.052	0.06	a	0.6	0.21	1.2	a	0.6	0.23	1.2	a
Stored UEBC	20	0.17	0.133	0.34	a	0.5	0.18	1	a	0.6	0.2	1.2	a
Sanergy Evergrow total N	20	0.05	0.007	0.1	a	0.9	0.36	1.8	a	1	0.36	2	a
Sanergy Evergrow PAN	20	0.02	0.035	0.04	a	0.8	0.16	1.6	a	0.9	0.16	1.8	a
SOIL Konpos Lakay total N	20	0.02	0.056	0.04	a	0.8	0.14	1.6	a	0.8	0.12	1.6	a
SOIL Konpos Lakay PAN	20	0	0	0	a	1.3	0.47	2.6	a	1.3	0.47	2.6	a
Unfertilized	20	0.22	0.136	0.44	a	0.6	0.13	1.2	a	0.9	0.13	1.8	a
Fresh Urine	30	0.6	0.422	1.2	a	1.3	0.57	2.6	a	1.9	0.67	3.8	a

Stored Urine	30	0.38	0.024	0.76	a	0.4	0.43	0.8	a	0.7	0.43	1.4	a
Fresh UEBC	30	0.88	0.332	1.76	a	0.1	0.12	0.2	a	1	0.28	2	a
Stored UEBC	30	2.96	1.479	5.92	**b**	0.4	0.26	0.8	a	3.4	1.36	6.8	a
Sanergy Evergrow total N	30	0.44	0.026	0.88	a	0.5	0.24	1	a	0.9	0.25	1.8	a
Sanergy Evergrow PAN	30	0.46	0.196	0.92	a	2	1.51	4	a	2.5	1.47	5	a
SOIL Konpos Lakay total N	30	0.47	0.231	0.94	a	0.1	0.1	0.2	a	0.6	0.25	1.2	a
SOIL Konpos Lakay PAN	30	0.63	0.558	1.26	a	0.5	0.16	1	a	1.1	0.54	2.2	a
Unfertilized	30	0.55	0.263	1.1	a	0.4	0.29	0.8	a	0.9	0.41	1.8	a
Fresh Urine	60	0	0	0	a	0.9	0.64	1.8	**ab**	0.9	0.64	1.8	**ab**
Stored Urine	60	0	0	0	a	1.8	1.22	3.6	**ab**	1.8	1.22	3.6	**ab**
Fresh UEBC	60	0	0	0	a	1.1	0.5	2.2	**ab**	1.1	0.5	2.2	**ab**
Stored UEBC	60	0	0	0	a	0.5	0.38	1	a	0.5	0.38	1	a
Sanergy Evergrow total N	60	0	0	0	a	18.3	10.66	36.6	**c**	18.3	10.66	36.6	**c**

Sanergy Evergrow PAN	60	0	0	0	a	1.3	1.06	2.6	**ab**	1.3	1.06	2.6	**ab**
SOIL Konpos Lakay total N	60	0	0	0	a	5.9	4.44	11.8	**b**	5.9	4.44	11.8	**b**
SOIL Konpos Lakay PAN	60	0	0	0	a	0.8	0.17	1.6	a	0.8	0.17	1.6	a
Unfertilized	60	0	0	0	a	1.1	0.4	2.2	**ab**	1.1	0.4	2.2	**ab**
Fresh Urine	90	0.42	0.078	0.84	**ab**	0.2	0.08	0.4	a	0.6	0.09	1.2	a
Stored Urine	90	0.44	0.124	0.88	**ab**	0.2	0.12	0.4	a	0.7	0.11	1.4	a
Fresh UEBC	90	0.38	0.035	0.76	a	1	2.02	2	a	1.3	2.01	2.6	a
Stored UEBC	90	0.46	0.109	0.92	**ab**	0.1	0.09	0.2	a	0.6	0.1	1.2	a
Sanergy Evergrow total N	90	0.54	0.173	1.08	**ab**	0.2	0.15	0.4	a	0.7	0.19	1.4	a
Sanergy Evergrow PAN	90	0.55	0.07	1.1	**ab**	0.2	0.1	0.4	a	0.8	0.07	1.6	a
SOIL Konpos Lakay total N	90	1.03	1.192	2.06	**b**	0.1	0.08	0.2	a	1.1	1.22	2.2	a
SOIL Konpos Lakay PAN	90	0.3	0.06	0.6	a	1.3	2.81	2.6	a	1.6	2.82	3.2	a

Unfertilized	90	0.42	0.055	0.84	**ab**	0.4	0.08	0.8	a	0.8	0.11	1.6	a

Table S3-2: $N_{min}t$, or the percentage of N mineralized from the amendment correcting for N mineralization in the unfertilized control, relative to the amount of N applied. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly. These results are expressed graphically in Figure S3-3.

Treatment	**Day**	**$N_{min}t$**	**SD**	**HSD**
Fresh Urine	0	68.802	6.895	**d**
Stored Urine	0	88.245	6.177	**e**
Fresh UEBC	0	32.210	6.212	**c**
Stored UEBC	0	29.397	8.135	**b**
Sanergy Evergrow total N	0	1.884	0.948	a
Sanergy Evergrow PAN	0	0.831	0.116	a
SOIL Konpos Lakay total N	0	1.285	1.208	a
SOIL Konpos Lakay PAN	0	0.738	0.062	a
Fresh Urine	5	40.071	4.938	**e**
Stored Urine	5	34.554	3.382	**d**
Fresh UEBC	5	13.924	1.401	**b**
Stored UEBC	5	18.554	7.390	**c**
Sanergy Evergrow total N	5	-0.103	0.363	a
Sanergy Evergrow PAN	5	0.121	0.096	a
SOIL Konpos Lakay total N	5	0.399	0.307	a
SOIL Konpos Lakay PAN	5	0.320	0.068	a
Fresh Urine	10	1.163	0.438	a
Stored Urine	10	0.201	0.223	a

Fresh UEBC	10	1.035	1.134	a
Stored UEBC	10	0.042	0.311	a
Sanergy Evergrow total N	10	0.589	0.410	a
Sanergy Evergrow PAN	10	0.034	0.014	a
SOIL Konpos Lakay total N	10	0.137	0.238	a
SOIL Konpos Lakay PAN	10	0.075	0.025	a
Fresh Urine	20	0.119	0.226	a
Stored Urine	20	0.171	0.409	a
Fresh UEBC	20	-0.274	0.247	a
Stored UEBC	20	-0.227	0.216	a
Sanergy Evergrow total N	20	0.127	0.354	a
Sanergy Evergrow PAN	20	0.000	0.013	a
SOIL Konpos Lakay total N	20	-0.052	0.168	a
SOIL Konpos Lakay PAN	20	0.028	0.032	a
Fresh Urine	30	0.970	0.724	**ab**
Stored Urine	30	-0.197	0.549	a
Fresh UEBC	30	0.056	0.458	a
Stored UEBC	30	2.449	1.311	**b**
Sanergy Evergrow total N	30	-0.033	0.440	a
Sanergy Evergrow PAN	30	0.107	0.099	a
SOIL Konpos Lakay total N	30	-0.350	0.441	a
SOIL Konpos Lakay PAN	30	0.010	0.044	a
Fresh Urine	60	-0.193	0.694	a
Stored Urine	60	0.754	1.191	a
Fresh UEBC	60	0.065	0.594	a

Stored UEBC	60	-0.474	0.507	a
Sanergy Evergrow total N	60	17.199	9.880	**c**
Sanergy Evergrow PAN	60	0.020	0.074	a
SOIL Konpos Lakay total N	60	4.794	4.125	**b**
SOIL Konpos Lakay PAN	60	-0.021	0.028	a
Fresh Urine	90	-0.157	0.135	a
Stored Urine	90	-0.128	0.146	a
Fresh UEBC	90	0.567	1.868	a
Stored UEBC	90	-0.195	0.137	a
Sanergy Evergrow total N	90	-0.034	0.203	a
Sanergy Evergrow PAN	90	0.000	0.009	a
SOIL Konpos Lakay total N	90	0.349	1.130	a
SOIL Konpos Lakay PAN	90	0.055	0.183	a

Table S3-3: Extracted PO_4-P for each BBP extractant by treatment and date. Kg ha^{-1} conversion is an average value. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly.

		CaCl2			**Citric Acid**			**Enzyme**			**HCl**		
Treatment	**Day**	mg PO_4-P kg dry soil $^{-1}$	kg PO_4-P ha^{-1}	HSD	mg PO_4-P kg dry soil $^{-1}$	kg PO_4-P ha^{-1}	HSD	mg PO_4-P kg dry soil $^{-1}$	kg PO_4-P ha^{-1}	HSD	mg PO_4-P kg dry soil $^{-1}$	kg PO_4-P ha^{-1}	HSD
Fresh Urine	0	0.16 ± 0.04	0.32	a	23 ± 1.7	46	a	5.5 ± 1.1	11	a	77 ± 14	154	**ab**
Stored Urine	0	0.46 ± 0.77	0.92	a	20 ± 2.3	40	a	3.9 ± 0.1	7.8	a	59 ± 7	118	a

Fresh UEBC	0	0.15 ± 0.05	0.3	a	21 ± 1.8	42	a	6.2 ± 0.5	12.4	a	47 ± 5	94	a
Stored UEBC	0	0.08 ± 0.04	0.16	a	19 ± 1.1	38	a	4.7 ± 0.3	9.4	a	49 ± 5	98	a
Sanergy Evergrow total N	0	1.06 ± 0.33	2.12	a	37 ± 2.7	74	a	10.3 ± 2.4	20.6	a	100 ± 6	200	**ab**
Sanergy Evergrow PAN	0	20.7 ± 2.7	41.4	**b**	343 ± 39.4	686	**c**	176.2 ± 26.4	352.4	**b**	164 ± 15	328	**c**
SOIL Konpos Lakay total N	0	1.67 ± 0.62	3.34	a	40 ± 10.4	80	a	6.3 ± 4.2	12.6	a	74 ± 17	148	**ab**
SOIL Konpos Lakay PAN	0	48.38 ± 4.39	96.76	**c**	257 ± 76.8	514	**b**	140.6 ± 37	281.2	**b**	135 ± 5	270	**bc**
Unfertilized	0	0.11 ± 0.04	0.22	a	11 ± 1.1	22	a	2.1 ± 0.2	4.2	a	41 ± 2	82	a
Fresh Urine	30	0.72 ± 0.13	1.44	a	20 ± 3.8	40	a	3.8 ± 0.4	7.6	a	53 ± 6	106	**ab**
Stored Urine	30	0.5 ± 0.18	1	a	19 ± 0.8	38	a	4.2 ± 0.5	8.4	a	47 ± 9	94	a
Fresh UEBC	30	0.75 ± 0.05	1.5	a	20 ± 0.8	40	a	5.8 ± 1.1	11.6	a	44 ± 6	88	a
Stored UEBC	30	2.11 ± 2.81	4.22	a	20 ± 3	40	a	4.3 ± 0.1	8.6	a	50 ± 4	100	**ab**

Sanergy Evergrow total N	30	1.57 ± 0.28	3.14	a	37 ± 6.7	74	a	3.8 ± 2.7	7.6	a	73 ± 11	146	**ab**
Sanergy Evergrow PAN	30	14.8 ± 9.8	29.6	a	176 ± 122.3	352	**b**	135 ± 106	270	**c**	110 ± 40	220	**b**
SOIL Konpos Lakay total N	30	1.35 ± 0.24	2.7	a	33 ± 16.2	66	a	4.7 ± 1.2	9.4	a	62 ± 12	124	**ab**
SOIL Konpos Lakay PAN	30	34.6 ± 2.64	69.2	**b**	315 ± 50	630	**c**	88.6 ± 6.1	177.2	**b**	252 ± 158	504	**c**
Unfertilized	30	0.36 ± 0.2	0.72	a	17 ± 2	34	a	3.3 ± 0.8	6.6	a	44 ± 7	88	a
Fresh Urine	60	0.38 ± 0.09	0.76	a	22 ± 1.1	44	a	2.4 ± 1.2	4.8	a	89 ± 2	178	a
Stored Urine	60	0.49 ± 0.19	0.98	a	22 ± 2.8	44	a	3.5 ± 0.7	7	a	94 ± 7	188	a
Fresh UEBC	60	0.48 ± 0.16	0.96	a	28 ± 0.7	56	a	4 ± 0.2	8	a	87 ± 3	174	a
Stored UEBC	60	0.48 ± 0.08	0.96	a	25 ± 0.9	50	a	4.7 ± 1.3	9.4	a	83 ± 5	166	a
Sanergy Evergrow total N	60	24.99 ± 8.05	49.98	**b**	41 ± 8.7	82	a	2.1 ± 2.2	4.2	a	121 ± 15	242	a

Sanergy Evergrow PAN	60	136.92 ± 58.51	273.84	**c**	330 ± 68.6	660	**b**	121.2 ± 19.8	242.4	**c**	479 ± 66	958	**b**
SOIL Konpos Lakay total N	60	23.39 ± 5.21	46.78	**b**	36 ± 5.2	72	a	0 ± 0	0	a	117 ± 21	234	a
SOIL Konpos Lakay PAN	60	149.06 ± 10.87	298.12	**c**	281 ± 40.7	562	**b**	73.6 ± 12.5	147.2	**b**	456 ± 63	912	**b**
Unfertilized	60	0.64 ± 0.54	1.28	a	21 ± 2.6	42	a	4.1 ± 1.6	8.2	a	80 ± 11	160	a
Fresh Urine	90	0.73 ± 0.04	1.46	a	21 ± 3	42	a	3.7 ± 0.6	7.4	a	77 ± 4	154	a
Stored Urine	90	0.44 ± 0.09	0.88	a	19 ± 2.1	38	a	2.2 ± 1.8	4.4	a	76 ± 17	152	a
Fresh UEBC	90	0.71 ± 0.23	1.42	a	23 ± 3.1	46	a	3.8 ± 1.9	7.6	a	76 ± 9	152	a
Stored UEBC	90	0.46 ± 0.19	0.92	a	16 ± 2.4	32	a	1.7 ± 0.4	3.4	a	63 ± 8	126	a
Sanergy Evergrow total N	90	6.38 ± 0.23	12.76	a	45 ± 9.5	90	a	0.1 ± 0.2	0.2	a	98 ± 18	196	a
Sanergy Evergrow PAN	90	15.71 ± 1.2	31.42	**ab**	321 ± 92.1	642	**c**	114.8 ± 22	229.6	**c**	507 ± 22	1014	**b**

SOIL Konpos Lakay total N	90	6.39 ± 0.19	12.78	a	47 ± 12	94	a	0.7 ± 0.7	1.4	a	111 ± 6	222	a
SOIL Konpos Lakay PAN	90	26.01 ± 7.78	52.02	**b**	247 ± 35	494	**b**	52.6 ± 4.5	105.2	**b**	496 ± 57	992	**b**
Unfertilized	90	0.56 ± 0.1	1.12	a	18 ± 3.6	36	a	4.1 ± 1.5	8.2	a	77 ± 23	154	a

Table S3-4: $P_{min}t$, or the percentage of P mineralized from the amendment correcting for P mineralization in the unfertilized control, relative to the amount of P applied. Letters show significant differences from a post hoc Tukey's HSD test at $p < 0.05$ between treatments for each extraction date. If treatments share a letter, they do not differ significantly. These results are expressed graphically in Figure S3-4.

		CaCl2			**Citric Acid**			**Enzyme**			**HCl**		
Treatment	Day	$P_{min}t$	sd	HSD	$P_{min}t$	sd	HSD	$P_{min}t$	Sd	HSD	$P_{min}t$	sd	HSD
Fresh Urine	0	0.9	0.9	a	226	33	a	60	19	a	633	233	a
Stored Urine	0	10.8	22.5	a	296	74	a	57	8	a	537	212	a
Fresh UEBC	0	0.6	0.7	a	116	22	a	44	5	a	63	52	a
Stored UEBC	0	-0.5	0.8	a	129	21	a	39	5	a	119	67	a
Sanergy Evergrow total N	0	509.1	167.8	**d**	14045	1446	**d**	4426	1222	**d**	31242	2884	**d**
Sanergy Evergrow PAN	0	773.8	94	**c**	12480	1372	**c**	6545	918	**c**	4608	525	**c**

SOIL Konpos Lakay total N	0	454.5	167.5	**c**	8599	2816	**c**	1479	1096	**c**	9589	4559	**c**
SOIL Konpos Lakay PAN	0	986.7	83	**b**	5034	1453	**b**	2831	701	**b**	1910	104	**b**
Fresh Urine	30	6.5	4	a	67	72	a	10	15	a	145	162	a
Stored Urine	30	4.5	7.8	a	76	63	a	31	28	a	87	337	a
Fresh UEBC	30	4.3	2.1	a	34	22	a	28	14	a	-6	96	a
Stored UEBC	30	32.7	40.1	a	55	50	a	16	12	a	84	119	a
Sanergy Evergrow total N	30	623.5	165	**c**	11053	3496	**c**	277	1384	**c**	15132	6408	**c**
Sanergy Evergrow PAN	30	637.8	290.4	**b**	5968	4257	**b**	4953	3688	**b**	2460	1417	**b**
SOIL Konpos Lakay total N	30	290	84.8	**b**	4635	4406	**b**	417	428	**b**	5245	3891	**b**
SOIL Konpos Lakay PAN	30	700	50.2	**b**	6092	947	**b**	1744	117	**b**	4233	3001	**b**
Fresh Urine	60	-4.8	9.1	a	18	47	a	-32	34	a	167	185	a
Stored Urine	60	-4.7	16.6	a	29	111	a	-21	49	a	439	371	a
Fresh UEBC	60	-1.8	5.7	a	75	27	a	-2	16	a	83	115	a

Stored UEBC	60	-2.6	7.7	a	58	38	a	8	28	a	38	169	a
Sanergy Evergrow total N	60	13072.4	4009	**e**	10817	4498	**e**	-839	1325	**e**	21943	9255	**e**
Sanergy Evergrow PAN	60	5122.1	2036	**d**	11611	2388	**d**	4398	692	**d**	14991	2338	**d**
SOIL Konpos Lakay total N	60	6642	1416	**c**	4480	1583	**c**	-1209	460	**c**	11907	6343	**c**
SOIL Konpos Lakay PAN	60	3033.9	205.9	**b**	5321	772	**b**	1421	239	**b**	7773	1308	**b**
Fresh Urine	90	3.1	1.8	a	48	77	a	-1	23	a	2	392	a
Stored Urine	90	-2.9	3.7	a	26	121	a	-33	60	a	-33	832	a
Fresh UEBC	90	1.7	2.5	a	52	48	a	1	23	a	-8	252	a
Stored UEBC	90	-1.5	3	a	-35	61	a	-30	18	a	-216	341	a
Sanergy Evergrow total N	90	3126.4	122.2	**c**	14313	5050	**c**	-1857	647	**c**	11381	14547	**c**
Sanergy Evergrow PAN	90	569.3	42	**c**	11388	3206	**c**	4175	769	**c**	16152	1108	**c**
SOIL Konpos Lakay total N	90	1702.1	58.5	**b**	8633	3394	**b**	-898	382	**b**	9540	6470	**b**
SOIL Konpos Lakay PAN	90	520.4	147.3	**b**	4679	667	**b**	999	89	**b**	8565	1160	**b**

Table S3-5: The ratio of N mineralized from PAN composts compared to total N composts for each feces-derived compost at each extraction date. Note that on day 60 no nitrate was extracted.

Treatment	Day	NO_3^-	NH_4^+	mineral N
Sanergy Evergrow	0	0.825	2.645161	2.351351
SOIL Konpos Lakay	0	1.079208	2.586207	2.367647
Sanergy Evergrow	5	2	2.75	2.5
SOIL Konpos Lakay	5	0.978723	4.5	3.411765
Sanergy Evergrow	10	1.2	0.833333	0.9375
SOIL Konpos Lakay	10	0.595238	2.571429	1.909091
Sanergy Evergrow	20	0.4	0.888889	0.9
SOIL Konpos Lakay	20	0	1.625	1.625
Sanergy Evergrow	30	1.045455	4	2.777778
SOIL Konpos Lakay	30	1.340426	5	1.833333
Sanergy Evergrow	60	NaN	0.071038	0.071038
SOIL Konpos Lakay	60	NaN	0.135593	0.135593
Sanergy Evergrow	90	1.018519	1	1.142857
SOIL Konpos Lakay	90	0.291262	13	1.454545

Table S3-6: Sorption capacity (mg g^{-1}) of walnut shell biochar for N and P from fresh and stored urine. Qe values were calculated using Eq. 1 in the main text.

Treatment	Qe N (mg-N g^{-1})	Qe P (mg-P g^{-1})
Fresh UEBC	6.224	0.391
Stored UEBC	5.404	0.253

Appendix C: Supplemental Information for Chapter 4

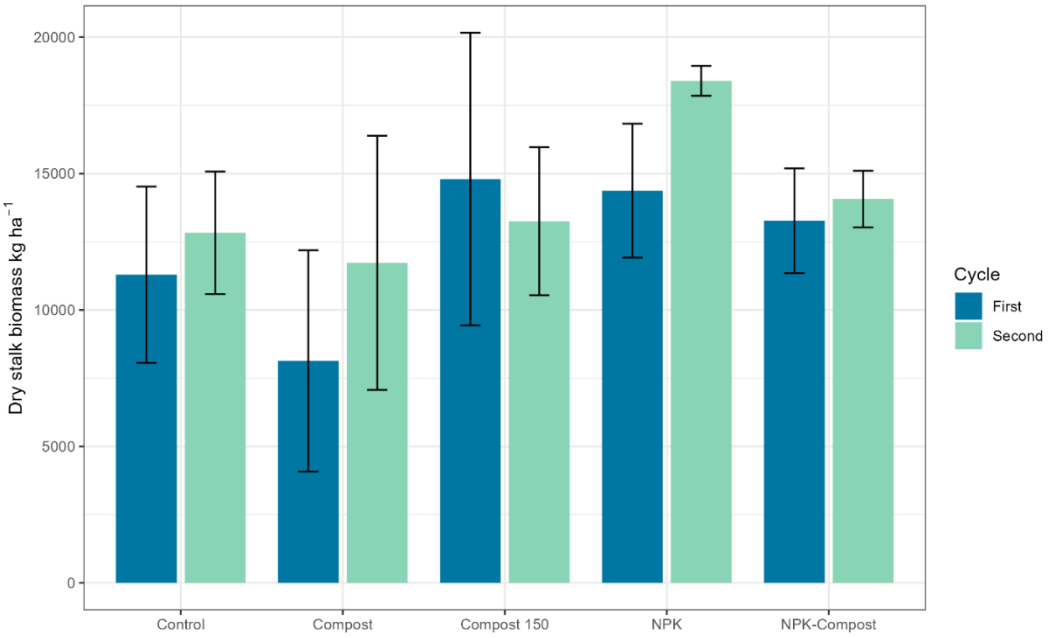

Figure S4-1: Dry stalk biomass for the first and second cycle. No significant differences were detected between treatments for either cycle.

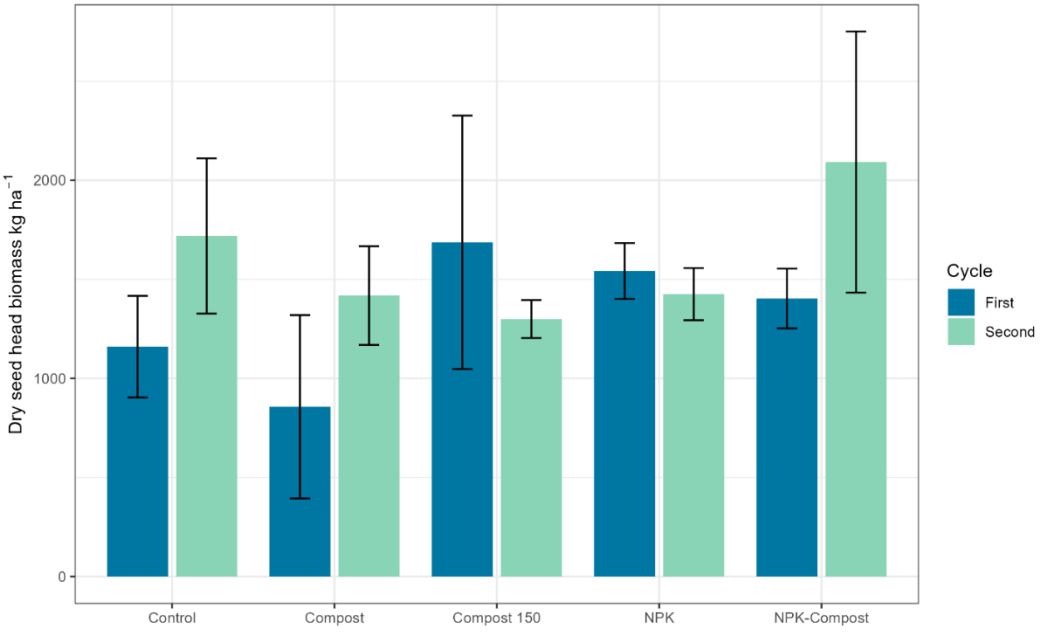

Figure S4-2: Dry seed head biomass for the first and second cycle. No significant differences were detected between treatments for either cycle.

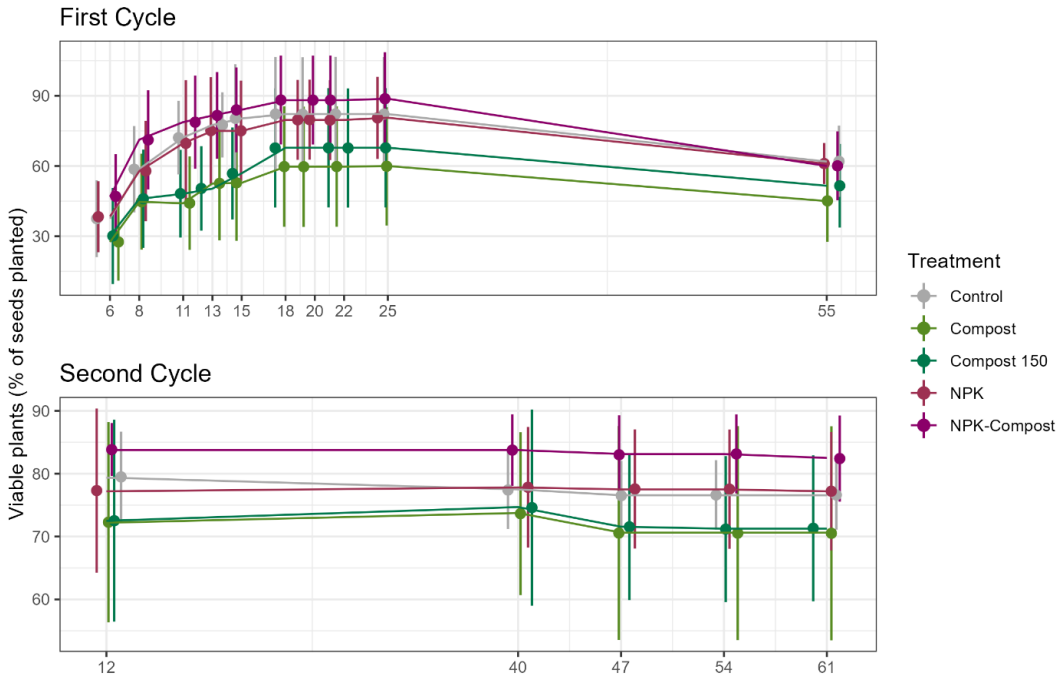

Figure S4-3: Average plant viability (± one standard deviation) as a percentage of seeds planted after thinning for the first and second cycle (n = 10). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

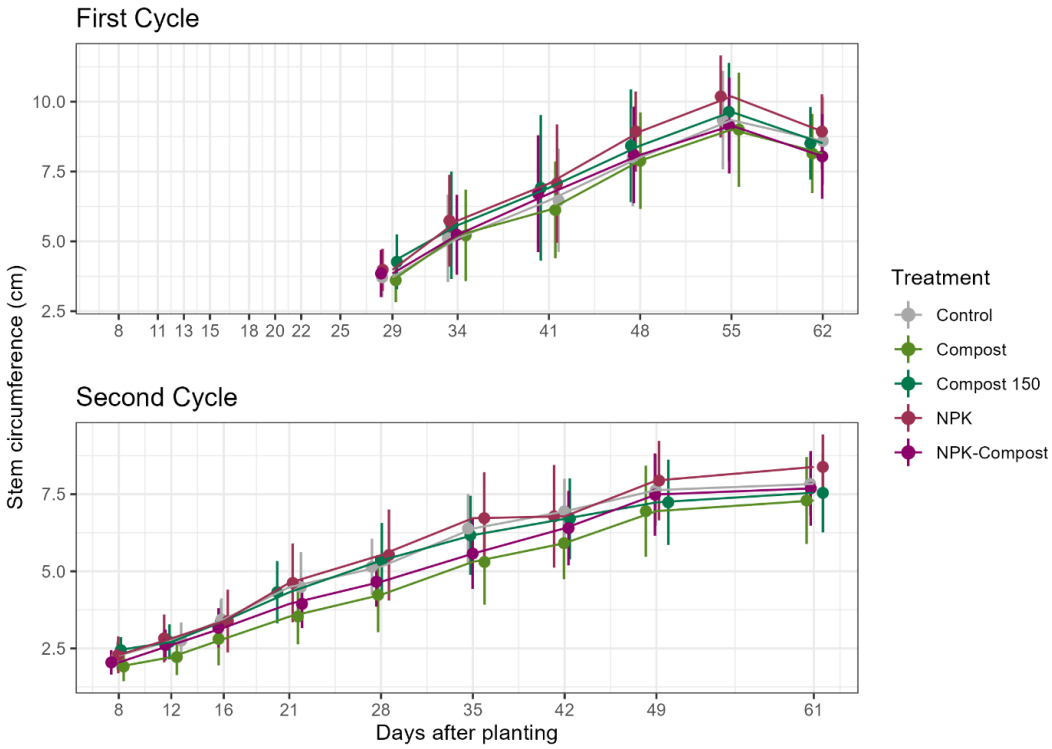

Figure S4-4: Average stem circumference (± one standard deviation) in the first and second cycle (n = 10). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

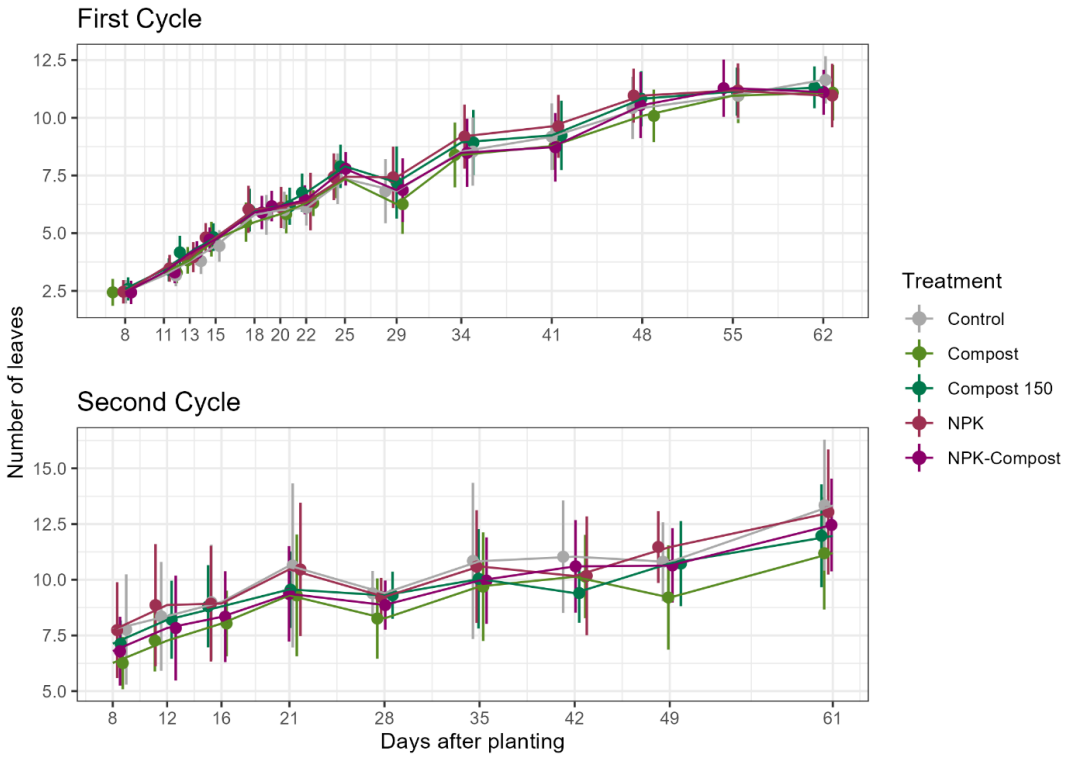

Figure S4-5: Average leaf number (± one standard deviation) in the first and second cycle (n = 10). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

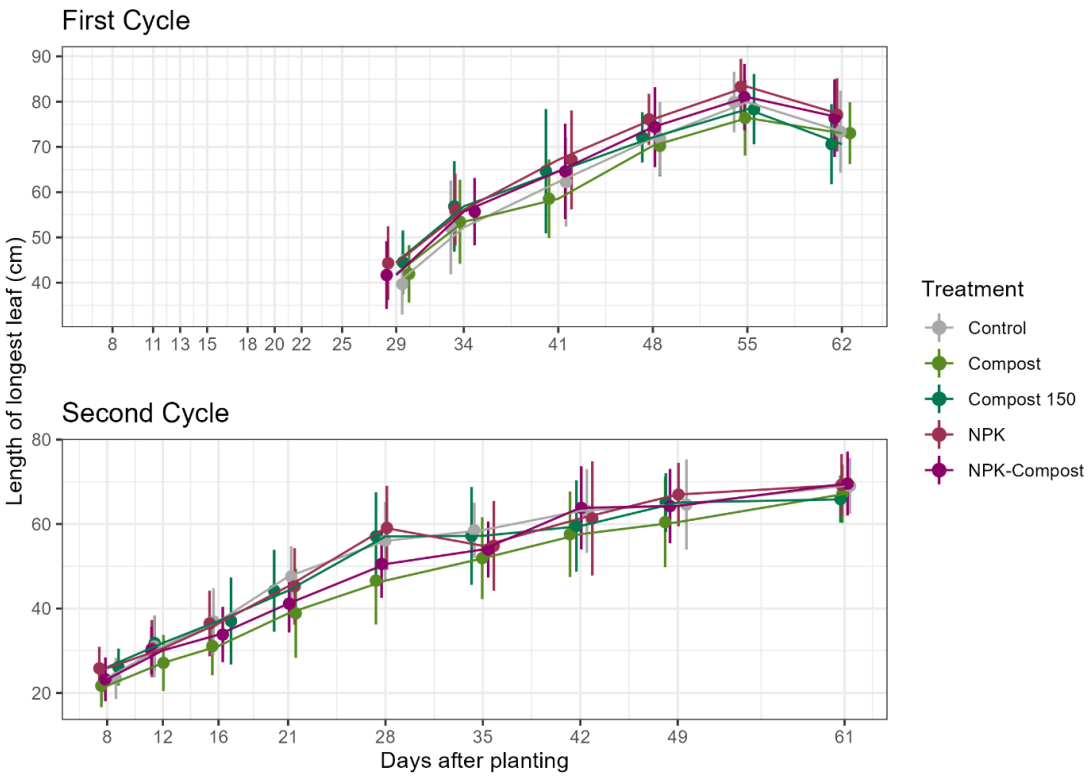

Figure S4-6: Average length of the longest leaf (± one standard deviation) in the first and second cycle (n = 10). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

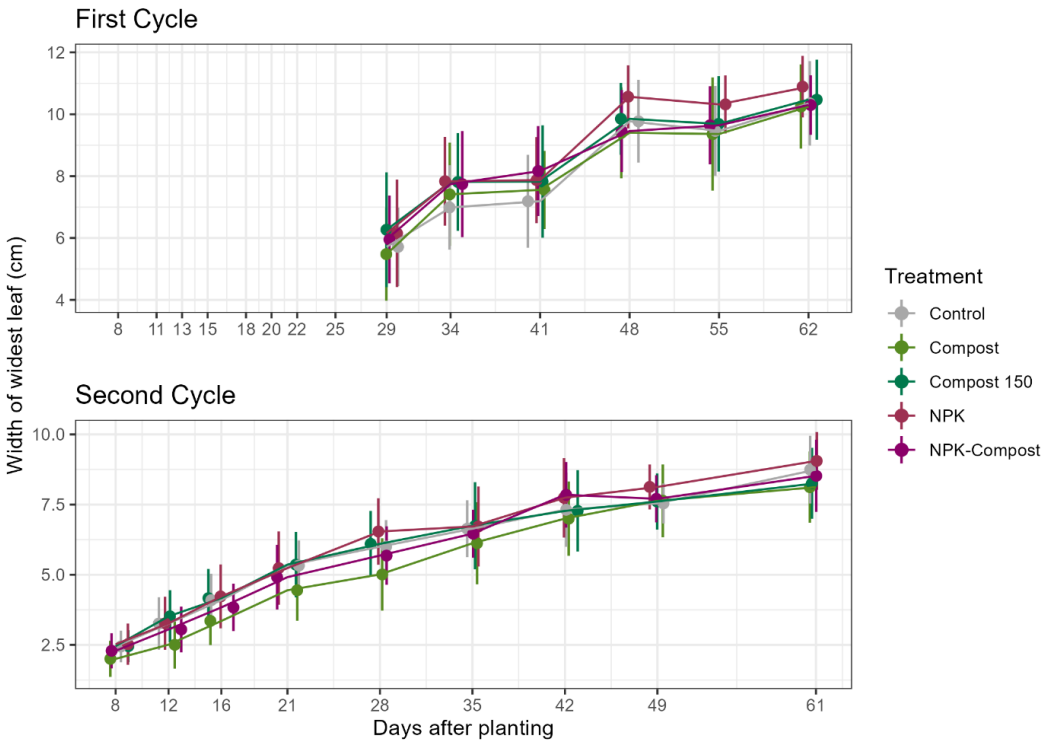

Figure S4-7: Average width of the widest leaf (± one standard deviation) in the first and second cycle (n = 10). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

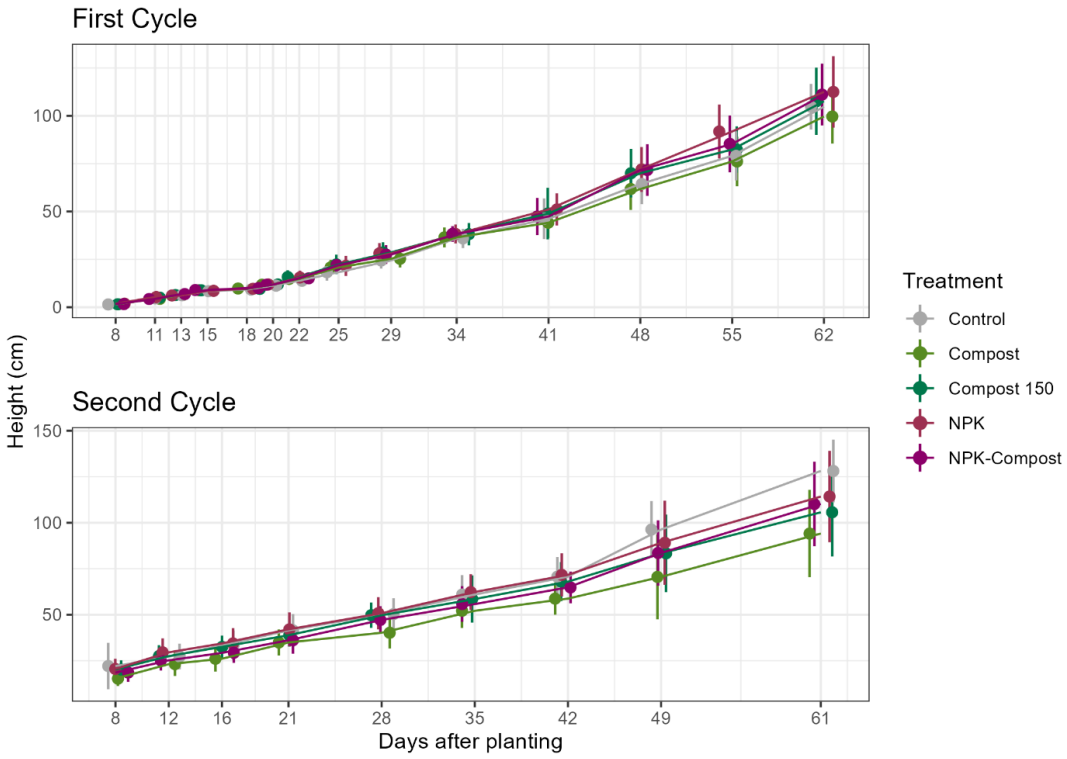

Figure S4-8: Average plant height (± one standard deviation) in the first and second cycle (n = 10). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

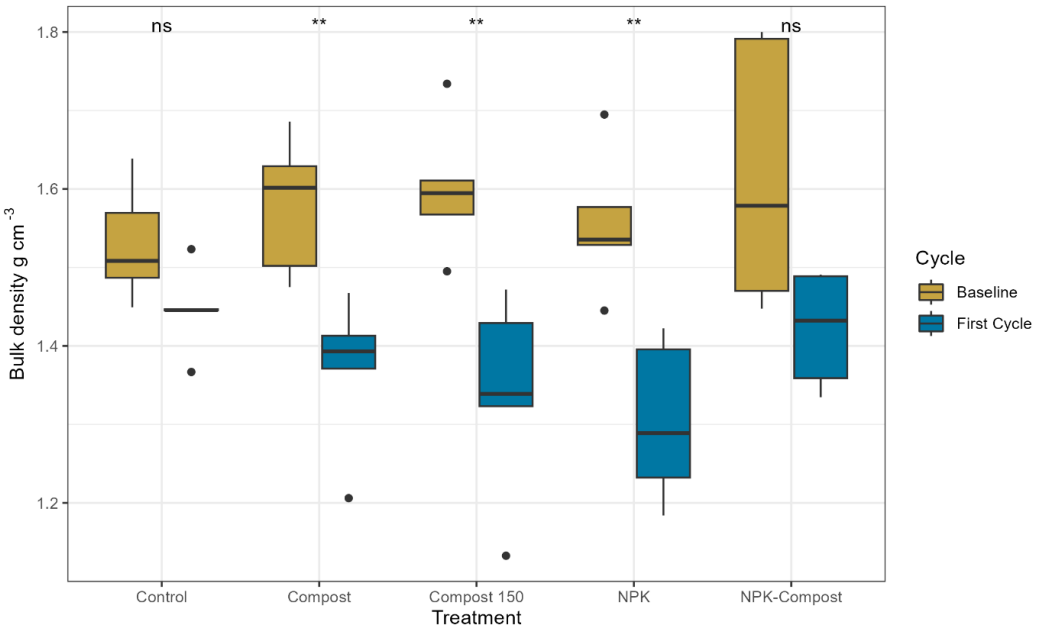

Figure S4-9: Change in soil bulk density between the baseline and first cycle. No significant differences were detected between treatments for either cycle. Stars show significance for a t-test at $p < 0.01$.

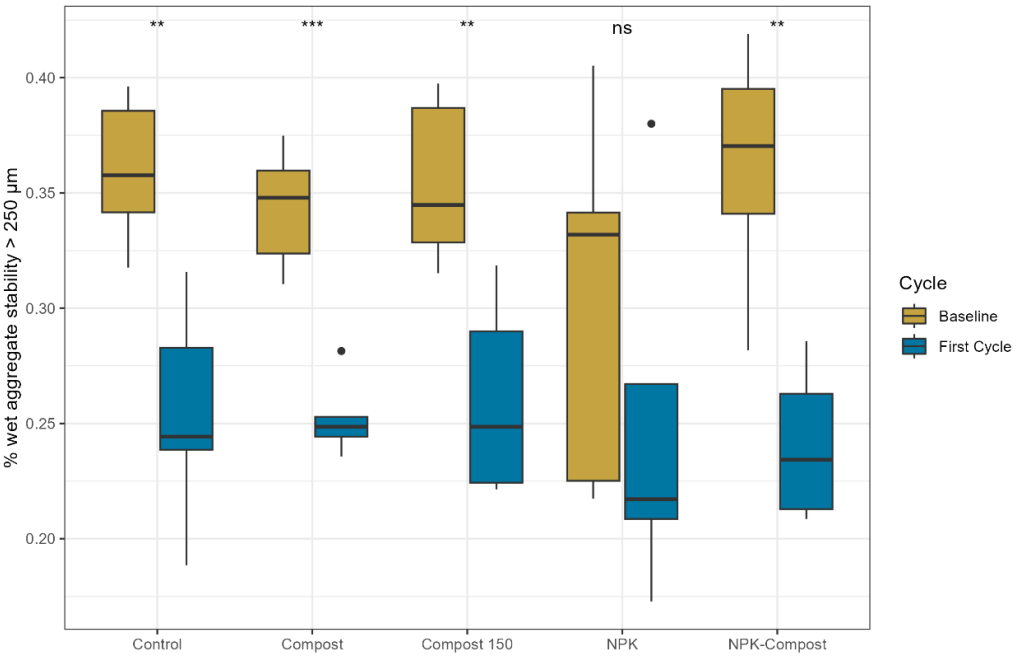

Figure S4-10: Change in soil aggregates less than 250 µm between the baseline and first cycle. No significant differences were detected between treatments for either cycle. Stars show significance for a t-test at $p < 0.01$.

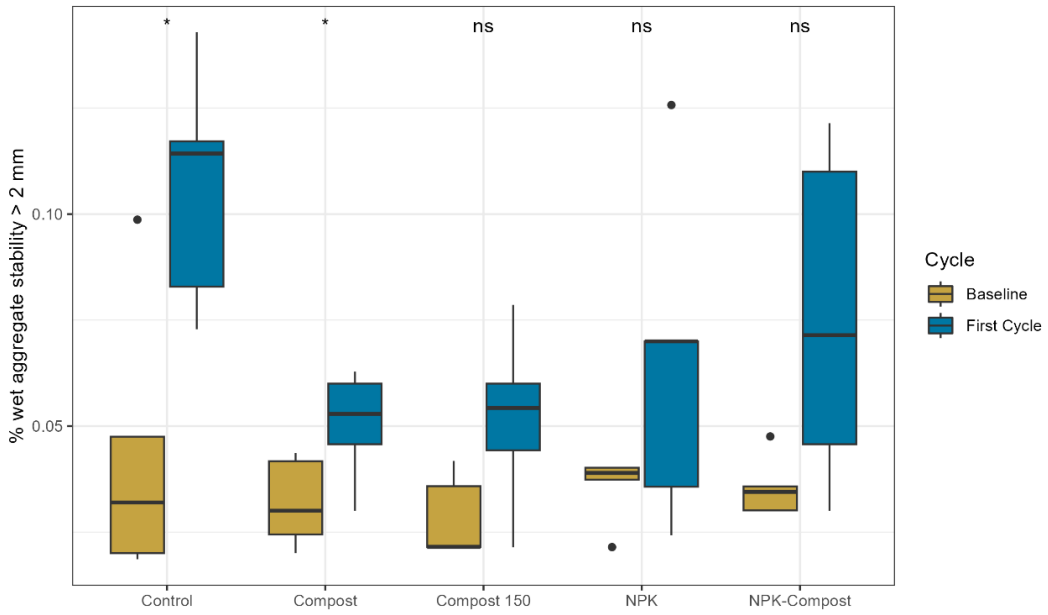

Figure S4-11: Change in soil aggregates greater than 2 mm between the baseline and first cycle. No significant differences were detected between treatments for either cycle. Stars show significance for a t-test at $p < 0.01$.

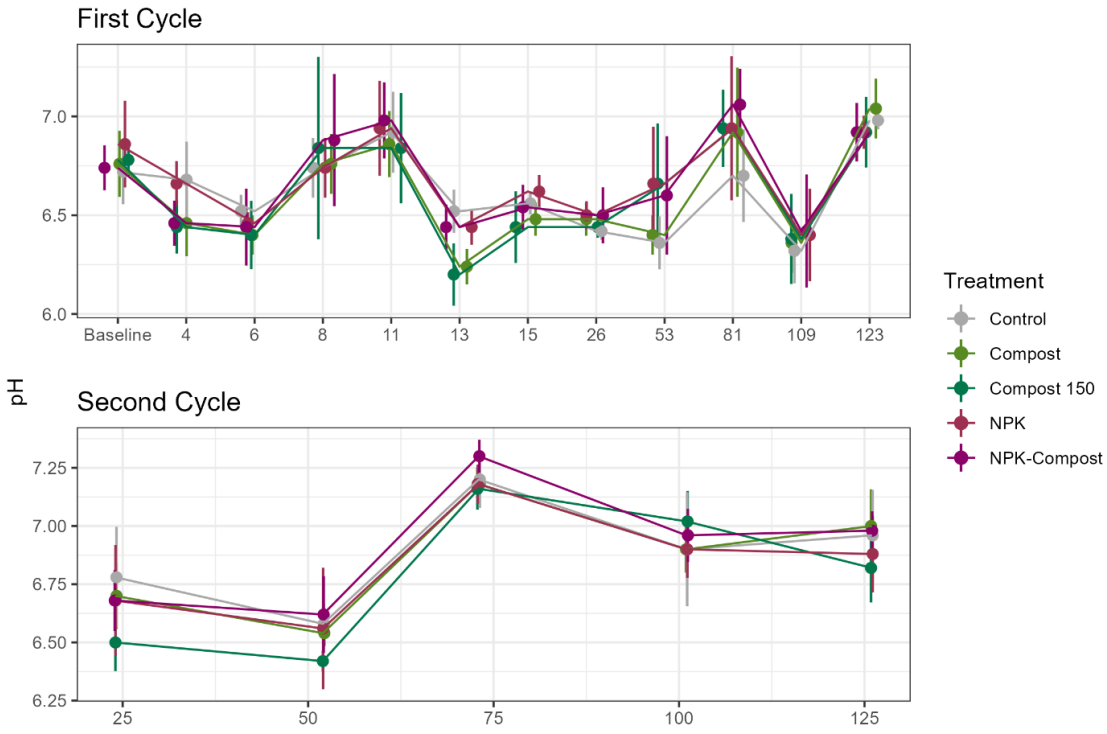

Figure S4-12: Average soil pH (± one standard deviation) in the first and second cycle (n = 5). Soil pH differed significantly between treatments in the second cycle. The x axis shows days after planting.

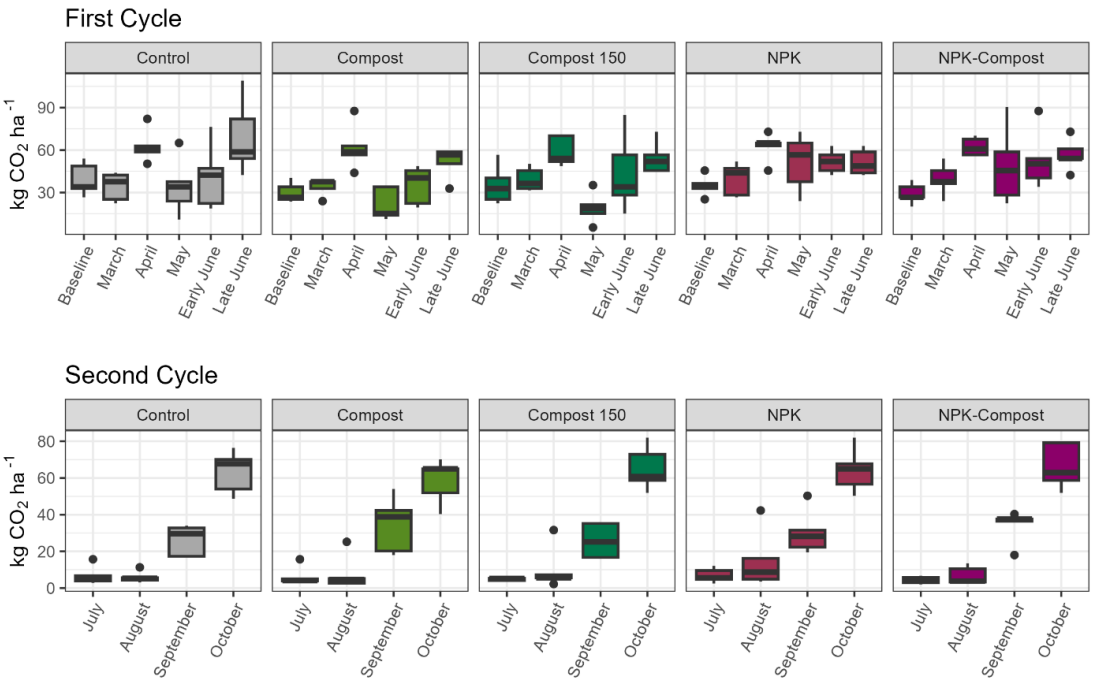

Figure S4-13: Monthly soil basal respiration flux for both cycles (n = 5). No significant differences were detected between treatments for either cycle.

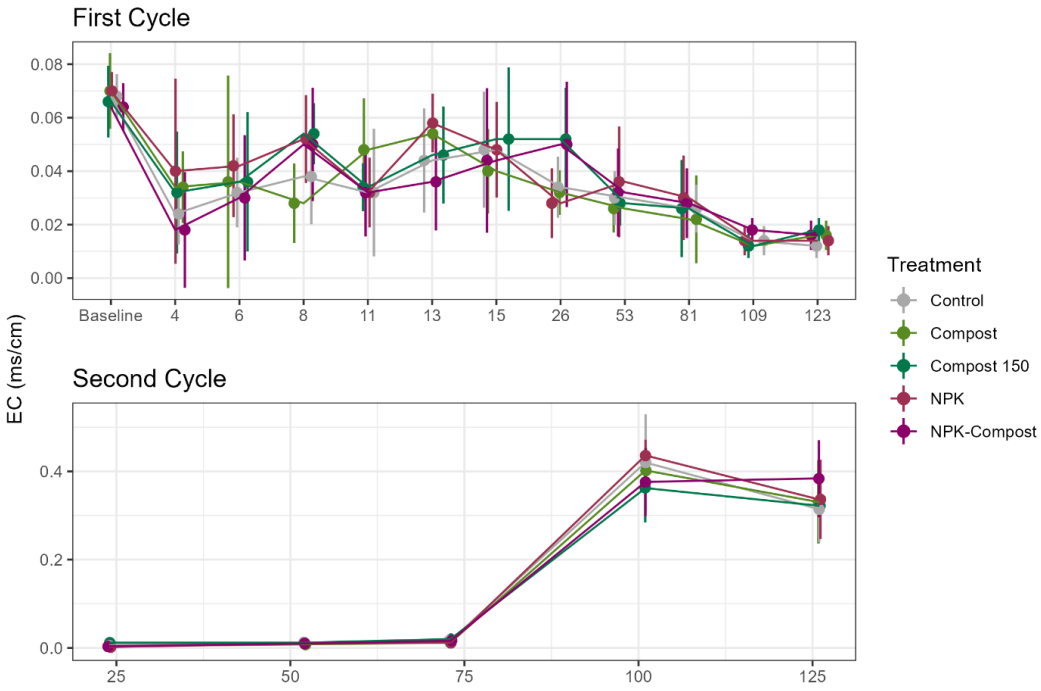

Figure S4-14: Monthly soil EC for both cycles (n = 5). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

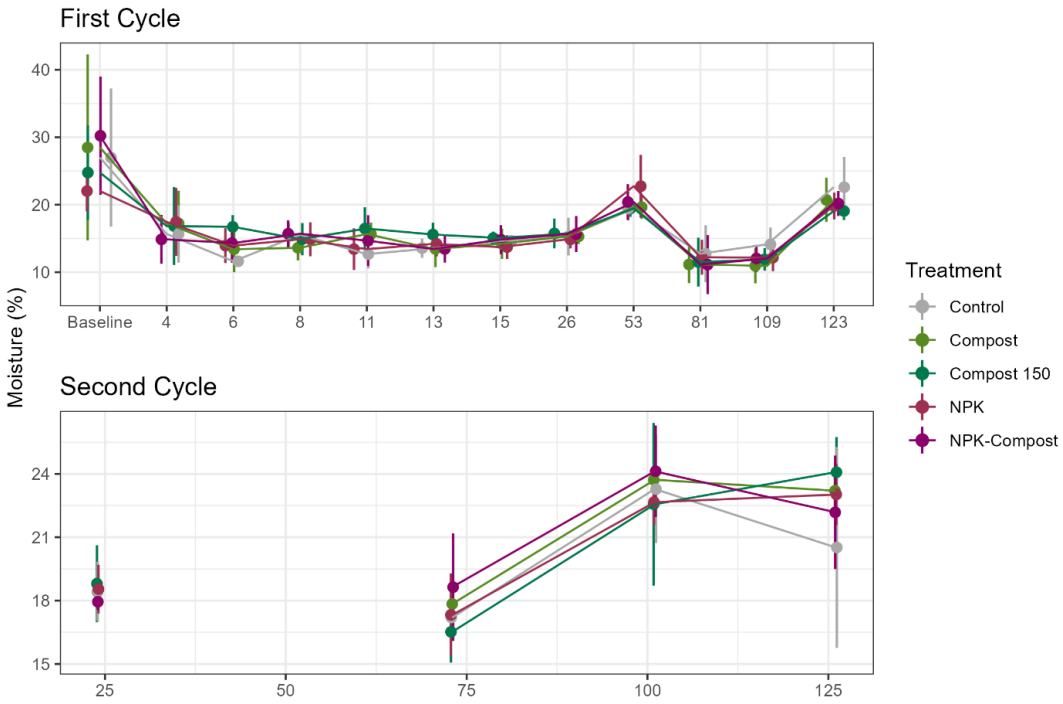

Figure S4-15: Monthly soil moisture for both cycles (n = 5). No significant differences were detected between treatments for either cycle. The x axis shows days after planting. Missing data in the second cycle is from a broken moisture probe.

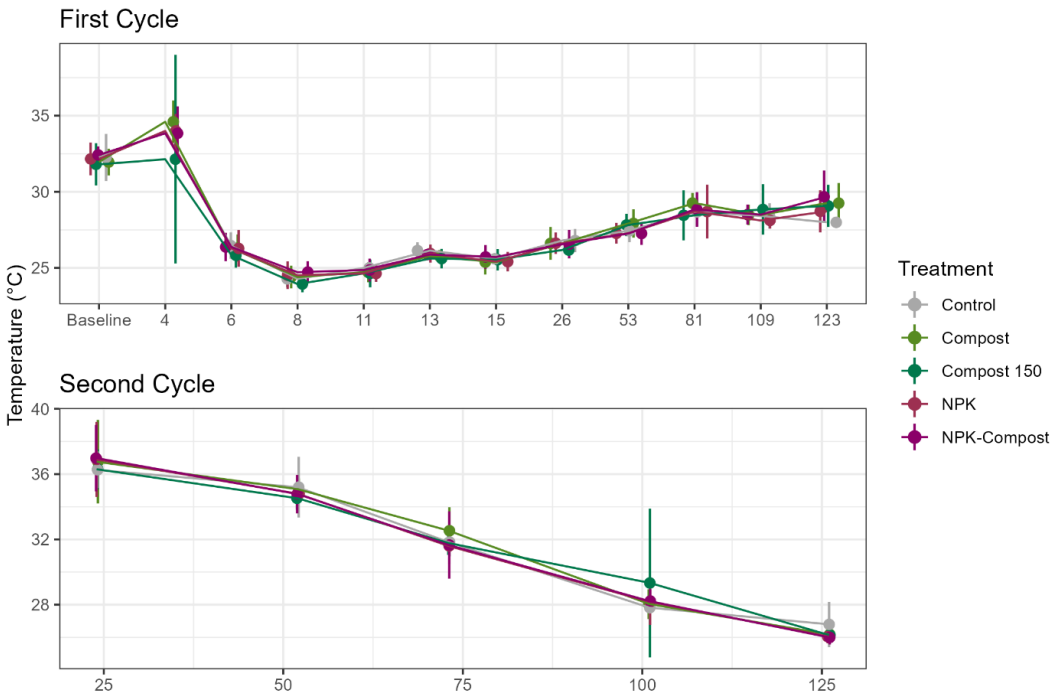

Figure S4-16: Monthly soil temperature for both cycles (n = 5). No significant differences were detected between treatments for either cycle. The x axis shows days after planting.

Bioavailable nutrients

Table S4-1: Results of a t-test for bioavailable nutrients between the first and second cropping cycle at $p < 0.05$.

Treatment	Nutrient	Group 1	Group 2	Statistic	DF	P value	Significance
Control	Al	First	Second	1.9	4.49	0.122	ns
Compost	Al	First	Second	1.32	5.97	0.234	ns
Compost 150	Al	First	Second	0.48	7.09	0.645	ns
NPK	Al	First	Second	1.58	4.51	0.182	ns
NPK-Compost	Al	First	Second	1.69	5.43	0.148	ns
Control	B	First	Second	1.75	4.66	0.144	ns

Compost	B	First	Second	1.5	4	0.207	ns
Compost 150	B	First	Second	-0.47	5.41	0.658	ns
NPK	B	First	Second	2.05	4	0.11	ns
NPK-Compost	B	First	Second	1.09	4.64	0.328	ns
Control	Ca	First	Second	0.71	7.56	0.499	ns
Compost	Ca	First	Second	0.83	4.91	0.443	ns
Compost 150	Ca	First	Second	1.24	7.9	0.25	ns
NPK	Ca	First	Second	2.65	4.23	0.054	ns
NPK-Compost	Ca	First	Second	-0.9	5.6	0.405	ns
Control	Cd	First	Second	NA	NA	NA	NA
Compost	Cd	First	Second	NA	NA	NA	NA
Compost 150	Cd	First	Second	NA	NA	NA	NA
NPK	Cd	First	Second	NA	NA	NA	NA
NPK-Compost	Cd	First	Second	1	4	0.374	ns
Control	Cu	First	Second	0.91	4.35	0.409	ns
Compost	Cu	First	Second	-2.52	4.4	0.06	ns
Compost 150	Cu	First	Second	-1.29	4.83	0.255	ns
NPK	Cu	First	Second	-0.58	6.13	0.582	ns
NPK-Compost	Cu	First	Second	-2.5	4.12	0.065	ns
Control	Fe	First	Second	-0.63	4.76	0.556	ns
Compost	Fe	First	Second	-2.18	4.09	0.093	ns

Compost 150	Fe	First	Second	-1.31	5	0.248	ns
NPK	Fe	First	Second	-1.51	4.1	0.204	ns
NPK-Compost	Fe	First	Second	-3.34	4.37	0.025	*
Control	K	First	Second	-0.75	6.03	0.481	ns
Compost	K	First	Second	-1.56	7.59	0.159	ns
Compost 150	K	First	Second	-2.23	4.9	0.077	ns
NPK	K	First	Second	0	5.02	> .999	ns
NPK-Compost	K	First	Second	-0.5	7.78	0.63	ns
Control	Mg	First	Second	0.7	7.74	0.504	ns
Compost	Mg	First	Second	1.13	5.68	0.304	ns
Compost 150	Mg	First	Second	1.58	7.94	0.154	ns
NPK	Mg	First	Second	3.32	4.61	0.024	*
NPK-Compost	Mg	First	Second	-0.08	6.34	0.939	ns
Control	Mn	First	Second	0.13	4.74	0.902	ns
Compost	Mn	First	Second	-2.42	4.02	0.073	ns
Compost 150	Mn	First	Second	-1.51	4.77	0.194	ns
NPK	Mn	First	Second	-1.38	4.22	0.237	ns
NPK-Compost	Mn	First	Second	-6.24	5.37	0.001	*
Control	NH4N	First	Second	-5.08	6.48	0.002	*
Compost	NH4N	First	Second	-5.48	4	0.005	*
Compost 150	NH4N	First	Second	-5.09	7.08	0.001	*

NPK	NH4N	First	Second	-6.4	6.63	< .001	*
NPK-Compost	NH4N	First	Second	-2.44	7.33	0.043	*
Control	NO3N	First	Second	1.74	4.27	0.153	ns
Compost	NO3N	First	Second	-1.26	7.83	0.245	ns
Compost 150	NO3N	First	Second	-0.4	7.81	0.702	ns
NPK	NO3N	First	Second	-0.05	6.42	0.96	ns
NPK-Compost	NO3N	First	Second	-0.58	7.14	0.579	ns
Control	P	First	Second	-1.05	7.01	0.33	ns
Compost	P	First	Second	-3.11	7.81	0.015	*
Compost 150	P	First	Second	-2.76	5.09	0.039	*
NPK	P	First	Second	-0.04	4.3	0.972	ns
NPK-Compost	P	First	Second	-3.56	4.04	0.023	*
Control	Pb	First	Second	1.49	7.62	0.177	ns
Compost	Pb	First	Second	-1.3	4.13	0.261	ns
Compost 150	Pb	First	Second	-0.53	5.8	0.614	ns
NPK	Pb	First	Second	-2.64	5.61	0.041	*
NPK-Compost	Pb	First	Second	-1.67	4.09	0.169	ns
Control	S	First	Second	-0.84	6.02	0.435	ns
Compost	S	First	Second	-2.72	7.97	0.026	*
Compost 150	S	First	Second	-2.2	5.65	0.072	ns
NPK	S	First	Second	0.71	5.05	0.511	ns

NPK-Compost	S	First	Second	-2.9	4.47	0.039	*
Control	Zn	First	Second	-3.47	4	0.026	*
Compost	Zn	First	Second	-5.72	4	0.005	*
Compost 150	Zn	First	Second	-2.07	4.77	0.096	ns
NPK	Zn	First	Second	-2.26	4	0.087	ns
NPK-Compost	Zn	First	Second	-2.54	4.14	0.062	ns

Table S4-2: All bioavailable nutrients in both cropping cycles. Significance of an ANOVA test within each cycle between treatments is shown at $p < 0.05$. Treatments that share a letter do not differ significantly. Significant results are bolded.

Treatment	Nutrient	Cycle	Mean	SD	SE	Significance
Control	Al	First	8.02	6.64	2.97	ns
Compost	Al	First	4.2	4.51	2.02	ns
Compost 150	Al	First	3.33	2.07	0.93	ns
NPK	Al	First	2.45	0.48	0.22	ns
NPK-Compost	Al	First	6.21	3.72	1.66	ns
Control	B	First	0.59	0.62	0.28	ns
Compost	B	First	0.27	0.4	0.18	ns
Compost 150	B	First	0.11	0.21	0.09	ns
NPK	B	First	0.05	0.06	0.03	ns
NPK-Compost	B	First	0.22	0.31	0.14	ns
Control	Ca	First	819	412.97	184.68	ns
Compost	Ca	First	785	384.65	172.02	ns
Compost 150	Ca	First	887.6	196.85	88.03	ns

NPK	Ca	First	822.8	232.39	103.93	ns
NPK-Compost	Ca	First	734.2	103.56	46.32	ns
Control	Cd	First	0	0	0	ns
Compost	Cd	First	0	0	0	ns
Compost 150	Cd	First	0	0	0	ns
NPK	Cd	First	0	0	0	ns
NPK-Compost	Cd	First	0.002	0.00447	0.002	ns
Control	Cu	First	0.24	0.34	0.15	ns
Compost	Cu	First	0.07	0.08	0.04	ns
Compost 150	Cu	First	0.19	0.23	0.1	ns
NPK	Cu	First	0.09	0.13	0.06	ns
NPK-Compost	Cu	First	0.07	0.08	0.03	ns
Control	Fe	First	2.18	2.15	0.96	ns
Compost	Fe	First	1.16	0.6	0.27	ns
Compost 150	Fe	First	2.46	2.61	1.17	ns
NPK	Fe	First	1.14	0.29	0.13	ns
NPK-Compost	Fe	First	1.29	0.66	0.3	ns
Control	K	First	14.2	3.03	1.36	ns
Compost	K	First	19	7.62	3.41	ns
Compost 150	K	First	25.2	6.1	2.73	ns
NPK	K	First	17.8	6.02	2.69	ns
NPK-Compost	K	First	18.2	5.45	2.44	ns
Control	Mg	First	321.2	128.7	57.55	ns
Compost	Mg	First	306.8	115.39	51.61	ns
Compost 150	Mg	First	357.4	55.2	24.69	ns

NPK	Mg	First	340.8	76.3	34.12	ns
NPK-Compost	Mg	First	308.8	47.28	21.15	ns
Control	Mn	First	1.65	1.42	0.64	ns
Compost	Mn	First	0.76	0.2	0.09	ns
Compost 150	Mn	First	1.7	1.9	0.85	ns
NPK	Mn	First	0.76	0.4	0.18	ns
NPK-Compost	Mn	First	0.64	0.27	0.12	ns
Control	NH4N	First	0.4	0.89	0.4	ns
Compost	NH4N	First	0	0	0	ns
Compost 150	NH4N	First	0.6	0.89	0.4	ns
NPK	NH4N	First	0.4	0.55	0.24	ns
NPK-Compost	NH4N	First	1.6	2.07	0.93	ns
Control	NO3N	First	41.2	35.23	15.76	ns
Compost	NO3N	First	50.2	33.45	14.96	ns
Compost 150	NO3N	First	68.8	43.93	19.65	ns
NPK	NO3N	First	29.4	22.2	9.93	ns
NPK-Compost	NO3N	First	45.8	27.24	12.18	ns
Control	P	First	0.76	0.57	0.26	ns
Compost	P	First	1.6	1.38	0.62	ns
Compost 150	P	First	2.13	1.67	0.75	ns
NPK	P	First	1.47	2.69	1.2	ns
NPK-Compost	P	First	0.79	0.17	0.07	ns
Control	Pb	First	0.06	0.04	0.02	ns
Compost	Pb	First	0.03	0.03	0.01	ns
Compost 150	Pb	First	0.11	0.17	0.07	ns

NPK	Pb	First	0.02	0.02	0.00917	ns
NPK-Compost	Pb	First	0.02	0.03	0.01	ns
Control	S	First	5.3	3.08	1.38	ns
Compost	S	First	6.8	4.37	1.95	ns
Compost 150	S	First	8.74	4.65	2.08	ns
NPK	S	First	8.78	6.18	2.76	ns
NPK-Compost	S	First	5.74	2.36	1.06	ns
Control	Zn	First	0	0	0	ns
Compost	Zn	First	0	0	0	ns
Compost 150	Zn	First	0.06	0.1	0.05	ns
NPK	Zn	First	0	0	0	ns
NPK-Compost	Zn	First	0.03	0.06	0.03	ns
Control	Al	Second	2.2	1.64	0.73	ns
Compost	Al	Second	1.2	2.31	1.03	ns
Compost 150	Al	Second	2.54	3.01	1.35	ns
NPK	Al	Second	1.06	1.91	0.85	ns
NPK-Compost	Al	Second	3.16	1.6	0.71	ns
Control	B	Second	0.08	0.18	0.08	ns
Compost	B	Second	0	0	0	ns
Compost 150	B	Second	0.22	0.49	0.22	ns
NPK	B	Second	0	0	0	ns
NPK-Compost	B	Second	0.06	0.09	0.04	ns
Control	Ca	Second	652.6	322.72	144.33	ns
Compost	Ca	Second	633.6	130.47	58.35	ns
Compost 150	Ca	Second	741	176.24	78.82	ns

NPK	Ca	Second	543.6	39.36	17.6	ns
NPK-Compost	Ca	Second	834.6	226.68	101.38	ns
Control	Cd	Second	0	0	0	ns
Compost	Cd	Second	0	0	0	ns
Compost 150	Cd	Second	0	0	0	ns
NPK	Cd	Second	0	0	0	ns
NPK-Compost	Cd	Second	0	0	0	ns
Control	Cu	Second	0.1	0.07	0.03	ns
Compost	Cu	Second	0.48	0.36	0.16	ns
Compost 150	Cu	Second	0.62	0.72	0.32	ns
NPK	Cu	Second	0.16	0.25	0.11	ns
NPK-Compost	Cu	Second	0.76	0.61	0.27	ns
Control	Fe	Second	2.82	0.66	0.3	ns
Compost	Fe	Second	6.7	5.65	2.53	ns
Compost 150	Fe	Second	7	7.3	3.27	ns
NPK	Fe	Second	2.9	2.59	1.16	ns
NPK-Compost	Fe	Second	5.98	3.07	1.37	ns
Control	K	Second	16.4	5.81	2.6	**b**
Compost	K	Second	27.6	9.66	4.32	**ab**
Compost 150	K	Second	44.2	18.07	8.08	**a**
NPK	K	Second	17.8	2.17	0.97	**b**
NPK-Compost	K	Second	19.8	4.6	2.06	**b**
Control	Mg	Second	268.8	106.84	47.78	ns
Compost	Mg	Second	242.4	54.2	24.24	ns
Compost 150	Mg	Second	304.6	50.6	22.63	ns

NPK	Mg	Second	223.2	21.12	9.45	ns
NPK-Compost	Mg	Second	312.2	83.29	37.25	ns
Control	Mn	Second	1.56	0.43	0.19	ns
Compost	Mn	Second	5.22	4.12	1.84	ns
Compost 150	Mn	Second	6	6.07	2.72	ns
NPK	Mn	Second	2.3	2.47	1.1	ns
NPK-Compost	Mn	Second	2.56	0.63	0.28	ns
Control	NH4N	Second	4.4	1.52	0.68	ns
Compost	NH4N	Second	3	1.22	0.55	ns
Compost 150	NH4N	Second	4.2	1.3	0.58	ns
NPK	NH4N	Second	3.4	0.89	0.4	ns
NPK-Compost	NH4N	Second	4.4	1.52	0.68	ns
Control	NO3N	Second	13.4	6.5	2.91	**b**
Compost	NO3N	Second	79	38.87	17.38	**a**
Compost 150	NO3N	Second	80.8	51.37	22.97	**a**
NPK	NO3N	Second	30	12.88	5.76	**ab**
NPK-Compost	NO3N	Second	58.2	39.1	17.49	**ab**
Control	P	Second	1.24	0.85	0.38	**b**
Compost	P	Second	4.54	1.61	0.72	**ab**
Compost 150	P	Second	8.04	4.48	2	**a**
NPK	P	Second	1.52	0.52	0.23	**b**
NPK-Compost	P	Second	4.82	2.53	1.13	**ab**
Control	Pb	Second	0.02	0.04	0.02	ns
Compost	Pb	Second	0.18	0.25	0.11	ns
Compost 150	Pb	Second	0.2	0.34	0.15	ns

NPK	Pb	Second	0.08	0.04	0.02	ns
NPK-Compost	Pb	Second	0.2	0.23	0.1	ns
Control	S	Second	6.6	1.6	0.72	**b**
Compost	S	Second	14.56	4.66	2.08	**ab**
Compost 150	S	Second	19.62	10.01	4.48	**a**
NPK	S	Second	6.7	2.26	1.01	**b**
NPK-Compost	S	Second	18.68	9.71	4.34	**ab**
Control	Zn	Second	0.34	0.22	0.1	ns
Compost	Zn	Second	0.28	0.11	0.05	ns
Compost 150	Zn	Second	0.38	0.33	0.15	ns
NPK	Zn	Second	0.28	0.28	0.12	ns
NPK-Compost	Zn	Second	0.52	0.43	0.19	ns

Harvest

First cycle statistics:

Table S4-3: Results of an ANOVA test with treatment as the predictor and dry total biomass as the response variable for the first cycle.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	111919510	27979877	1.78	0.209
Residuals	10	157222230	15722223		

Table S4-4: Results of an ANOVA test with treatment as the predictor and dry stalk biomass as the response variable for the first cycle.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	89373432	22343358	1.711	0.224
Residuals	10	130593991	13059399		

Table S4-5: Results of an ANOVA test with treatment as the predictor and dry seed head biomass as the response variable for the first cycle.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	1291059	322765	2.206	0.141
Residuals	10	1463102	146310		

Second cycle statistics:

Table S4-6: Results of an ANOVA test with treatment as the predictor and dry total biomass as the response variable for the second cycle.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	79065699	19766425	2.47	0.112
Residuals	10	80020685	8002069		

Table S4-7: Results of an ANOVA test with treatment as the predictor and dry stalk biomass as the response variable for the second cycle.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	0.8218	0.20546	2.419	0.117
Residuals	10	0.8493	0.08493		

Table S4-8: Results of an ANOVA test with treatment as the predictor and dry seed head biomass as the response variable for the second cycle.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	0.01228	0.003071	1.182	0.376
Residuals	10	0.02598	0.002598		

Comparing both cycles:

Table S4-9: Results of a t-test for total biomass between the first and second cropping cycle at $p < 0.05$.

Treatment	Group 1	Group 2	N 1	N 2	statistic	df	p
Control	First	Second	3	3	-0.83677	3.69976	0.453
Compost	First	Second	3	3	-1.0793	3.971442	0.342
Compost 150	First	Second	3	3	0.510569	2.740794	0.648
NPK	First	Second	3	3	-2.56289	2.193445	0.114
NPK-Compost	First	Second	3	3	-1.0993	3.693419	0.338

Table S4-10: Results of a t-test for stalk biomass between the first and second cropping cycle at $p < 0.05$.

Treatment	Group 1	Group 2	N 1	N 2	statistic	df	p
Control	First	Second	3	3	-0.6775	3.565702	0.539
Compost	First	Second	3	3	-1.00732	3.924521	0.372
Compost 150	First	Second	3	3	0.444974	2.961365	0.687
NPK	First	Second	3	3	-2.7732	2.198504	0.0983
NPK-Compost	First	Second	3	3	-0.63278	3.075543	0.571

Table S4-11: Results of a t-test for stalk biomass between the first and second cropping cycle at $p < 0.05$.

Treatment	Group 1	Group 2	N 1	N 2	statistic	df	p
Control	First	Second	3	3	-2.06586	3.4486	0.119
Compost	First	Second	3	3	-1.85124	3.068599	0.159
Compost 150	First	Second	3	3	1.037926	2.089712	0.404
NPK	First	Second	3	3	1.046032	3.981184	0.355
NPK-Compost	First	Second	3	3	-1.7646	2.209374	0.208

Growth indicators

<u>Plant viability</u>

Table S4-12: Results of an ANOVA test on a linear mixed effect model with number of viable plants as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	Num DF	Den DF	F value	Pr(>F)
Date	17771.23	2221.4	8	176	74.16	2.39E-52
Treatment	37.7	9.42	4	176	0.31	0.87
Date:Treatment	1664.51	52.02	32	176	1.74	0.01

Table S4-13: Results of an ANOVA test on a linear mixed effect model with number of viable plants as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	Num DF	Den DF	F value	Pr(>F)
Date	51.35	17.12	3	76	2.46	0.07
Treatment	4.2	1.05	4	76	0.15	0.96
Date:Treatment	70.9	5.91	12	76	0.85	0.6

Table S4-14: Results of a t-test for plant viability between the first and second cropping cycle at $p < 0.05$.

Treatment	Group 1	Group 2	n1	n2	statistic	p	p.adj	p.adj.signif
Control	First	Second	5	5	1.581139	0.113846	0.113846	ns
Compost	First	Second	5	5	1.780997	0.074913	0.074913	ns
Compost 150	First	Second	5	5	1.566699	0.117185	0.117185	ns
NPK	First	Second	5	5	2.193378	0.02828	0.02828	*
NPK-Compost	First	Second	5	5	2.108185	0.035015	0.035015	*

Stem circumference

Table S4-15: Results of an ANOVA test on a linear mixed effect model with stem circumference as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	636.1	159.03	4	639	124.21	2.14E-78
Treatment	3.46	0.87	4	639.82	0.68	0.61
Date:Treatment	16.75	1.05	16	639.02	0.82	0.67

Table S4-16: Results of an ANOVA test on a linear mixed effect model with stem circumference as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	76.33	10.9	7	1160	17.08	1.34E-21
Treatment	2.01	0.5	4	1160	0.79	0.53
Date:Treatment	32.53	1.16	28	1160	1.82	5.80E-03

Leaf number

Table S4-17: Results of an ANOVA test on a linear mixed effect model with number of leaves as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	620.92	51.74	12	1693	58.18	9.34E-118
Treatment	0.65	0.16	4	1693	0.18	0.95
Date:Treatment	66.76	1.39	48	1693	1.56	8.57E-03

Table S4-18: Results of an ANOVA test on a linear mixed effect model with number of leaves as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	742.01	106	7	1160	18.51	1.71E-23
Treatment	2.06	0.52	4	1160	0.09	0.99
Date:Treatment	244.79	8.74	28	1160	1.53	0.04

Length of the longest leaf

Table S4-19: Results of an ANOVA test on a linear mixed effect model with length of the longest leaf as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	25821.97	6455.49	4	641	89.2	2.92E-60
Treatment	276.49	69.12	4	641	0.96	0.43
Date:Treatment	1234.67	77.17	16	641	1.07	0.38

Table S4-20: Results of an ANOVA test on a linear mixed effect model with length of the longest leaf as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	8630.08	1232.87	7	1160	15.96	4.00E-20
Treatment	104.27	26.07	4	1160	0.34	0.85
Date:Treatment	5193.2	185.47	28	1160	2.4	6.41E-05

Width of the widest leaf

Table S4-21: Results of an ANOVA test on a linear mixed effect model with width of the widest leaf as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	473.73	118.43	4	638.99	90.11	1.01E-60

Treatment	1.33	0.33	4	639.82	0.25	0.91
Date:Treatment	49.04	3.07	16	639.02	2.33	2.34E-03

Table S4-22: Results of an ANOVA test on a linear mixed effect model with width of the widest leaf as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	58.29	8.33	7	1158	7.17	2.04E-08
Treatment	1.08	0.27	4	1158	0.23	0.92
Date:Treatment	61.4	2.19	28	1158	1.89	3.56E-03

Plant height

Table S4-23: Results of an ANOVA test on a linear mixed effect model with plant height as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	83295.78	6941.31	12	1557.98	258.34	0
Treatment	166.93	41.73	4	1558.46	1.55	0.18
Date:Treatment	2253.91	46.96	48	1557.98	1.75	1.31E-03

Table S4-24: Results of an ANOVA test on a linear mixed effect model with plant height as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	51469.84	7352.83	7	1158	78.75	1.79E-93
Treatment	1549.92	387.48	4	1158	4.15	2.42E-03
Date:Treatment	4835.36	172.69	28	1158	1.85	4.70E-03

Table S4-25: Results of post hoc Tukey's HSD on a linear mixed effect model with plant height as the predictor variable and treatment and date as fixed effects for the second cycle.

1	estimate	SE	df	t.ratio	p.value
Compost 150 – Compost	0.82	0.88	1158	0.93	0.885
Compost 150 - Control	-2.58	0.88	1158	-2.93	**0.029**
Compost 150 - NPK	-1.03	0.88	1158	-1.17	0.768
Compost 150 - NPK-Compost	-0.78	0.88	1158	-0.88	0.903
Compost - Control	-3.4	0.88	1158	-3.86	**0.001**
Compost - NPK	-1.85	0.88	1158	-2.1	0.22
Compost - NPK-Compost	-1.6	0.88	1158	-1.81	0.367
Control - NPK	1.55	0.88	1158	1.76	0.4
Control - NPK-Compost	1.8	0.88	1158	2.04	0.245
NPK - NPK-Compost	0.25	0.88	1158	0.29	0.998

Soil physiochemical data

Table S4-26: Results of a t-test for soil bulk density between the baseline and first cycle at $p < 0.05$.

Treatment	Group 1	Group 2	n1	n2	statistic	df	p	sig
Control	Baseline	First Cycle	5	5	2.05	7.39	0.078	ns
Compost	Baseline	First Cycle	5	5	3.53	7.91	0.008	*
Compost 150	Baseline	First Cycle	5	5	3.72	6.95	0.008	*
NPK	Baseline	First Cycle	5	5	4.1	7.89	0.004	*
NPK-Compost	Baseline	First Cycle	5	5	2.38	5.4	0.06	ns

<u>pH</u>

Table S4-27: Results of an ANOVA test on a linear mixed effect model with soil pH as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	13.27	1.21	11	236	34.35	5.25E-43
Treatment	0.32	0.08	4	236	2.25	0.06
Date:Treatment	1.59	0.04	44	236	1.03	0.43

Table S4-28: Results of an ANOVA test on a linear mixed effect model with soil pH as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	6.63	1.66	4	96	95	1.65E-32
Treatment	0.22	0.06	4	96	3.22	**0.02**
Date:Treatment	0.33	0.02	16	96	1.19	0.29

Table S4-29: Results of post hoc Tukey's HSD on a linear mixed effect model with soil pH as the predictor variable and treatment and date as fixed effects for the second cycle.

1	estimate	SE	df	t.ratio	p.value
Compost - Compost 150	0.08	0.04	96	2.14	0.212
Compost - Control	-0.02	0.04	96	-0.54	0.983
Compost - NPK	0.02	0.04	96	0.64	0.968
Compost - (NPK-Compost)	-0.04	0.04	96	-1.18	0.764
Compost 150 - Control	-0.1	0.04	96	-2.68	0.065
Compost 150 - NPK	-0.06	0.04	96	-1.5	0.566
Compost 150 - (NPK-Compost)	-0.12	0.04	96	-3.32	**0.011**
Control - NPK	0.04	0.04	96	1.18	0.764
Control - (NPK-Compost)	-0.02	0.04	96	-0.64	0.968
NPK - (NPK-Compost)	-0.07	0.04	96	-1.82	0.368

Basal respiration

Table S4-30: Results of an ANOVA test on a linear mixed effect model with soil basal respiration flux as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Treatment	2172.33	543.08	4	116	2.71	**0.03**
Date	17441.94	3488.39	5	116	17.39	8.07E-13
Treatment:Date	5050.36	252.52	20	116	1.26	0.22

Table S4-31: Results of post hoc Tukey's HSD on a linear mixed effect model with soil basal respiration flux as the predictor variable and treatment and date as fixed effects for the first cycle.

Comparison	estimate	SE	df	t.ratio	p.value
Control - Compost	7.82	3.66	116	2.14	0.211
Control - Compost 150	5.03	3.66	116	1.38	0.644
Control - NPK	-1.65	3.66	116	-0.45	0.991
Control - (NPK-Compost)	-1.47	3.66	116	-0.4	0.994
Compost - Compost 150	-2.78	3.66	116	-0.76	0.941
Compost - NPK	-9.47	3.66	116	-2.59	0.079
Compost - (NPK-Compost)	-9.29	3.66	116	-2.54	0.089
Compost 150 - NPK	-6.68	3.66	116	-1.83	0.363
Compost 150 - (NPK-Compost)	-6.51	3.66	116	-1.78	0.39
NPK - (NPK-Compost)	0.18	3.66	116	0.05	> .999

Table S4-32: Results of an ANOVA test on a linear mixed effect model with soil basal respiration flux as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Treatment	150.83	37.71	4	76	0.45	0.77
Date	52763.25	17587.75	3	76	210.49	1.01E-36
Treatment:Date	689.92	57.49	12	76	0.69	0.76

Table S4-33: Results of a t-test for soil basal respiration flux between the first and second cycle at $p < 0.05$.

Comparison	Treatment	estimate	SE	df	t.ratio	p.value
First - Second	Compost	12.18	6.17	236	1.97	**0.05**
First - Second	Compost 150	15.32	6.17	236	2.48	**0.014**
First - Second	Control	21.3	6.17	236	3.45	**< .001**
First - Second	NPK	19.35	6.17	236	3.13	**0.002**
First - Second	NPK-Compost	20.49	6.17	236	3.32	**0.001**

Electrical conductivity (EC)

Table S4-34: Results of an ANOVA test on a linear mixed effect model with soil EC as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	0.06	5.46E-03	11	240	19.81	3.83E-28
Treatment	1.20E-03	3.01E-04	4	240	1.09	0.36
Date:Treatment	9.32E-03	2.12E-04	44	240	0.77	0.85

Table S4-35: Results of an ANOVA test on a linear mixed effect model with soil EC as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	3.89	0.97	4	96	382.12	5.00E-58
Treatment	3.01E-03	7.53E-04	4	96	0.3	0.88
Date:Treatment	0.03	1.95E-03	16	96	0.77	0.72

Moisture

Table S4-36: Results of an ANOVA test on a linear mixed effect model with soil moisture as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	4854.55	441.32	11	240	30.85	5.29E-40
Treatment	12.07	3.02	4	240	0.21	0.93
Date:Treatment	484.36	11.01	44	240	0.77	0.85

Table S4-37: Results of an ANOVA test on a linear mixed effect model with soil moisture as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	633.28	211.09	3	80	46.12	2.08E-17
Treatment	10.92	2.73	4	80	0.6	0.67
Date:Treatment	48.67	4.06	12	80	0.89	0.56

Temperature

Table S4-38: Results of an ANOVA test on a linear mixed effect model with soil temperature as the predictor variable and treatment and date as fixed effects for the first cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	2251.65	204.7	11	236	156.92	7.29E-102

Treatment	6.47	1.62	4	236	1.24	0.29
Date:Treatment	30.31	0.69	44	236	0.53	0.99

Table S4-39: Results of an ANOVA test on a linear mixed effect model with soil temperature as the predictor variable and treatment and date as fixed effects for the second cycle.

Fixed Effects	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Date	1894.69	473.67	4	96	198.54	1.65E-45
Treatment	0.72	0.18	4	96	0.08	0.99
Date:Treatment	14.99	0.94	16	96	0.39	0.98

Soil C

Table S4-40: Results of an ANOVA test on soil C between treatments grouped by soil sampling event and depth increment.

Sampling	Depth (cm)	F	p	sig	ges
Baseline	0-10	1.72	0.186	ns	0.26
First Cycle	0-10	1.07	0.398	ns	0.18
Second Cycle	0-10	0.83	0.52	ns	0.14
Baseline	10-30	0.93	0.468	ns	0.16
First Cycle	10-30	0.29	0.879	ns	0.06

Table S4-41: Results of a t-test for soil C in the 10-30 cm depth increment between the first and second cropping cycle at $p < 0.05$.

Treatment	Group 1	Group 2	n1	n2	statistic	df	p	sig
Control	Baseline	First Cycle	5	5	1.75	5.11	0.139	ns
Compost	Baseline	First Cycle	5	5	2.35	6.6	0.053	ns
Compost 150	Baseline	First Cycle	5	5	1.39	7.47	0.206	ns

NPK	Baseline	First Cycle	5	5	3.04	7.94	**0.016**	*
NPK-Compost	Baseline	First Cycle	5	5	0.99	7.99	0.353	ns

Soil N

Table S4-42: Results of an ANOVA test on soil N between treatments grouped by soil sampling event and depth increment.

Sampling	Depth (cm)	F	p	sig	ges
Baseline	0-10	2.02	0.13	ns	0.29
First Cycle	0-10	0.93	0.465	ns	0.16
Second Cycle	0-10	1.36	0.282	ns	0.21
Baseline	10-30	1.21	0.337	ns	0.2
First Cycle	10-30	0.36	0.831	ns	0.07

Table S4-43: Results of a t-test for soil N in the 10-30 cm depth increment between the first and second cropping cycle at $p < 0.05$.

Treatment	Group 1	Group 2	n1	n2	statistic	df	p	sig
Control	Baseline	First Cycle	5	5	1.87	5.97	0.11	ns
Compost	Baseline	First Cycle	5	5	2.34	5.51	0.061	ns
Compost 150	Baseline	First Cycle	5	5	1.63	7.45	0.144	ns
NPK	Baseline	First Cycle	5	5	3.36	7.28	**0.011**	*
NPK-Compost	Baseline	First Cycle	5	5	0.89	7.96	0.4	ns

Appendix D: Supplemental Information for Chapter 5

Translation of Creole words used in text

Gadyen: Caretaker

Ijans: Emergency or crisis

Ijans nivo oranj: Emergency level orange

Questions for the research team (English)

1. What did you enjoy about this project? What did you not enjoy about the project?
2. What did you think about the scientific objectives of the project?
3. What did you learn from this project?
4. What did you think about the protocols? Were they effective? We changed the protocols and scope of the experiment to account for the insecurity in Cap-Haitien. Do you agree with the changes made? Would you have made any additional changes?
5. What role did the guardian play in the project?
6. What were your expectations of the work requirements? How did the workload change due to the protests?
7. How did this research project relate to your personal academic and career goals? Did you achieve them? Why is this research important and interesting in general?
8. How did the protests affect your work? Did the protests impact your perspective on the importance of this research?
9. Did your relationship with the project team (fellow interns, Patrick & SOIL staff, Elena) change before the crisis and after the crisis? If so, how so?
10. How did communication and management change after Patrick left Haiti?
11. What were the biggest challenges for you from September through November? Why?
12. What do you feel was accomplished by the project and what do you believe was not accomplished by the project? Why?
13. If you were in charge, what would you have done differently?

14. (To Frantz) How did your experience differ between the first and second cycles?

15. (To Frantz) How did your role as a leader change due to the crisis?

16. What else would you like others to know about this project in general? What would you like others to know about working amidst a crisis?

17. Do you have other comments?

Questions for the research team (Haitian Creole)

1. Kisa ou te renmen nan pwojè sa a? Kisa ou pat renmen nan pwojè sa a?

2. Kijan ou dekri objektif syantifik pwojè a?

3. Kisa ou te aprann nan pwojè sa a?

4. Ki sa ou te panse sou pwotokòl/itinerè teknik yo? Èske yo te efikas? Nou te chanje pwotokòl yo akoz de ensekirite nan Okap. Èske w dakò ak chanjman ki fèt yo? Èske ou ta fè nenpòt chanjman adisyonèl?

5. Ki wòl gadyen an te jwe nan pwojè a?

6. Ki sa ou te espere nan kondisyon travay yo? Ki jan kantite travay la te chanje akòz manifestasyon yo?

7. Ki jan pwojè rechèch sa a te gen rapò ak objektif pèsonèl ou akademik ak karyè? Èske ou te reyalize yo? Poukisa ou panse rechèch sa a enpòtan jeneralman?

8. Ki jan manifestasyon yo te afekte travay ou a? Èske manifestasyon yo te afekte pèspektiv ou sou enpòtans rechèch sa a?

9. Èske relasyon w ak ekip pwojè a chanje (lòt estajyè yo, Patrick & moun SOIL, Elena, BRANA) anvan kriz la ak apre kriz la? Si se konsa, kòman sa?

10. Ki jan kominikasyon ak jesyon chanje apre Patrick te kite Ayiti?

11. Ki sa ki te pi gwo defi pou ou ant septanm e novanm? Poukisa? (Risk ak sekirite, kominikasyon, transpò, elatriye)

12. Kisa ou santi ou te akonpli pa pwojè a? E kisa ou kwè pat akonpli pa pwojè a? Poukisa?

13. Si w te responsab, kisa w t ap fè nan yon jan diferan?

14. (Pou Frantz) Ki jan eksperyans ou te diferan ant premye ak dezyèm sik la?

15. (Pou Frantz) Kòman wòl ou kòm lidè chanje akòz kriz la?

16. Ki lòt bagay ou ta renmen lòt moun konnen sou pwojè sa a an jeneral? Ki sa ou ta renmen lòt moun konnen sou travay nan mitan yon kriz?

17. Ou gen lòt kòmantè?