Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2022:48

# On-site treatment options for recovery of nutrients in dry toilets

## Lokale behandlingsmuligheter for gjenvinning av næringsstoffer i tørrtoaletter

Mariya Evgenieva Kelova

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Kelova, M., Ali, A., Dörsch, P., Kallenborn, R., Jenssen, P., 2021.
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**Paper III**

**Human excreta as a resource in agriculture – Evaluating the fertilizer potential of different composting and fermentation-derived products.** Kelova, M., Eich-Greatorex, S., Krogstad, T., 2021.
Resources, Conservation and Recycling 175.
https://doi.org/10.1016/j.resconrec.2021.105748

# Summary

Animal and human excreta had a major role in the development of agriculture in the past, but their role was lost with the development of the modern waterborne sanitation and inorganic fertilizers. To re-envision human excreta as a resource is a way to off-set some of the fertilizer needs and reduce the pressure of nutrient pollution to fresh water and marine environments. Among the existing sanitation systems, dry systems are more interesting from a resource-oriented management standpoint because they minimize the use of water and the volume of waste along with concentrating the nutrients. These toilets are commonly associated with a simple, rudimentary type of latrine but goals like ensuring availability and sustainable management of water and sanitation for all (SDG 6, UN) and to eliminate waste, circulate materials and regenerate nature require rethinking this perception. However, household level treatment options for dry sanitation systems are limited and there is insufficient understanding about how effective they are in a real-life context. Therefore, the main goal of this research was to improve the understanding of on-site treatment in dry toilets and how the nutrients can be recirculated to agriculture. Three main objectives were formulated: 1) to investigate which existing treatment options can be used for resource recovery, 2) to understand how treatment can be improved by comparing composting and lactic acid fermentation followed by vermicomposting, and 3) to evaluate and compare the nutrient content and fertilizer potential of the treatment products derived from the waste. The objectives were approached through a literature review (1) and lab scale experiments (2 and 3).

The literature review (Paper I) identified composting, vermicomposting, lactic-acid fermentation, chemical disinfection, and source separation of urine and feces as methods used to recover excreta-derived products in dry toilets with on-site treatment. Furthermore, new directions in treatment methods use high temperatures to transform the wet biomass of excreta to solid fuels and chars through processes such as drying, pelletizing, hydrothermal carbonization, and pyrolysis. Other developments use novel biological treatment methods such as anaerobic digestion, bioelectrochemical processes, or black soldier fly larvae composting. By defining the processes and the products with focus on practical considerations, the review illustrated that both the treatment methods and the excreta-derived products should be considered within a

complexity of external factors arising from particular environmental and socio-economic contexts. Dry toilets with on-site treatment are household-level technology and as such have a small size, little controlled treatment conditions, and are therefore limited by the prevailing environmental conditions. The treatment process and the products differ in space and time due to the variability in the composition of excreta, additional input materials, how the treatment is managed, and the local context. The products can be valuable resources locally but must be desired and their application regulated.

Lab experiments were used to understand how treatment can be improved and to evaluate the fertilizer potential of the treatment products. A controlled lab experiment was designed where excreta from a dry toilet were used in three mixtures, each composted or lactic acid fermented at three different ambient temperatures (7˚C, 20˚C, 38˚C), and then vermicomposted (Paper II). To evaluate the potential of the resulting products as fertilizer, they were applied as organic amendments in two experiments (Paper III). A soil incubation experiment was used to understand the mineralization dynamics of nitrogen and phosphorus, and a pot experiment with barley to demonstrate differences in yield and nutrient uptake under controlled conditions by comparing amendments, amendments supplemented with additional nitrogen, mineral fertilizer, and no fertilizer.

The lab scale comparison of composting and lactic acid fermentation illustrated that the degradation process is limited at low ambient temperatures (7˚C), whereas higher ambient temperatures (38˚C) during the composting supported higher microbial activity and active decomposition, resulting in lower numbers of fecal indicators and lower concentrations of pharmaceutical residues. The lactic acid fermentation was not affected by the difference in ambient temperatures (7˚C, 20˚C, 38˚C) and resulted in a substrate that had retained the initial high $NH_4$ content, but showed higher numbers of fecal indicators and pharmaceutical residues compared to the active composting at 38˚C. However, in comparison with the unsuccessful composting at 7˚C, lactic acid fermentation resulted in significantly lower numbers of *E. coli*. In our study, the vermicomposting was used as a secondary treatment after composting or fermentation, and the earthworm activity resulted in further stabilization and conditioning of the materials. Changes were more evident in the treatments where worms established

themselves better, which were those that were less successfully composted at 7°C or 20°C.

An evaluation of the fertilizer potential of the resulting organic amendments showed that the ranges of the concentrations of nutrients correspond to the ranges reported for other organic amendments such as municipal waste compost, and composted or vermicomposted manures. In a fertile loam soil, an application rate of 20 t $ha^{-1}$ would result in low application of immediately plant-available nitrogen (2 to 40 kg N $ha^{-1}$) and slow mineralization of organic nitrogen within 90 days, but substantial application of total phosphor and potassium. In a greenhouse experiment with barley as a test crop, an application rate of 20 t $ha^{-1}$ of the amendments resulted in yields which were higher than treatments without fertilizer but lower in comparison to conventional amounts of mineral fertilizer. The variation in yield between the amendments was highly correlated with initially available nitrogen, which was highest for those composted at high ambient temperatures (38°C). Our findings reveal that treatment method has a major effect on nitrogen retention and only a minor effect on other nutrients.

The outcomes of this work show that composting is limited at low temperature and lactic acid fermentation and/or vermicomposting could be alternatives or additions, particularly when composting is not successful. Our findings contribute towards better agronomic value quantification of products from on-site dry sanitation systems and the effect of treatment method on the availability of nitrogen. Given how common dry sanitation systems are in rural areas and areas without infrastructure, the results may contribute towards more sustainable management of the resources from those systems.

# Sammendrag

Ekskrementer fra dyr og mennesker hadde en stor rolle i utviklingen av landbruket tidligere, men deres rolle gikk tapt med utviklingen av moderne vannbaserte sanitærsystemer og mineralgjødsel. Å på nytt se for seg menneskelige ekskrementer som en ressurs er en måte å dekke noe av gjødselbehovet på og redusere faren for næringsforurensning i ferskvann og marine miljøer. Blant de eksisterende sanitærsystemene er tørre systemer mer interessante fra et ressursorientert forvaltningssynspunkt fordi de minimerer vannforbruket og avfallsvolumet samtidig som næringsstoffene konsentreres. Disse toalettene er ofte assosiert med en enkel, rudimentær type latrine, men mål som å sikre tilgjengelighet og bærekraftig forvaltning av vann og sanitær for alle (SDG 6, FN) og å eliminere avfall, sirkulere materialer og regenerere naturen krever en ny vurdering av denne oppfatningen. Imidlertid er behandlingsalternativer på husholdningsnivå for tørre sanitærsystemer begrenset, og det er manglende forståelse av hvor effektive de er i virkeligheten. Derfor var hovedmålet med denne forskningen å forbedre forståelsen av behandling av avfall fra tørrtoaletter på stedet og hvordan næringsstoffene kan resirkuleres til landbruket. Tre hovedmål ble formulert: 1) å undersøke hvilke eksisterende behandlingsalternativer som kan brukes for ressursgjenvinning, 2) å forstå hvordan behandlingen kan forbedres ved å sammenligne kompostering og melkesyregjæring etterfulgt av vermikompostering, og 3) å evaluere og sammenligne næringsstoffinnhold og gjødselpotensial i behandlingsproduktene som kommer fra avfallet. Målene ble nådd gjennom en litteraturgjennomgang (1) og forsøk i laboratorieskala (2 og 3).

Litteraturgjennomgangen (Paper I) identifiserte kompostering, vermikompostering, melkesyregjæring, kjemisk desinfeksjon og kildeseparasjon av urin og avføring som metoder brukt for å gjenvinne produkter fra ekskrementer i tørrtoaletter med behandling på stedet. Videre brukes høy temperatur i nye typer behandlingsmetoder for å omdanne den våte ekskrementmassen til fast brensel og kull gjennom prosesser som tørking, pelletering, hydrotermisk karbonisering og pyrolyse. Annen utvikling bruker nye biologiske behandlingsmetoder som anaerob fordøyelse, bioelektrokjemiske prosesser eller kompostering ved hjelp av svarte soldatfluelarver. Ved å definere prosessene og produktene med tanke på praktiske hensyn, viste review-

artikkelen at både behandlingsmetodene og produktene basert på ekskrementer bør vurderes innenfor komplekse eksterne faktorer som oppstår fra spesielle miljømessige og sosioøkonomiske kontekster. Tørrtoaletter med behandling på stedet er teknologi på husholdningsnivå og har som sådan liten størrelse, lite kontrollerte behandlingsforhold, og er derfor begrenset av de rådende miljøforholdene. Behandlingsprosessen og produktene er forskjellige i rom og tid på grunn av variasjonen i sammensetningen av ekskrementer, tilleggsmaterialer, hvordan behandlingen håndteres og den lokale konteksten. Produktene kan være verdifulle ressurser lokalt, men må være ønsket og anvendelsen må være regulert.

Laboratorieforsøk ble brukt for å forstå hvordan behandlingen kan forbedres og for å evaluere gjødselpotensialet til behandlingsproduktene. I et laboratorieforsøk under kontrollerte forhold ble ekskrementer fra et tørrtoalett brukt i tre blandinger, kompostert eller melkesyregjæret hver ved tre forskjellige omgivelsestemperaturer (7˚C, 20˚C, 38˚C), og deretter vermikompostert (Papir II). For å evaluere gjødselpotensialet til de resulterende produktene, ble de tilført som organisk gjødsel i to forsøk (Paper III). Et inkubasjonsforsøk i jord ble brukt for å forstå mineraliseringsdynamikken til nitrogen og fosfor, og et potteeksperiment med bygg for å demonstrere forskjeller i avling og næringsopptak under kontrollerte forhold ved å sammenligne produktene med eller uten ekstra nitrogen, mineralgjødsel og ingen gjødsel.

Sammenlikningen av kompostering og melkesyregjæring på laboratorieskala illustrerte at nedbrytningsprosessen er begrenset ved lave omgivelsestemperaturer (7˚C), mens høyere omgivelsestemperaturer (38˚C) under komposteringen støttet høyere mikrobiell aktivitet og aktiv nedbrytning, noe som resulterte i lavere antall fekale indikatorer og lavere konsentrasjoner av farmasøytiske rester. Melkesyregjæringen ble ikke påvirket av forskjellen i omgivelsestemperaturer (7˚C, 20˚C, 38˚C) og resulterte i et substrat som hadde beholdt det opprinnelige høye $NH_4$-innholdet, men viste høyere antall fekale indikatorer og farmasøytiske rester sammenlignet med aktiv kompostering ved 38˚C. Sammenlignet med den mislykkede komposteringen ved 7˚C, resulterte imidlertid melkesyregjæring i betydelig lavere antall *E. coli*. I vårt forsøk ble vermikomposteringen brukt som en sekundær behandling etter kompostering eller gjæring, og meitemarkaktiviteten resulterte i ytterligere stabilisering og kondisjonering

av materialene. Endringer var mer tydelige i behandlingene der meitemark etablerte seg bedre, som var de hvor komposteringen var mindre vellykket ved 7˚C eller 20˚C.

En evaluering av gjødselpotensialet til de resulterende organiske produktene viste at nivået av næringsstoffkonsentrasjon samsvarer med nivået rapportert for andre organiske gjødselmidler som kompost basert på kommunalt avfall og kompostert eller vermikompostert husdyrgjødsel. En påføringsmengde på 20 t $ha^{-1}$ til en fruktbar leirjord vil resultere i lav tilførsel av umiddelbart plantetilgjengelig nitrogen (2 til 40 kg N ha-1) og langsom mineralisering av organisk nitrogen i løpet av 90 dager, men betydelig påføring av totalt fosfor og kalium. I veksthusforsøket med bygg som prøvevekst, resulterte en tilførselsmengde på 20 t $ha^{-1}$ ekskrementprodukt i avlinger som var høyere enn behandlinger uten gjødsel, men lavere sammenlignet med konvensjonelle mengder mineralgjødsel. Variasjonen i avling mellom produktene var sterkt korrelert med opprinnelig tilgjengelig nitrogen, som var høyest for produktene fra kompostering ved høye omgivelsestemperaturer (38˚C). Våre funn viser at behandlingsmetode har stor effekt på nitrogenretensjon og kun en liten effekt på andre næringsstoffer.

Resultatene av dette arbeidet viser at kompostering er begrenset ved lav temperatur og melkesyregjæring og/eller vermikompostering kan være alternativer eller tilleggsbehandlinger, spesielt når kompostering ikke er vellykket. Våre funn bidrar til bedre agronomisk verdikvantifisering av produkter fra tørre sanitetssystemer med lokal behandling og effekten av behandlingsmetode på tilgjengeligheten av nitrogen. Gitt hvor vanlige tørre sanitærsystemer er i landlige områder og områder uten infrastruktur, kan resultatene bidra til mer bærekraftig forvaltning av ressursene fra disse systemene.

# Synopsis

# 1 Introduction

In September 2015, world leaders at the UN unanimously adopted the Sustainable Development Goals (SDGs), a set of goals and targets to guide all countries in addressing the world's most pressing challenges. Among them are SDG-6 which aspires for availability and sustainable management of water and sanitation for all and SDG-2 which aims to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture. Yet, in 2020 the Joint Monitoring Programme reported that nearly half the world's population still lack safely managed sanitation services (WHO/UNICEF, 2021). In rural areas the sanitation coverage is lower with only 44 % covered by safely managed services. Simultaneously, in 2020 30 % of the global population were affected by moderate or severe food insecurity (FAO, 2021a) and the invasion of the Russian federation in Ukraine could amplify the problem. While the causes are complex, including poverty, inequalities and COVID - 19, they are inevitably linked to food production and distribution systems. The greatest possibility for higher agricultural outputs globally is determined by the development and produce of the rural smallholder farmers (Giller et al., 2021; Lowder et al., 2016). There is a premise that improving the resource management in rural locations could bring many benefits and address multiple sustainability targets.

One of the guaranteed and often ignored resources are human excreta. Animal and human excreta had a major role in the development of agriculture in the past, but their role was lost with the development of the modern waterborne sanitation and inorganic fertilizers (Ferguson, 2014). That way the nutrients were taken away from the soils, shifted to the water bodies and substituted with inorganic fertilizers. The intensive crop production which is sustaining the growing population today is unthinkable without the inorganic sources of N, P and K but their availability and price is governed by limited geological resources and geopolitics (Camprubi, 2015; Cordell et al., 2009; Manning, 2015), and their use is associated with decreasing organic matter contents of soils and associated deterioration/negative consequences (Menšík et al., 2018). At the same time, wastewater treatment plants employ resources to remove the N and P before effluent discharge. But not all wastewater is treated beyond primary screening, estimations show that 44 % of the household wastewater is discharged without treatment (UN,

2021) and have resulted in the deterioration of multiple aquatic ecosystems (Lapointe et al., 2015; Nyenje et al., 2010). Substantial parts of the sanitation efforts and research so far aimed at limiting the nutrient and pollution load of the effluent, but research and policy attention is shifting from pollutant removal to resource-oriented management (Domenech and Bahn-Walkowiak, 2019; Harder et al., 2019). To envision human excreta as a resource is a way to off-set some of the fertilizer needs and reduce the pressure of nutrient pollution to fresh water and marine environments.

The prevailing paradigm in sanitation is centralized with sewers and treatment plants but the importance of on-site sanitation is increasingly recognized. Estimations on safely managed sanitation coverage show that in 2020 more people are serviced by on-site system in comparison to those serviced by sewered sanitation (WHO/UNICEF, 2021). These people are predominantly in rural locations and in low- and middle-income countries (WHO/UNICEF, 2021). The on-site sanitation systems and the management of the collected fecal sludge have gained more visibility in the last two decades which is reflected in the increased research and policy interest (Klingel et al., 2002; Ministry of UrbanDevelopment-India, 2017; Strande and Brdjanovic, 2014; Velkushanova et al., 2021). This highlighted challenges due to data collection, variability, and also the need for an integrated approach and regulation for the management of on-site systems but the main focus has been on urban centralized or semi-centralized management (Strande and Brdjanovic, 2014). The "Reinvent the toilet challenge", funded by the Bill & Melinda Gates foundation, has spurred research into innovative solutions for technologies combining collection, treatment within the latrine or close by, and valuable products as outputs (Bill & Melinda Gates Foundation, 2013). However, those solutions are still at lab and pilot scale, and the proposed advanced technologies could be challenged when put in real context, therefore, so far not providing viable alternatives to what is currently in use.

Among the existing sanitation systems, dry systems are more interesting from a resource-oriented management standpoint. They minimize the use of water and the volume of waste along with concentrating the nutrients. Water is a precious resource that is under increasing pressure (Mekonnen and Hoekstra, 2016) and it holds more value clean than recycled (Larson, 2020). Similarly, there is a clear argument that dilution of excreta with water is counteractive to its management and recycling. Using

drinking water to convey the excreta is a convenience that is not achievable and sustainable everywhere.

However, household level treatment options for dry sanitation systems are limited and there is insufficient understanding about how effective they are in a real-life context. Among the reasons are the variability of the input (excreta and amendments), the variety of designs and treatment methods that can be utilized, and what is the impact of different environmental conditions (Bhagwan et al., 2008; McKinley et al., 2012a; Rose et al., 2015). Accordingly, the safety, quantity, and quality of the derived products will vary from one application of the sanitation technology to another. Research on the utilization of excreta-derived products is scarce and even though they are commonly used as a soil conditioner or fertilizer, little is known about how different treatments compare, the effects of treatment conditions outside of the optimum ranges for successful treatment, and what could determine or limit their fertilizer value. The current evidence suggests a positive effect from applications, but the focus has been on quantification of macronutrients and yield (Moya et al., 2019a; Sangare et al., 2015; Winker et al., 2009). The research gaps limit the confidence of institutions concerning the safe management of excreta and accordingly the products' value, and short- and long-term consequences from their utilization (McConville et al., 2017). Better understanding how environmental conditions affect treatment, what can limit the effectiveness, and how that is reflected in the product and its fertilizer value is a step towards building the confidence of institutions, sanitation and agriculture practitioners, and users in the excreta-derived products.

# 2 Aims and objectives

The main goal of the research was to improve the understanding of on-site treatment in dry toilets and the application of the resulting materials to agriculture. From the main goal three main objectives were formulated. The first objective was to investigate what treatment options exist that can be used for resource recovery on-site in dry toilets. The second objective was to understand how treatment can be improved by comparing composting and LAF followed by vermicomposting. The third objective was to evaluate and compare the nutrient content and fertilizer potential of the treatment products derived from the dry toilet waste.

The objectives were approached through lab scale experiments and a literature review. To achieve the objectives, research focused on:

- Critical review of the existing methods for resource recovery on-site in dry toilets with focus on practical considerations and challenges that need to be addressed to implement them more successfully with long-lasting results (Paper I)
- Examining and comparing biological transformations of human excreta through composting and lactic acid fermentation (LAF), followed by vermicomposting. (Paper II)
- Investigating the importance of the ambient temperature for the processes. (Paper II)
- Assessing and comparing the product characteristics in terms of stability, nutrient content, and safety (fecal indicators, pharmaceutical residues). (Paper II)
- Evaluating the fertilizer potential of the products. (Paper III)

# 3 Background

## 3.1 On-site sanitation

Sanitation can be broadly defined as a barrier between humans and their waste that can be established through facilities and services, a process, or conditions (Tilley et al., 2014; WHO, 2018), which should provide a hygienic environment and protect human health. The management of excreta is part of the wastewater management which includes collection, transport or conveyance, treatment, and disposal or/and reuse of liquid wastes and specifically wastewater. The conventional domestic wastewater management is centralized with a sewer system for collection and transport, a treatment plant for removal of pollutants and effluent discharge to a water body. Such systems, however, serve only 34 % of the world population (WHO/UNICEF, 2021). On-site sanitation is used to describe technology or systems in which the wastewater is managed (collected, stored, emptied, or treated) on the plot where it is generated (WHO, 2018).

Due to a large fraction of the population without "safe" sanitation, it is expected that there will be an increase in on-site facilities rather than centralized sanitation with sewer connections (WHO/UNICEF, 2021). For rural areas and in low- and middle-income countries without high coverage of sewer connections, decentralized and on-site sanitation are more common and practical. It would not be reasonable to consider sewers and centralized treatment a feasible option for the regions that are currently behind in sanitation coverage. Estimates show that those regions are predominantly in low and low-middle income countries and prevalent in rural locations (WHO/UNICEF, 2021). On-site sanitation facilities are predominant also in developed countries where the infrastructure is missing, unfunctional, or does not make economic sense, as often is the case in rural locations.

In contrast to the conventional sewer systems, where all wastewater streams (domestic and communal) are mixed, on-site facilities can be more easily designed or re-designed to separate the flows because they are independent of sewer infrastructure and can separately convey and treat specific wastewater streams. Source separation enables better treatment and more possibilities for recycling of the waste (Larsen et al., 2009).

The domestic wastewater streams are two – water used for washing (sinks, shower, laundry etc.) and toilet wastewater. Depending on the input, the toilet waste can be characterized as black water (human urine, feces, and menstrual blood mixed with flush and cleansing water), brown water (separately collected feces mixed with flush and cleansing water), and yellow water (separately collected urine mixed with flush or cleansing water), excreta (human urine, feces, and menstrual blood), or urine and feces.

Recent efforts towards greater sustainability also resulted in better recognition of the resources in sanitation systems, from reduction of inputs such as water, infrastructure, and transport, to recovery of resources. Reducing the amount of water for conveyance as well as the infrastructure and transport are ways to increase the sustainability and first principle in the circular economy. Recoverable resources from wastewater are water, nutrients, organic matter, energy, minerals and trace elements, and can be found in different waste streams (Figure 1). On-site sanitation and separation of the flows could promote more efficient recovery. A detailed review of the processes and pathways for recovery can be found in Harder et al. (2019). From the variety of possible products, however, nutrients and organic matter have been identified to have greater significance towards achieving the SDGs (Trimmer et al., 2017) and to long-term soil, food, and nutrient security (Harder et al., 2020).

## 3.2 Dry toilets

Dry toilets are the interface of sanitation systems that do not use flush water to convey the human excreta. Dry toilets are still common in rural areas, areas without infrastructure, and in regions with water scarcity. Those toilets are commonly associated with a simple, rudimentary type of latrine but goals like ensuring availability and sustainable management of water and sanitation for all (SDG 6, UN) and to eliminate waste, circulate materials and regenerate nature (Ellen MacArthur Foundation, 2022) require rethinking this perception due to its sustainability. Dry toilets with containment and treatment/safe disposal on-site are the least demanding infrastructure and investment, which makes them a more feasible (i.e., easily available) option for those currently lacking sanitation. Moreover, water is a resource that is under increased pressure due to changing climate and the flush toilet is among the big consumers of drinking water at a household level (Bradley, 2004). Diluting the excreta with water increases the burden of fecal contamination and eutrophication of

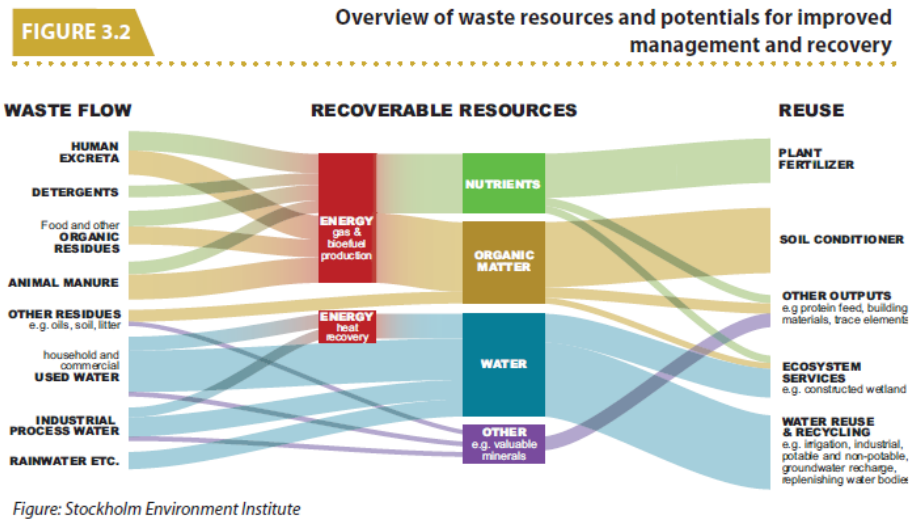



Figure 1. Overview of waste resources and potentials for improved management and recovery. From: Andersson and Rosemarin, (2016): Sanitation, Wastewater Management and Sustainability: from Waste Disposal to Resource Recovery. Nairobi and Stockholm: United Nations Environment Programme and Stockholm Environment Institute.

water ecosystems due to planned and accidental discharges in the environment (Lapointe et al., 2015; Van Drecht et al., 2009). The volume of human excreta is a small fraction of the average volume of black water. On average a human excretes urine in the range of 0.6 to 2.6 L $cap^{-1}$ $day^{-1}$ and feces within the range of 51 to 796 g $cap^{-1}$ $day^{-1}$ (Rose et al., 2015), whereas the flush water is three to six liters per flush. It is, therefore, easier to manage, to treat, to store and recycle separated excreta and close the loop by returning nutrients and organic matter back to the environment for better soil quality plant or food production.

The dry toilet is only the user interface of the system, and it could be combined with a variety of technologies for conveyance, treatment, and disposal or application. The collected excreta can be managed in a central way by off-site treatment of the fecal sludge or by the recently developed management model of container-based sanitation, a service which provides sealable, removable containers for collection of human excreta on a regular basis and transport them to treatment facilities (Tilmans et al., 2015).

The existing technology options for treatment on-site in dry toilets are pit latrines, or single or double vault latrines for collection with disposal; Arborloos and Fossa Alterna for collection with partial treatment; and composting and urine diversion dry toilets (UDDTs) for collection, on-site treatment, and resource recovery (Tilley et al., 2014). The most common technology combined with dry toilets is a pit (pit latrine) (Nasim et al., 2022; Strande et al., 2014) where the material is stored and accumulated and once full can be emptied and treated off-site. Rudimentary alternatives such as Arborloo and Fossa Alterna are technologies in which the material is left in the soil to degrade, therefore limiting the disposal to water bodies but without intentional treatment or a product that can be utilized. More recent methods for treatment of excreta include vermicomposting and lactic acid fermentation (LAF; e.g., Terra Preta sanitation), as well as disinfection with lime and urea (Buzie-Fru, 2010; Factura et al., 2010; Niwagaba et al., 2009a; Nordin et al., 2009b). Furthermore, increased attention to the resources in excreta has driven innovations in treatment options for resource recovery such as electrochemical systems, anaerobic digestion, black soldier fly larvae composting, and thermal treatments (Andriessen et al., 2019; Lalander et al., 2013a; Lansing et al., 2017; Leicester et al., 2020). The Bill and Melinda Gates funding the "Reinvent the Toilet" challenge also incentivized innovations like the use of microwaves, electrochemical processes, and pyrolysis for on-site treatment within the latrine, but their development is still at lab or pilot scale (Bill & Melinda Gates Foundation, 2013).

Dry composting toilets are considered one of the best current options for on-site treatment in terms of resource recovery (J. R. McConville et al., 2020; Orner and Mihelcic, 2018). However, composting is not always successful, and the resulting material is usually neither stabilized nor sanitized (Hill et al., 2013b; Niwagaba et al., 2009b). A few studies have addressed this important aspect by examining how to improve the treatment physically or chemically by e.g., solar heating (Redlinger et al., 2001), different bulking materials (Hashemi et al., 2019; McKinley et al., 2012b) and/or amendments such as biochar (Hijikata et al., 2015) or urea (Vinnerås, 2007). Others have focused on modifying the treatment by vermicomposting (Yadav et al., 2011), lactic acid fermentation (LAF) (Andreev et al., 2018) or fly larvae composting (Lalander et al., 2013a).

Combinations of treatments are considered promising. Integrated composting-vermicomposting has been investigated for a variety of organic wastes (Lim et al., 2016) including fecal slurry (Yadav et al., 2012). The material is first sanitized by thermophilic composting and conditioned further with earthworms to improve the quality of the end product. Pre-composting facilitates better conditioning because earthworms are vulnerable to thermophilic temperatures and toxic compounds in the organic wastes (Yadav et al., 2012). Another treatment combination for on-site sanitation is pre-treatment with LAF followed by thermophilic composting (Andreev et al., 2016) or vermicomposting (De Gisi et al., 2014). LAF is easy to manage and reduces quickly fecal pathogens, while the organic matter and nutrients are retained (Odey et al., 2018a). However, LAF alone does not sufficiently stabilize and sanitize fecal matter and further treatment is needed before application as soil conditioner or fertilizer (Andreev et al., 2018). The combination of LAF and vermicomposting is part of the Terra Preta sanitation concept, which is inspired by ancient practices of organic waste management for soil fertility in the Amazon region (De Gisi et al., 2014). Central to the Terra Preta sanitation concept is the addition of carbonaceous pyrogenic material, i.e., biochar to retain nutrients and increase the product value for improving soil health and fertility. Biochar amendment in organic waste treatment has been shown to have benefits for agricultural application, with respect to retention of nutrients and pollution remediation (Wu et al., 2017). However, the efficiency for pollutant removal has not yet been assessed.

## 3.3 Linking sanitation and agriculture

One of the big challenges now and in the future is the provision of adequate food for the growing population. The UN goals of eradicating hunger and achieving food security are, among other factors, most recently challenged by the COVID-19 pandemic and the restrictive prices and availability of mineral fertilizers (FAO, IFAD, UNICEF, 2021).

Mineral fertilizers have been essential for the increase in crop production during the last decades (increase of 53 % of the production of primary crops from 2000 to 2019 according to (FAO, 2021b), and their use has increased with 40 % from year 2000 to 2019, totaling 189 million tones (as total N, $P_2O_5$, $K_2O$)(FAO, 2021b). The use of mineral fertilizers, however, is linked with two burdens: risks of environmental degradation and pollution, and food and economic insecurities due to fluctuating availability and prices.

On the one hand, the production of mineral fertilizers is based on mining and fossil fuels and the excessive application has altered the natural biochemical flows to an extent that some consider exceeds the planet threshold (Steffen et al., 2015). On the other hand, the raw materials in the production are limited resources and as such their availability and price is governed by complex factors including geopolitics. The high energy prices, trade restrictions and policies were among the main drivers for the sharp increase in the price of inorganic fertilizers during 2021 (Cross, 2022). The current invasion of Russia in Ukraine can drive the demand and prices even higher, as Russia is among the top five producers of N, P and K (FAO, 2021c) Futureproofing agriculture requires investment in better management and distribution of nutrients together with smart management of soil and water resources for healthy agro-ecosystems that can sustain better production without deterioration of the environment. A step towards addressing those challenges is to utilize the nutrients in our excreta.

Only a small part of the nutrient content of the food is retained by the organism of an adult, and the nitrogen and phosphorus excretion is at or near 100 % of the intake (Jönsson et al., 2004; Rose et al., 2015). Returning these nutrients back to the soil and food production is important for nutrient cycling and soil quality. Currently, only a small part is recycled to land application, while the majority is disposed of as a waste driven to landfills or as an effluent discharged to water bodies (Harder et al., 2019; Trimmer et al., 2017; Xu et al., 2020). As a result, the nutrients and the organic matter create a burden to the receiving environments, while they are lost to soils and food production. The recycling of human excreta not only provides reduction of waste and pollution but could also close the loop between agriculture and sanitation and could address future challenges for fertilizer availability and better nutrient management.

## 3.4 Human excreta as fertilizer

Human excreta have been utilized extensively in the past to maintain soil fertility in rural regions (Ferguson, 2014; Rockefeller, 1998), and their potential as fertilizer has been well identified (Heinonen-Tanski and Van Wijk-Sijbesma, 2004; Jönsson et al., 2004; Musazura and Odindo, 2022). Today the prevailing paradigm is waterborne and centralized sanitation where the recognition of the resources in excreta have led to the development of different products that can be utilized in agriculture, among which are single and multi-nutrient solutions, precipitates, sorbents, and sludge products such as

biosolids, biogas digestate, compost, fodder (BSFL), or chars (Harder et al., 2019). Advanced engineering and expert control over the treatment processes, therefore, yield a variety of products with predictable quality and parameters. However, those are less applicable for dry toilets with on-site treatment, especially in rural and poor areas. On the one hand the management and operation are in the hands of the user, and on the other hand the product depends on local practice, diet, and amendments.

Most of the food and water consumed by a person is excreted as urine and feces and their composition varies according to the diet and the water balance in the human body. Considerable variation in the concentration of elements in excreta have been reported and ranges for the major elements are presented in Rose et al., (2015). The essential plant macronutrients N, P and K are excreted in greater quantities with urine, whereas the feces are high in organic matter and richer in P, Ca, and Mg (Jönsson et al., 2004; Krause et al., 2021; Rose et al., 2015). Human excreta are established as nutrient-rich raw material with fertilizer potential (Jönsson et al., 2004; Krause et al., 2021; Tran-Thi et al., 2017). But turning them into organic fertilizer as a mixed stream (urine and feces), requires considerations regarding the salt content, as well as an additional carbon source to achieve an appropriate C:N ratio and prevent N loss through leaching or volatilization (Krause et al., 2021). Additional C rich additives are commonly used to facilitate degradation or composting, to sorb liquids or prevent odors and flies.

Human excreta also contain hazards like pathogens and environmental contaminants such as heavy metals and organic pollutants. A large percentage of the excreta dry matter is bacterial mass (25-54 %) including pathogens (Krause et al., 2021). One gram of feces can contain $10^9$ infectious virus particles without the human host necessarily exhibiting clinical signs (Feachem et al., 1984). Therefore, an important part of the management of excreta is minimizing the risk of pathogen transmission to the environment. Pathogenic organisms are eliminated mainly through the treatment, but the risks can be prevented or reduced at multiple points of the management including the handling and the way the excreta-derived products are utilized or disposed of (WHO, 2006). Heavy metals and microplastics are generally found in lower concentrations in human excreta compared to wastewater or other organic wastes and fertilizers. For this reason, their concentrations are not a cause of concern in products from dry toilets and their application as fertilizer (Krause et al., 2021). The organic pollutants that can be

found in human excreta are predominantly pharmaceutical residues. They are pollutants which include a multitude of compounds with a fate that is complex to determine as it depends on the properties of the compound, the environmental matrix and conditions. There is not sufficient knowledge currently to evaluate the risks, but it has been shown that for most compounds, the degradation in soils is better than in water, and only relatively small amounts have been found taken up by plants, which would not pose risk to human health (Krause et al., 2021; Viskari et al., 2018).

When human excreta are applied to soil to supply plant nutrients they are used as an organic fertilizer. Direct application is associated with high risk of pathogen transmission and environmental contamination, but it should be noted that pathogens are excreted mainly with feces and the risk of using source-separated urine is low (WHO, 2006). Traditionally used methods to transform the excreta into organic fertilizers are based on biological degradation such as composting and fermentation, similar to what are the most common and established methods for organic wastes and animal manures (Heinonen-Tanski and Van Wijk-Sijbesma, 2004; Li et al., 2020; Zabaleta et al., 2020). The most common traditional treatment method used in on-site dry sanitation systems is composting, other methods for biological degradation and stabilization with links to practices from the past are vermicomposting and lactic acid fermentation (De Gisi et al., 2014). During such processes, the organic matter composition is altered through the metabolism of the microorganisms and/or invertebrates (worms). The agronomic value of the resulting products can be expected to differ, and an important determinant is the fate of the nutrients.

Among all essential plant nutrients, N is the element with the highest turnover during organic matter degradation, during treatment, and after application to soils. Aerobic treatment methods are associated with high N losses due to ammonia volatilization and nitrate leaching, but the N fate is dependent of multiple factors such as temperature, pH, available carbon, and microbial activity (Tiquia, 2002). Those factors can increase the losses or be used to prevent them. Lactic acid fermentation, on the other hand, preserves N and the end product has a higher $NH_4$ concentration, but the product requires further treatment to become suitable for agricultural application, and the process is often combined with composting or vermicomposting (Andreev et al., 2018). For other elements that are not a main part of the microbial metabolic respiration, aerobic organic

matter degradation typically results in a concentration effect due to the C mineralization of organic matter, with marginal losses through volatile compounds or leaching (Bernal et al., 2009; Eneji et al., 2001). However, the extent of the nutrient changes in biological transformation is dependent of multiple factors such as inputs and management, and environmental conditions. The availability and intake by plants is even more complexly regulated and dependent on soil type, crop demands, environmental conditions, and time of application.

# 4 Methods

## 4.1 Treatment of excreta from dry toilets

Two strategies were chosen to understand the limitations and improve on-site treatment. The first strategy was to improve on-site composting by understanding how it is influenced by ambient temperature and the second to compare it to selected alternatives: addition of biochar, LAF, and a follow-up step of vermicomposting. The temperatures were chosen to represent cold climate (7˚C), warm climate (20˚C), and additional heating (38˚C). Composting, LAF, and vermicomposting are established methods for organic waste transformations and traditionally used to transform animal manures and human excreta into organic fertilizers, and are established methods for organic wastes and animal manures. Therefore, direct comparison can evaluate their efficiency and quality of the product at different temperature under controlled conditions.

An initial pre-experiment was used to design and evaluate the suitability of the reactors, establish ratio of materials, C/N ratio, and moisture for optimal microbial degradation and to identify the period of peak microbial activity. Based on the results of the initial study, a controlled lab experiment was designed where excreta from a dry toilet were used in three mixtures, one for composting (C), one for composting with biochar (CB) and one for LAF (F). Each mix was composted/fermented at three different temperatures for 71 days, then vermicomposted at room temperature for 77 days (Figure 2). To condition the material for vermicomposting, an intermediate step of 15 composting days at room temperature was used before the vermicomposting.

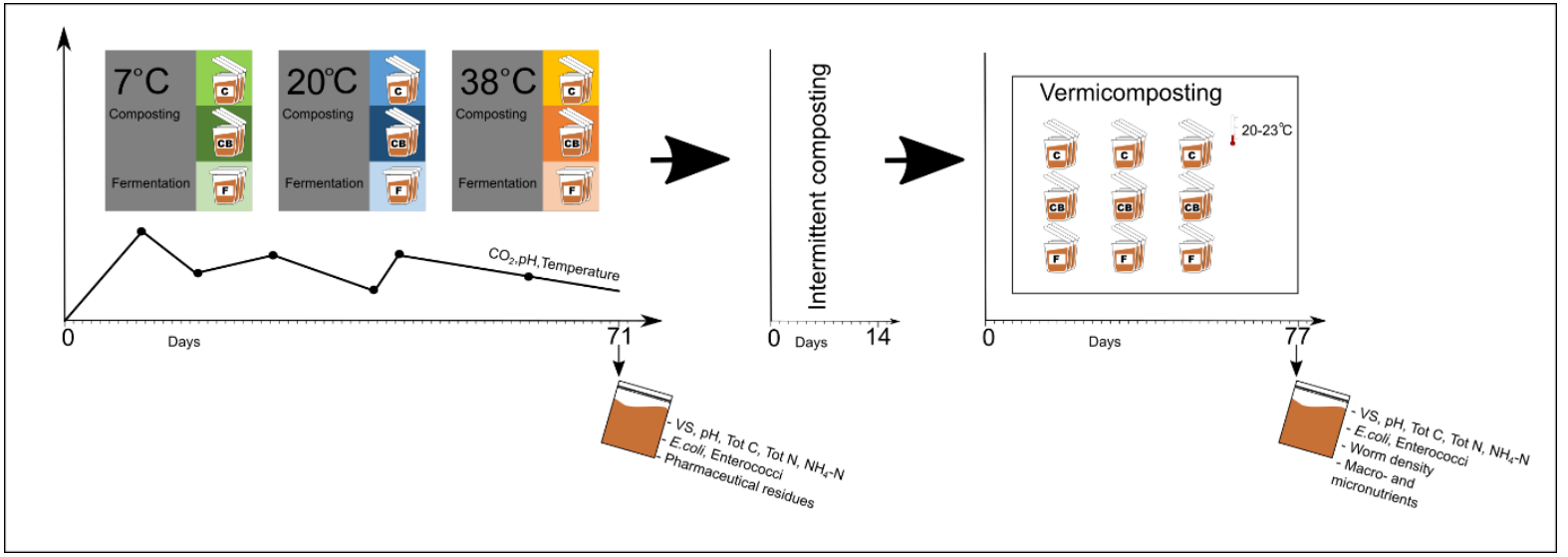



Figure 2. Composting/LAF of excreta followed by vermicomposting - overview of the experimental setup, treatments, and sampling time.

### 4.1.1 Initial mixtures, materials

The fecal matter was acquired from one collection compartment servicing five dry toilets at Åstjern cabin complex, Bleiken, Norway (Figure 3). The fecal sludge was accumulated over two years with daily fresh inputs until the day of collection. The urine was collected separately from a nearby farm household and stored in closed containers prior to use in the experiment. The sanitary bark was a finely cut (0-15mm) coniferous bark from a commercial producer (Nordic Garden, Steinshold, Norway). The biochar was collected from a Pyreg pilot pyrolysis plant (established as part of the "Stockholm Biochar Project," (2018) and using garden waste as a substrate for the process) and was added to the corresponding mixtures at approximately 5 % of the volume of the total volume. In the composting mixtures, compost from a preliminary trial with fecal matter, bark and food waste was used as inoculant, whereas in the fermentation treatment, sauerkraut juice was used as inoculant. The substrates were combined and mixed in a cement mixer (Atika, model Comet 130 S, Ahlen, Germany) (Figure 3) and divided by weight in the reactors (Table 1). The resulting mixtures were labeled with C for compost, CB for composting with biochar, and F for fermentation.

Table 1. Initial substrate composition presented as average wet weight per reactor. Table 1 from Paper II.

| | **Fecal matter and sanitary bark (C)** | **Fecal matter, sanitary bark and biochar (CB)** | **Fecal matter, sanitary bark and biochar, for fermentation (F)** |
|---|---|---|---|
| Fecal material (kg) | 3.2 | 3 | 3.2 |
| Sanitary bark (kg) | 1.7 | 1.6 | 1.4 |
| Compost inoculant (kg) | 0.13 | 0.13 | 0 |
| Urine (L) | 0.75 | 0.75 | 0.7 |
| Water (L) | 0.5 | 0.47 | 0.25 |
| Biochar (kg) | - | 0.28 | 0.3 |
| Lactic acid bacteria inoculant - Sauerkraut juice (L) | - | - | 0.3 |
| Total (kg) | 6.3 | 6.2 | 6.25 |


Figure 3. Left – the dry toilet collection compartment. Right – Mixing the substrates in a cement mixer.

### 4.1.2 Composting reactors and experimental set-up

The reactor size was chosen, as a compromise between real-world size for on-site sanitation from a single toilet and the need for multiple replicates under controlled conditions. The experimental reactors were modified 16 L bokashi bins. The modification for the composting reactors was to connect the drainage bottom compartment to a liquid trap and to a vacuum pump and to facilitate measurements of $CO_2$ production (Figure 4). LAF reactors were not modified. The composting substrate was subjected to a negative pressure aeration with a vacuum pump (Mini Diaphragm Vacuum Pump LABOPORT, model N86 KN.18, KNF, Freiburg, Germany), which was operated on a time regime of 15 min on, followed by 30 min off. For LAF the reactors were incubated statically without aeration and with a closed lid. The moisture in the composting substrate was maintained by periodically returning the leachate collected in the liquid traps and by sprinkling with tap water. The reactors were placed in three climate-controlled rooms, maintaining ambient temperatures of 7, 20, and 38˚C. At each temperature there were nine reactors, three replicates of each treatment mix. After 71 days, the material from each reactor was emptied into another container, thoroughly hand-mixed with gardening tools and sub-sampled for analyses.

Before vermicomposting, the reactors were mixed and sampled and left open for two weeks to compost at room temperature without forced aeration to increase pH and remove some of the $NH_3$. Thereafter, 150 red wiggler worms *Eisenia fetida*, provided by the industrial waste treatment and recycling facility Lindum, Drammen (Norway), were placed in each reactor. The reactors were kept moist, open and at room temperature (23˚C). After 77 days, the material from each reactor was emptied into another container, the earthworms were counted and removed, the material was thoroughly mixed with gardening tools and sampled. The method of counting did neither differentiate between development stages of the earthworms nor include eggs.

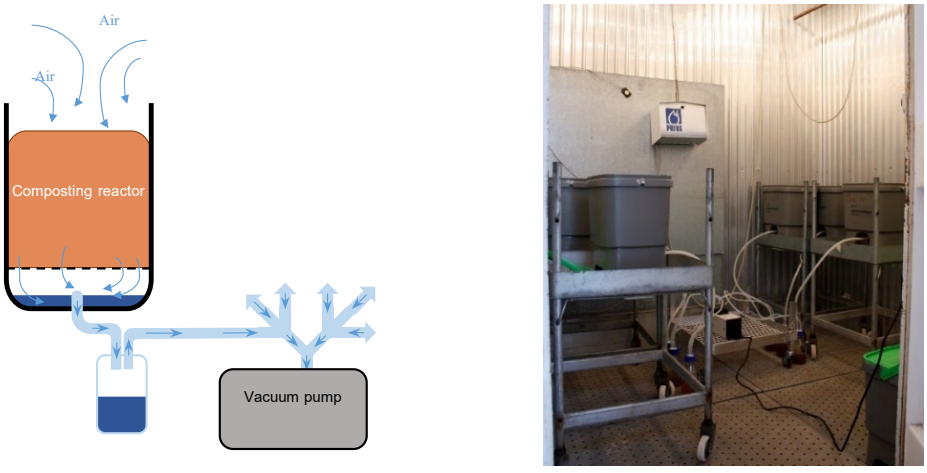



Figure 4. Left - Schematic representation of the composting reactor setup, modified Figure 1 from Paper II. Right – Photo of the reactors' setup in a climate-controlled room

### 4.1.3 Microbial activity during composting

Substrate temperature and $CO_2$ production were used as indicators for microbial activity during composting in treatments C and CB. The temperature was measured in the center of the composting matrix by a HOBO Pendant Temperature Data Logger (Onset, Bourne, USA, 0.5˚C accuracy). $CO_2$ production was used as an indicator for respiration and measured as $CO_2$ accumulation in the reactor headspace using a

portable $CO_2$ infrared gas analyzer (EGM-5, PP-systems, Amesbury, USA, dynamic range 0-100000 ppm). It was measured daily for the first 15 days, and every second day for the remainder of the 71-day period. During measurements, the gas analyzer was sequentially connected to each reactor while keeping the lock closed and with headspace airflow of ca. 0.5 L $min^{-1}$. The $CO_2$ production rate was estimated from the increase of $CO_2$ concentration over time by a linear regression of on average 200 s from the middle of the 6 min measurement period and expressed as mg $CO_2$-C $reactor^{-1}$ per unit time using Equation 1:

$$mgCO_2 - C_{reactor^{-1}\,hour^{-1}} = \frac{ppmCO_2 s^{-1} * 10^{-6} * V}{V_m * M * 3600 * 1000} \quad (1)$$

where ppm $CO_2$ $s^{-1}$ is the change in $CO_2$ concentration, V is the volume of the headspace (L) (V was adjusted three times to accommodate for changes in the headspace due to reduction of the substrate during treatment), Vm the molar volume (L $mol^{-1}$) at each temperature and M is the molecular weight of C in $CO_2$ (12 g $mol^{-1}$).

The total amount of C respired during composting was derived for each replicate by cumulating the average of each two adjacent measurements before averaging the values per treatment. The amount of cumulatively respired $CO_2$-C was expressed per kg initial C in each reactor.

### 4.1.4 Fecal indicators

Composite samples taken in duplicates after the composting/fermentation and after the vermicomposting were stored at approximately 4°C and analyzed within 78 h of collection. A subsample of 10 g was used for analysis and prepared by dilution in 90 mL maximum recovery diluent (purchased from Sigma-Aldrich) and mechanically homogenized by a stomacher for 2 min. The presence of *E. coli* and *enterococci* was determined according to the method of enumeration by a defined substrate most probable number (MPN) technique (APHA, 2005) using Colilert 18 test kits (IDEXX Laboratories Inc., Westbrook, ME, USA). The cell numbers were determined according to the IDEXX Quanti-Tray/2000 MPN table and expressed per g of dry solid.

### 4.1.5 Pharmaceuticals

For the quantification of targeted analytes in this study, a previously optimized analytical method was adopted with some modifications (Ali et al., 2019). The selection of the compounds was based on their high rates of production and prescription in addition to their frequent detection in contaminated environmental samples in Norway.

### 4.1.6 Physicochemical characteristics and macro- and micronutrient concentrations

Composite samples were collected after the initial mixing of the substrates and at the end of the 71-day composting and 77-day vermicomposting, after thorough mixing with gardening tools. The samples were analyzed for dry matter (DM), volatile solids (VS), pH, total carbon (tot C), total nitrogen (tot N), $NH_4$-N, and total concentrations of P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B, Ni and Na. Dry matter was determined by drying the samples at 60˚C for 48 h. Volatile solids were determined by combustion of dry samples at 500˚C for 3 – 4 h in a muffle furnace. The pH was measured in deionized water with a substrate to water ratio of 1:1.5 (v/v) in fecal matter and the derived products and with a soil to water ratio of 1:2.5 (v/v) in soils and amended soils. Total C was determined in crushed samples by dry combustion (Nelson, D.W., and Sommers, 1982) at 1050°C using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA). Total N was measured on the same instrument according to the Dumas method (Bremner, J.M. and Mulvaney, 1982). Ammonium ($NH_4$-N) was measured by flow injection analysis (FIA, Tecator FIAstar 5010 Analyzer, Hillerød, Denmark) after extraction with 2M KCl in both fresh and dry samples. The difference in the concentration of $NH_4$-N between fresh and dry samples was used to correct the tot N for the $NH_4$-N loss as $NH_3$ during drying. The elemental concentrations were determined in dried (48h, 60˚C) and ground samples by inductively coupled plasma mass spectrometry (Agilent ICP-MS 8800 TripleQ, Santa Clara, USA) after ultraclave digestion with concentrated, double-distilled $HNO_3$. In addition, duplicate samples from each treatment product were analysed for total N by the Kjeldahl method.

## 4.2 Fertilizer potential

To evaluate the potential of the treatment products as fertilizer, they were applied as organic amendments in a soil incubation experiment and in a pot experiment with barley. The application to soil under controlled temperature and moisture was used to understand the mineralization dynamics of N and the easily available P over a period of three months, which approximately represents the time barley would take up nutrients from the soil. The pot experiment was carried out to demonstrate differences in yield and nutrient uptake under controlled conditions by comparing amendments, amendments supplemented with additional N, mineral fertilizer, and no fertilizer (Figure 5).

### 4.2.1 Soil incubation

Soil amended with the nine vermicomposts and a control (without amendment) was incubated for 90 days at 15˚C, under dark and aerobic conditions and at 60 % WHC. The moisture level was adjusted twice a week according to weight loss. For the incubation, the equivalent of 10 g dry soil was weighed into 50 ml plastic tubes with holes in the lids to allow for gas exchange. The soil used was a loam (20 % clay, 37 % silt, 38 % sand) collected from the top layer (0 - 20 cm) of an agricultural field in south-eastern Norway. The soil organic matter content was 4.5 % (SD = 0.08), total N content was 1963 mg $kg^{-1}$ (SD = 73). The soil pH was 6.1.

Air-dried, finely ground samples from each human excreta-derived organic amendment were weighed and added to the soil at a rate equivalent to 150 mg Kjeldahl-N $kg^{-1}$ soil (DW). For each treatment, 27 tubes were incubated and three replicates per treatment were destructively sampled at nine time points: Days 0, 1, 3, 7, 14, 29, 42, 60, and 90, respectively. Concentrations of N and P were measured in 2M KCl extracts, which were prepared by adding 25 ml 2M KCl to the sample, shaking for 30 min, and filtration through 125 mm Blue ribbon paper filters. The extracts were stored at 4˚C and analysed for $NO_3$-N and $NH_4$-N by flow injection analysis (FIA, Tecator FIAstar 5010 Analyzer, Hillerød, Denmark). The concentration of P was determined with the molybdenum blue method according to Murphy and Riley (1962), measured spectrophotometrically at 882 nm (Agilent Cary 60, Santa Clara, USA).

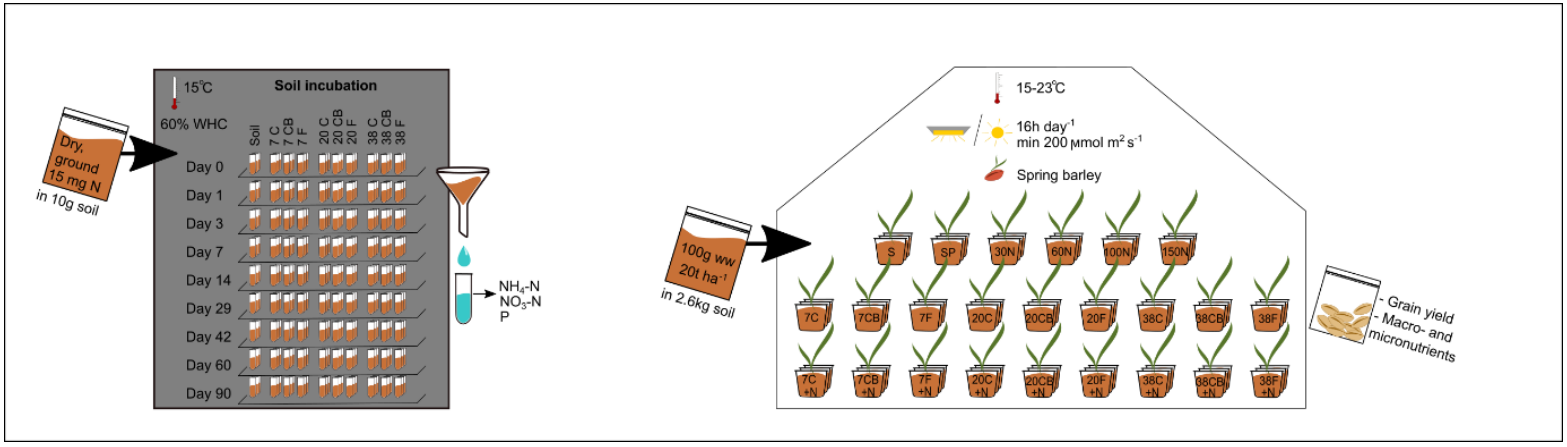



Figure 5. Soil incubation and greenhouse experiment - overview of the experimental setup, treatments, and conditions.

### 4.2.2 Pot experiment

A pot growth experiment was carried out in a greenhouse with two-row spring barley (*Hordeum vulgare*) of the variety Salome as a test crop (Figure 6). During the experiment, the temperature was controlled in the range of 15 to 23˚C and artificial light was used for a period of 16 h per day.

For the experiment, 3 L pots were filled with 2.6 kg soil (dry weight) and adjusted to 60 % WHC. The soil used was the same as in the soil incubation. Altogether, 24 treatments with three replicates each were prepared. Two were without any fertilization, four were with mineral fertilizer covering a range of N levels, nine with only amendments and an additional nine with amendments supplemented by mineral N. The unfertilized controls were only soil (S) and soil with peat (SP), where the peat was matching the amount of the added organic amendments (100 g wet weight). The peat was natural, brown sphagnum peat, low in nutrients, and with a pH of 4.0 measured in deionized water. The mineral fertilizer treatments also included peat (100 g wet weight). The mineral fertilizer was added per pot as 0.075 g P (as $Ca(H_2PO_4)_2$), 0.3 g K (as $K_2SO_4$), 0.031 g Mg (as $MgSO_4$), and N (as $Ca(NO_3)_2$) corresponding to 30, 60, 100 and 150 kg N $ha^{-1}$ (30N, 60N, 100N, and 150N), which amounted to 0.045, 0.09, 0.15 and 0.225 g N, respectively. To assess the fertilizer potential of the nine organic amendments, 100 g wet weight of each amendment were added to two sets of nine treatments; one set was fertilized only with an amendment and one set was fertilized with an amendment and additional mineral N fertilizer corresponding to 60 kg $ha^{-1}$. As the moisture level for the different amendments was similar and approximated 70 %, the 100 g wet weight was considered to correspond to an application of 20 t (DW) $ha^{-1}$. The application rate of the fertilizers and amendments, as well as yield, were converted to area based on the assumption that one hectare has 2 000 000 L topsoil in the upper 20 cm.

Twelve barley seeds were sown per pot, which after germination were thinned to eight plants. The plants were harvested after maturation (after approximately 3.5 months) by cutting 2 – 3 cm above the soil. The collected aboveground biomass was weighed and dried at 60˚C for 48h. The plants were threshed, and the grains counted and weighed.

After drying and grinding, the grains were analyzed for P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B, Ni, and Na. The samples were digested by concentrated $HNO_3$ under pressure using

an Ultraclave high performance microwave reactor (Milestone, Shelton, USA). The elemental concentration in the extracts was determined by inductively coupled plasma mass spectrometry (Agilent ICP-MS 8800 TripleQ, Santa Clara, USA). The total N in the grain was determined by the same method used for the excreta-derived substrates.

Figure 6. Greenhouse experiment with spring barley.

### 4.2.3 Calculations

The mineral nitrogen released from the amendments during the incubation was estimated based on Equation 2:

$$minN_{amendment}\ mg\ kg^{-1} = \left(minN_{measured}\ (mg) - minN_{soil}\ (mg)\right) * 100 \qquad (2)$$

Where $minN_{measured}$ is the measured concentration of $NH_4$-N + $NO_3$-N mg in KCl extracts from the amended treatments, and the $minN_{soil}$ is the measured concentration of $NH_4$-N + $NO_3$-N mg in KCl extracts from the soil control treatment. 100 is a conversion factor from 10 g sample to concentration per kg soil.

The percentage of mineral N as a fraction of the total N in the amendments was estimated based on Equation 3:

$$Mineral\ N\ \% = \frac{minN\ \ mg\ kg^{-1}}{Total\ N\ mg\ kg^{-1}} * 100 \quad (3)$$

The estimation of the available mineral N in each treatment in the greenhouse experiment was based on Equations 4 and 5.

$$minN\ mg\ kg^{-1} = \frac{minN_{soil}\ \ mg\ kg^{-1} + minN_{added}}{Dry\ weight_{\ soil}\ (kg)} \quad (4)$$

For the amendments:

$$minN_{added}\ mg\ kg^{-1} = \frac{(minN_{measured}\ \ (mg) - minN_{\ soil}\ (mg))}{Dry\ weight_{\ amendment}\ (g)} * \%\ DM_{amendment} \quad (5)$$

## 4.3 Statistics

Analysis of variance was used to compare the variation between the different treatments. The assumptions were checked with Levene's test for homogeneity of variances and the Shapiro-Wilk test for normality. Two-way ANOVA was used when the assumptions were met. To evaluate differences of means per factor, the ANOVA was followed by Tukey's post hoc comparison of means ($p < 0.05$). When the normality assumption was violated, the Kruskal-Wallis rank sum test was used for analysis. Differences between cumulative C-mass losses between the composting with and without biochar and initial and post-treatment concentrations of pharmaceutical compounds were evaluated with one tail, unequal variance t-test. Statistical analyses were carried out using the R statistical package version 1.3.959 under the GNU public license (Boston, MA, USA).

# 5 Results

## 5.1 Review of on-site treatment options for resource recovery in dry toilets (Paper I)

The review of the literature (Paper I) identified several on-site treatment options in practice and innovative methods that are being tested and developed. From the methods which are currently in use for treatment at household level, four where selected for detailed review - composting, vermicomposting, LAF and chemical disinfection. In addition, source-separation with treatment by storage (urine) and desiccation (feces) was also discussed. Those methods were selected because they include transformation of the material trough treatment, a product that can be utilized, and were considered feasible as in-latrine treatment or on-site. Relevant practical considerations that were identified in the literature for when those methods are used in on-site sanitation technologies at household level are listed in Table 2.

Composting and vermicomposting provide stabilized products with a degree of humification of organic matter, with reduced volume and higher concentration of nutrients in comparison to the raw substrate. The resulting compost can be used as an organic amendment or fertilizer. However, average treatment time spans over months, requires space, specific design, and knowledge. Chemical disinfection methods and LAF have a timespan of hours to weeks and the main effects of the treatments are disinfection and reduction of odors. Therefore, LAF and chemical disinfection are more suitable for pretreatment or in an emergency context (Anderson et al., 2015).

New developments in treatment methods can be divided into two main categories – thermal and biological treatment methods. Thermal treatments use high temperatures to transform the wet biomass of excreta to solid fuels and chars (Andriessen et al., 2019). Typical processes are drying, pelletizing, hydrothermal carbonization, and pyrolysis (Andriessen et al., 2019). Novel biological treatment methods for on-site small-scale treatment use diverse processes in three main directions: anaerobic digestion (Forbis-Stokes et al., 2016; Lansing et al., 2017; Regattieri et al., 2018); bioelectrochemical processes (Leicester et al., 2020); and digestion by larvae in the case of black soldier fly larvae (BSFL) composting (Lalander et al., 2018, 2013a).

Table 2. Lists of general, design and product considerations identified in the literature for the current on-site treatment options in dry toilets with mixed collection of urine and feces, which generate products that can be valorized.

| | Practical considerations | Design considerations | Products considerations | References |
|---|---|---|---|---|
| **Composting** | - Commonly known process<br>- Require some degree of control and knowledge<br>- Require time and space<br>- Ambient temperature has an effect<br>- Sanitization depends on high temperatures or long treatment/storage time<br>- Require additional inputs: bulking material, water, energy | - Drainage for leachate<br>- Space for the bulking agent and the extended treatment time<br>- Insulation and / or heating<br>- Mixing/turning mechanism<br>- Shielding from insects and rodents<br>- Affected by ambient temperatures<br>- Assessment of successful process can be by temperature, observation, Solvita® | - The products are compost or partially degraded and stabilized sludge and leachate<br>- Variability, depending on design, management, and environmental conditions<br>- Concentration effect of nutrients<br>- Higher humic content<br>- Restricted application to edible crops<br>- Leachate can pose risk of disease transmission to surface or ground water if not treated | Anand and Apul, (2014)<br>Berger et al., (2011)<br>Del Porto and Steinfeld, (1998)<br>Epstein, (1997)<br>Funamizu, (2018)<br>Hijikata et al., (2015)<br>McConville et al., (2020)<br>Nasri et al., (2019)<br>Niwagaba et al., (2009b)<br>Vinnerås, (2007) |
| **Lactic acid fermentation** | - Require additional inputs: LAF inoculant, sugars or molasses<br>- Used for pre-treatment<br>- Cheap and independent of location<br>- Not many applications for treatment of excreta yet but widely used in food conservation, so many people are familiar with domestic use of the process<br>- Quick in comparison to other bio-based processes<br>- Ambient temperature can have an effect<br>- So far, little is known about survival of pathogens, especially specific groups as viruses and protozoa | - Little to no oxygen, should be in closed or sealed container<br>- Treatment time is one to several weeks<br>- Assessment of successful process can be done with pH and LA strip tests<br>- Little to no odor | - The product is organic material similar to the input with low pH,<br>- Little to no odor, and high concentration of ammonium<br>- Require further processing and treatment to be utilized in agriculture<br>- Shown to have good effect on plant growth after further treatment | Anderson et al., (2015)<br>Andreev, (2017)<br>Andreev et al., (2016)<br>De Gisi et al., (2014)<br>Factura et al., (2010)<br>Odey et al., (2018b), (2018a) |
| **Vermicomposting** | - Require additional inputs: bulking material, water, worms.<br>- Commonly known process<br>- Require some degree of control and knowledge<br>- Require time and space. Treatment time –weeks, months<br>- Ambient temperature has an effect<br>- Sanitization depends on pre-treatment, post-treatment, or long treatment/storage time<br>- It is easy to visually distinguish between the undigested substrate and the vermicasts | - Drainage for leachate<br>- Space for the bulking agent and the extended treatment time. Extra space for more surface area<br>- Insulation, because it is affected by ambient temperatures<br>- Require pre-treatment to prevent high temperatures and high concentration of ammonium<br>- Shielding from insects and rodents<br>- Assessment of successful process can be done by observation – transformation to vermicasts | - The product is vermicompost, worms and leachate.<br>- Concentration effect of nutrients<br>- Restricted application as it might not be sufficiently sanitized<br>- Leachate can pose risk of disease transmission to surface or ground water if not treated<br>- The worms have to be removed | Buzie-Fru, (2010)<br>Dominguez and Edwards, (2004)<br>Hill and Baldwin, (2012)<br>Lalander et al., (2013b)<br>Lim et al., (2016)<br>Monroy et al., (2009)<br>Nigussie et al., (2016)<br>Tognetti et al., (2005)<br>Yadav et al., (2012), (2010) |
| **Chemical disinfection** | - Require additional inputs: ash / lime / additional urine / urea<br>- Independent of location but dependent on conditions such as volume, temperature, concentration<br>- Quick | - Treatment time – quick, depending on dose and conditions<br>- Require mixing<br>- Ammonia based- Must be closed or sealed container | - The product is disinfected excreta with high pH and if the disinfection is trough ammonia – High ammonia concentration and ammonia odor.<br>- High pH can limit application<br>- Possibility for regrowth of pathogens<br>- No studies on utilization as fertilizer or soil conditioner. | Anderson et al., (2015)<br>Fidjeland et al., (2013)<br>Niwagaba et al., (2009a)<br>Nordin et al., (2009a), (2009b)<br>Ogunyoku et al., (2016)<br>B. Vinnerås et al., (2003);<br>Vinnerås, (2007) |

## 5.2 Comparison of treatments (Paper II)

### 5.2.1 Composting, composting with biochar and lactic acid fermentation (LAF)

The results of the experimental work showed that the composting process was more efficient and active under higher ambient temperatures (Table 3). The microbial activity was used as an indicator for assessing the process dynamics and demonstrated a more active process at higher ambient temperature. The core temperature of the reactors was higher than ambient temperature for the first 6 to 10 days. The peak temperatures in each treatment were approximately 10˚C above the ambient and were registered shortly after mixing, i.e., in the first three days (Table 3: 7˚C – no peak, 20˚C – 3$^{rd}$, 38˚C – 2$^{nd}$). Because the material was mixed and prepared at room temperature, no distinct peak could be determined for the treatments at 7˚C. The $CO_2$ evolution corroborated that the highest activity was at 38˚C and lowest at 7˚C (Figure 7). Total cumulative loss of $CO_2$-C showed significant differences in the respired C for the period of the measurements (71 days) as a result of the different ambient temperatures.

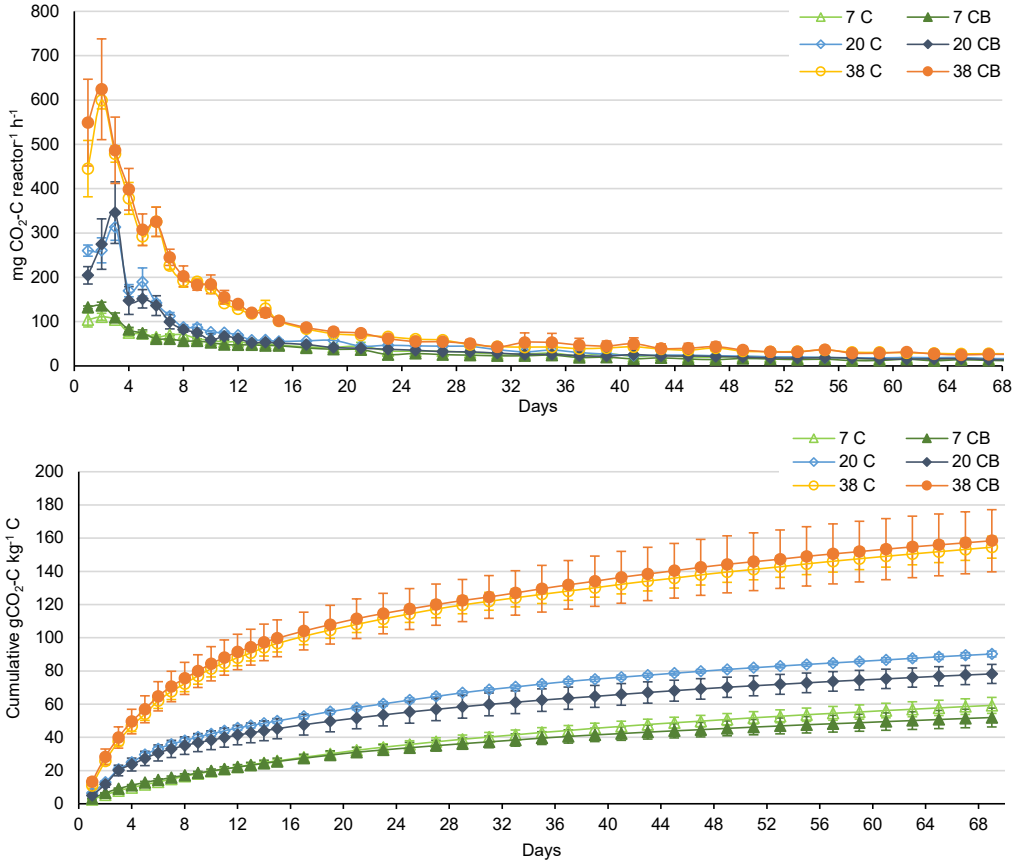
**Physicochemical parameters**

The physicochemical parameters between the nine treatments after composting/fermentation were compared with ANOVA and significant differences were identified for VS ($p<0.1$), tot. N ($p<0.001$) and C/N ($p<0.001$) (Table 3). Tukey comparison distinguished 38C and 38CB as significantly different to other treatments due to the effect of ambient temperature.

Significant variation as a result of the different initial mixture composition and composting versus fermentation treatment were found for VS, tot C, Tot. N, C/N and $NH_4$. A probable explanation could be the higher recalcitrant carbon content in the mixtures with biochar – CB and F and the higher $NH_4$ preserved in the F treatments.

Table 3. Composting and LAF peak temperatures, cumulative C mass, physicochemical characteristics (n=3, (SD)), and enumeration of *E. coli* (n=2, (SD))

| | | Peak temperature | day | Cumulative C mass, $gCO_2$-C $day^{-1}$ $kg^{-1}$ initial C | VS % | pH | Tot. C % | Tot. N % | C/N | $NH_4$-N mg $g^{-1}$ | *E.coli* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| After composting | 7 C | / | / | 59 (4.8) | 88 (0.2) | 7.2 | 47.6 (0.24) | 1.78 (0.15) | 27 (2.5) | 0.86 (0.03) | 4.4E+08 (3.3E+08) |
| | 7 CB | / | / | 52 (5.7) | 89 (0.3) | 7.2 | 49.6 (0.58) | 1.83 (0.02) | 27 (0.1) | 0.79 (0.05) | 4.3E+08 (3.3E+08) |
| | 7 F | / | / | / | 89 (0.1) | 6.8 | 51.6 (0.58) | 1.83 (0.08) | 28 (1.1) | 3.91 (0.05) | 3.5E+04 (2.9E+04) |
| | 20 C | 31 (1.3) | 3rd | 90 (1.7) | 89 (2.6) | 7.1 | 47.6 (0.33) | 1.76 (0.06) | 27 (1.1) | 0.10 (0.06) | 7.4E+04 (3.8E+04) |
| | 20 CB | 32 (1.7) | 3rd | 78 (5.7) | 86 (1.0) | 7.2 | 49.4 (0.60) | 1.82 (0.06) | 27 (0.7) | 0.09 (0.02) | 5.1E+04 (2.2E+04) |
| | 20 F | / | | / | 89 (0.5) | 6.8 | 49.9 (0.42) | 1.81 (0.03) | 28 (0.7) | 4.12 (0.29) | 9.6E+03 (6.5E+03) |
| | 38 C | 50 (1.6) | 2nd | 154 (6.6) | 85 (0.7) | 7.3 | 46.8 (0.10) | 2.28 (0.05) | 21 (0.5) | 0.87 (0.15) | 7.3E+02 (4.9E+02) |
| | 38 CB | 51 (1.9) | 2nd | 158 (18.7) | 79 (3.6) | 7.0 | 49.7 (1.44) | 2.19 (0.04) | 23 (1.1) | 0.46 (0.17) | 1.7E+03 (1.9E+03) |
| | 38 F | / | | / | 89 (0.8) | 7.5 | 49.9 (0.78) | 1.70 (0.04) | 29 (1.0) | 4.24 (0.04) | 1.1E+04 (5.7E+03) |
| | | | | | K-W test | K-W test | | | | K-W test | |
| Temperature | | | | | . | ns | ns | *** | *** | ns | |
| Mix | | | | | *** | ns | *** | *** | ** | *** | |
| Temperature*Mix | | | | | / | / | ns | *** | *** | / | |
| Significance codes: | '***' 0.001 , '**' 0.01, '*' 0.05 , '.' 0.1 ns = not significant | | | | | | | | | | |




| | 7C | 7CB | 20C | 20CB | 38C | 38CB |
|---|---|---|---|---|---|---|
| *Total $CO_2$-C g kg$^{-1}$ C* | 59 (4.8) | 52 (5.7) | 90 (1.7) | 78 (5.7) | 155 (6.6) | 158 (18.7) |

Figure 7. $CO_2$ emission rates (top) and mean cumulative $CO_2$ emission (bottom) (n=3, (SD)) during composting at three different ambient temperatures (7, 20 and 38°C). C: composting; CB: composting with addition of biochar. Cumulative $CO_2$ emission estimation is based on periodic $CO_2$ production measurements in the headspace and adjusted for initial total C. The average values with standard deviations for three replicates for total cumulative $CO_2$-C g kg$^{-1}$C are given bellow in the table. Figure 4 from Paper II.

**Fecal indicators**

The *E. coli* and enterococci abundance in the samples showed that higher ambient temperatures or more active composting corresponded to lower numbers in the composting treatments – C and CB (Figure 8). Four to five orders of magnitude higher numbers of *E. coli* were detected for the composting at 7˚C (C and CB) in comparison to the other treatments, indicating that composting at 7˚C did not result in reduction of *E. coli*. Relative differences with the fermentation treatment indicated better elimination of *E. coli* at 7˚C for LAF compared to composting.

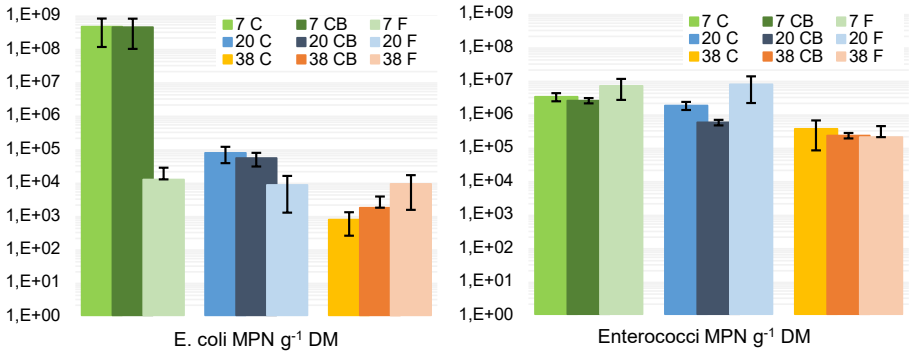



Figure 8. Enumeration of the indicator organisms *E. coli* (left) and enterococci (right) in samples after composting/fermentation at 7, 20 and 38˚C ambient temperature. C: composting; CB: composting with addition of biochar. The error bars represent standard deviation. Figure 6 from Paper II.

**Pharmaceutical residues**

From the ten pharmaceutical compounds that were tested (Table 4), Ibuprofen, sulfamethoxazole, and diclofenac had unreliable recoveries and the results are regarded as semi-quantitative. The concentrations detected after composting/fermentation for caffeine, atorvastatin, losartan, diclofenac, and warfarin were lower for treatments subjected to higher ambient temperatures. Highest concentrations for those compounds were detected in the materials treated at 7˚C and lowest in those treated at 38˚C. In comparison between composting and LAF, the post treatment concentrations were lower for composting for caffeine, carbamazepine, metoprolol, acetaminophen, and

Table 4. Concentrations of acetaminophen, atorvastatin, caffeine, diclofenac, losartan, metoprolol, and warfarin detected in the mixtures before (C, CB, F) and after composting/fermentation (n=3, (SD)).

| | Acetaminophen µg kg$^{-1}$ | Atorvastatin µg kg$^{-1}$ | Caffeine µg kg$^{-1}$ | Carbamazepine µg kg$^{-1}$ | Diclofenac* µg kg$^{-1}$ | Losartan µg kg$^{-1}$ | Metoprolol µg kg$^{-1}$ | Warfarin µg kg$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| LOD | 0.028 | 0.064 | 0.025 | 0.002 | 0.306 | 0.247 | 0.005 | 0.004 |
| LOQ | 0.093 | 0.215 | 0.082 | 0.006 | 1.020 | 0.823 | 0.016 | 0.012 |
| MDL | 0.171 | 0.215 | 0.341 | 0.007 | 1.020 | 0.823 | 0.424 | 0.012 |
| Recovery | 38.4 (9.0) | 119.0 (37) | 62.9 (10) | 119.0 (8) | 195.0 (5) | 107.0 (46) | 112.0 (9) | 124.0 (6) |
| C | 0.0 (0.0) | 324.4 (150.3) | 5577 (862) | 421.5 (164.3) | 0.89 (0.85) | 111.1 (11.2) | 340 (35) | 0.06 (0.02) |
| BC | 5.2 (5.8) | 93.2 (131.7) | 5201 (1432) | 351.4 (168.4) | 0.31 (0.35) | 30.8 (42.8) | 214 (51) | 0.09 (0.01) |
| F | 32.7 (18.7) | 92.6 (66.2) | 5579 (829) | 866.1 (640.5) | 0.24 (0.34) | 67.8 (82.5) | 411 (95) | 0.08 (0.01) |
| 7C | 0.0 (0.0) | 75.6 (16.4) | 2202 (514) | 391.5 (107.9) | 12.01 (3.91) | 85.2 (12.7) | 119 (24) | 0.15 (0.01) |
| 7BC | 0.0 (0.0) | 62.0 (13.1) | 1204 (251) | 245.9 (20.0) | 5.80 (4.53) | 65.0 (37.7) | 83 (21) | 0.19 (0.08) |
| 7F | 12.7 (6.9 | 14.1 (15.6) | 4395 (59) | 515.5 (358.4) | 0.38 (0.54) | 36.1 (45.4) | 570 (56) | 0.20 (0.15) |
| 20C | 0.0 (0.0) | 25.3 (2.8) | 628 (78) | 323.8 (94.0) | 5.66 (0.35) | 51.9 (7.6) | 115 (5) | 0.07 (0.01) |
| 20BC | 1.7 (2.3) | 54.6 (46.4) | 912 (328) | 285.3 (22.8) | 0.54 (0.77) | 45.4 (43.9) | 117 (70) | 0.14 (0.08) |
| 20F | 28.9 (20.3) | 1.3 (0.3) | 4694 (729) | 977.9 (532.7) | 1.12 (0.88) | 5.8 (4.6) | 594 (102) | 0.34 (0.05) |
| 38C | 0.0 (0.0) | 7.4 (3.4) | 129 (115) | 445.0 (137.3) | 3.31 (2.72) | 36.8 (15.1) | 119 (31) | 0.02 (0.01) |
| 38BC | 0.0 (0.0) | 2.8 (1.6) | 72 (47) | 262.7 (30.3) | 0.00 (0.00) | 11.0 (2.5) | 116 (78) | 0.00 (0.00) |
| 38F | 18.6 (7.7) | 14.8 (6.5) | 1399 (753) | 661.7 (517.0) | 0.00 (0.00) | 13.8 (5.9) | 384 (73) | 0.11 (0.04) |

*semi-quantitative

warfarin. By contrast, atorvastatin, losartan, and diclofenac were detected in lower concentrations after LAF.

Carbamazepine, losartan, metoprolol, atorvastatin, caffeine, and acetaminophen showed a general trend of reduction after treatment (Figure 9). The cross comparison of the concentrations after treatment indicated a pattern of better removal of pharmaceutical compounds under higher ambient temperature and for composting and composting with biochar. The comparison is only relative, however, based on the pharmaceutical residues detected in the mixtures and subject to uncertainties due to the comparison between complex matrixes with different parameters.

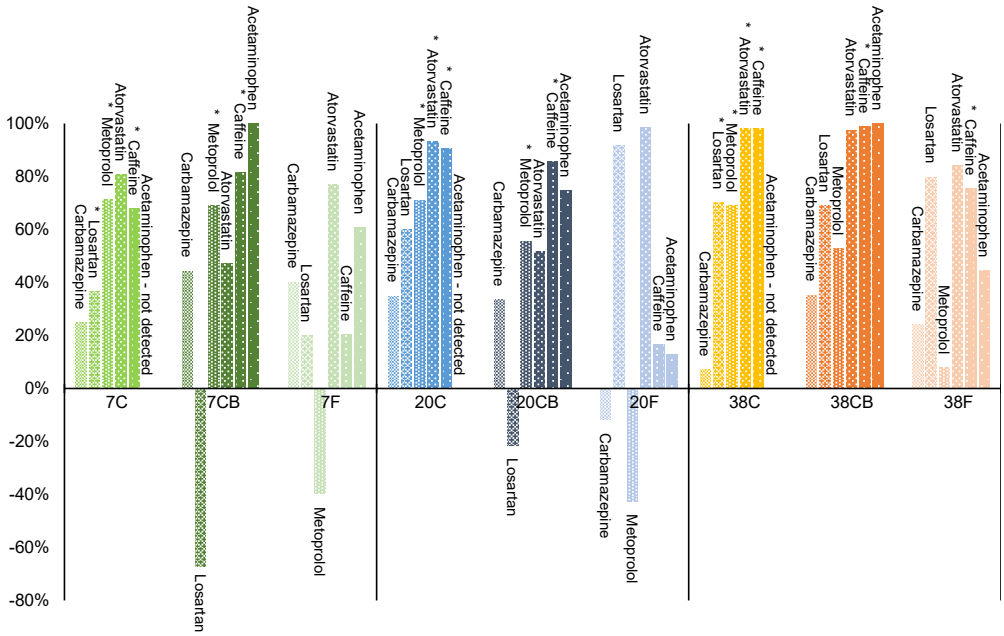



Figure 9. Removal of carbamazepine, losartan, metoprolol, atorvastatin, caffeine, acetaminophen (in % of initial) after 71 days of composting/fermentation at 7, 20 and 38˚C. The asterisk indicates the cases in which T-test showed statistically significant removal at $p>0.05$. C: composting; CB: composting with addition of biochar, F: lactic acid fermentation. Figure 8 from Paper II.

### 5.2.2 Composting and LAF followed by vermicomposting

The additional treatment step of vermicomposting resulted in further stabilization and conditioning of the materials. An ANOVA for the physicochemical parameters before and after vermicomposting identified significant differences in VS, tot C, Tot. N, C/N and $NH_4$ (Table 5). The reduction in VS and $NH_4$-N suggests mineralization of organic matter and further stabilization. Even after the conditioning with worms for 77 days, the material that was previously composted at 38˚C (C and CB) was still standing out with higher tot.N, lower C/N ratio, $NH_4$-N and pH. A change was observed in the numeration of *E. coli* for 7C and 7CB, which were four to five orders of magnitude lower in numbers compared to samples before vermicomposting. On the other hand, the mean values for 20C, 20F, 38C and 38F were on average 1 to 2 $log^{10}$ higher.

Table 5. Physicochemical characteristics, enumeration of *E. coli*, and mean density of *E. fetida* after vermicomposting.

| | | pH | Tot. C % | Tot. N % | C/N | $NH_4$-N mg $g^{-1}$ | *E.coli* | Mean density *E. Fetida* $g^{-1}$ DM |
|---|---|---|---|---|---|---|---|---|
| After vermicomposting | 7 C | 7.1 | 46.6 (0.51) | 1.98 (0.39) | 25 (3.2) | 0.026 (0.005) | 8.3E+02 (7.2E+02) | 0.85 (0.26) |
| | 7 CB | 7.2 | 48.7 (0.58) | 1.70 (0.01) | 29 (0.6) | 0.024 (0.004) | 4.4E+04 (3.1E+04) | 1.01 (0.42) |
| | 7 F | 7.8 | 48.3 (0.99) | 1.78 (0.07) | 27 (1.5) | 0.033 (0.002) | 1.1E+04 (4.5E+03) | 0.36 (0.08) |
| | 20 C | 7.0 | 46.6 (0.33) | 1.67 (0.01) | 28 (0.1) | 0.020 (0.003) | 1.2E+04 (1.6E+04) | 0.80 (0.20) |
| | 20 CB | 7.4 | 48.3 (0.40) | 1.69 (0.08) | 29 (1.3) | 0.020 (0.002) | 4.4E+03 (3.0E+03) | 1.21 (0.30) |
| | 20 F | 7.5 | 48.5 (0.27) | 1.86 (0.10) | 26 (1.3) | 0.040 (0.007) | 2.0E+05 (3.7E+04) | 0.38 (0.20) |
| | 38 C | 5.9 | 45.9 (0.50) | 2.19 (0.09) | 21 (1.0) | 0.018 (0.001) | 8.6E+04 (1.2E+05) | 0.12 (0.01) |
| | 38 CB | 5.8 | 47.9 (0.31) | 2.27 (0.08) | 21 (0.9) | 0.015 (0.003) | 2.2E+03 (1.1E+03) | 0.25 (0.08) |
| | 38 F | 7.4 | 49.9 (1.43) | 1.75 (0.02) | 28 (1.1) | 0.039 (0.008) | 1.1E+05 (8.8E+04) | 0.32 (0.13) |
| | | | K-W test | | | | | |
| Treatment | | | ** | ** | *** | *** | | |
| Temperature | | | ns | *** | ** | ** | | |
| Mix | | | *** | *** | *** | *** | | |
| Temperature*Mix | | | / | *** | *** | . | | |
| Significance codes: | | '***' 0.001 , '**' 0.01, '*' 0.05 , '.' 0.1 ns = not significant | | | | | | |

## 5.3 Fertilizer potential (Paper III)

### 5.3.1 Macro- and micronutrient content

The concentrations of the micro- and macronutrients in the organic amendments derived from the excreta are listed in Paper III, Table 2. In comparison to the values detected initially in the substrates, the concentrations for all nutrients except for N and Na increased after composting/LAF followed by vermicomposting. During composting and vermicomposting, the organic matter is mineralized, resulting in a concentration effect for most elements. The variations in the concentrations of nutrients due to a

different treatment method were low, except for N, which was detected in notably higher concentrations in the 38C and 38CB. To distinguish which differences were due to the variations in organic matter mineralization, the treatment products/amendments were also compared per ash content (Paper III, Table S1). The comparison per ash content only indicated significant variation in the cases of total N and Na where the Na concentrations reflect differences in the initial mixtures (given as g $kg^{-1}$ in Paper III, Table 2).

### 5.3.2 N, P and K after application to soil

The plant-available nitrogen contribution from the amendments in the incubated and amended soil was determined by the concentrations of $NH_4$-N and $NO_3$-N, excluding the contribution from the soil. $NH_4$-N release from the amendments throughout the period was negligently small, varying from below the limit of detection to 1.13 mg $kg^{-1}$ (Paper III, Table S3). Accordingly, most of the mineral N contribution was in the form of $NO_3$-N. The concentrations of $NO_3$-N in the amended soils were in the range of 20 to 52 mg $kg^{-1}$, of which the soil-derived $NO_3$-N amounted to 54 to 97 %. For all amendments, the pattern of N mineralization for 90 days (Figure 10A) indicated slow mineralization for the first 60 days and a slight increase between Day 60 and Day 90. The main differences between the amendments were in the amount of total and mineral N (Table 6). Both total and mineral N were highest in the 38C and 38CB treatments. Interestingly, they also had the highest percentage of mineral N as fraction of the total N. The rest of the amendments had similar concentrations of total N but differed significantly regarding what percentage was mineral. Given the total N and the estimated % of mineral N, if the amendments were applied at 20 t $ha^{-1}$ that would result in immediately plant available N of 2 to 40 kg N $ha^{-1}$ and within three months the mineral N would increase to 16 to 55 kg N $ha^{-1}$ (Table 7). The lower values correspond to the 7CB and 20F and higher correspond to 38C and 38CB. In addition, the most easily available fraction of P was followed for 90 days (Figure 10B). The amendments added KCl - extractable P to the soil but in very small amounts in the range of 0.05 to 0.6 mg $kg^{-1}$. Most of that additional P was adsorbed by the soil within 30 days as indicated by the 90-day incubation.

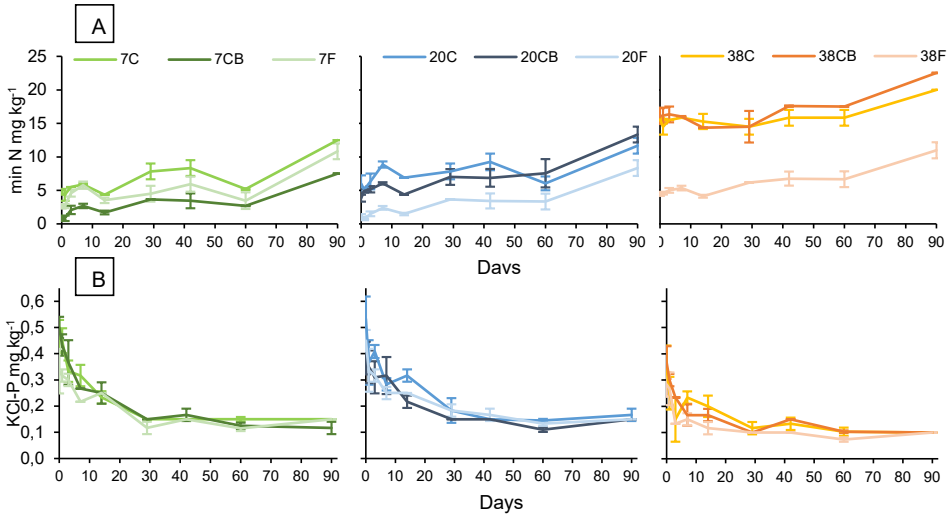



Figure 10. Changes in KCl-extractable mineral N (Part A) and P (Part B) attributed to the different amendments applied to soil at 150 mg Kjeldahl-N $kg^{-1}$ during 90 days of incubation at 15˚C (n=3, (SD)). Figure 1 from Paper III.

Table 6. N, P, and K in the amendments (n=3, (SD)). Total concentrations in the amendments and the fractions of mineral N and KCl-extractable P at Day 0 and Day 90 of the soil incubation.

| | N | | | P | | | K |
|---|---|---|---|---|---|---|---|
| Treatment | Total N g $kg^{-1}$ | % N miner from tot N | % N miner from tot N | Total P g $kg^{-1}$ | % KCL P from tot P | % KCL P from tot P | Total K g $kg^{-1}$ |
| | | Day 0 | Day 90 | | Day 0 | Day 90 | |
| 7C | 19.8 (3.9) | 2.7 (0.2) | 7.1 (0.0) | 10.3 (0.5) | 0.51 (0.05) | 0.17 (0.01) | 9.0 (0.3) |
| 7CB | 17.0 (0.1) | 0.5 (0.1) | 4.7 (0.0) | 9.5 (0.5) | 0.56 (0.07) | 0.13 (0.02) | 8.8 (0.3) |
| 7F | 17.8 (0.7) | 1.6 (0.1) | 7.0 (0.8) | 9.5 (0.7) | 0.4 (0.07) | 0.18 (0.01) | 9.0 (0.1) |
| 20C | 16.7 (0.1) | 3.9 (0.8) | 7.5 (0.8) | 10.1 (0.3) | 0.57 (0.11) | 0.18 (0.03) | 9.2 (0.1) |
| 20CB | 16.9 (0.8) | 2.7 (0.4) | 9.3 (0.8) | 9.9 (0.5) | 0.46 (0.06) | 0.18 (0.01) | 9.1 (0.4) |
| 20F | 18.6 (1.0) | 0.7 (0.1) | 4.8 (0.7) | 10.0 (0.7) | 0.48 (0.01) | 0.16 (0.01) | 9.1 (0.2) |
| 38C | 21.9 (0.9) | 9.2 (0.1) | 11.8 (0.0) | 10.3 (1.0) | 0.43 (0.06) | 0.13 (0.01) | 8.3 (0.3) |
| 38CB | 22.7 (0.8) | 8.6 (0.6) | 12.1 (0.0) | 10.2 (0.6) | 0.44 (0.08) | 0.12 (0.01) | 8.6 (0.2) |
| 38F | 17.5 (0.2) | 3.0 (0.2) | 7.5 (0.8) | 9.3 (0.6) | 0.37 (0.06) | 0.13 (0.01) | 8.9 (0.2) |

Table 7. Estimations of the total amounts of N, P and K that would be applied per ha with application rate of 20 t $ha^{-1}$ amendment with additional estimations of the mineral N and KCl-extractable P as at Day 0 and Day 90.

| | | N | | | P | | K |
|---|---|---|---|---|---|---|---|
| Treatment | Tot N kg $ha^{-1}$ | Mineral N kg $ha^{-1}$ | Mineral N kg $ha^{-1}$ | Tot P kg $ha^{-1}$ | KCl-P kg $ha^{-1}$ | KCl-P kg $ha^{-1}$ | Tot K kg $ha^{-1}$ |
| | | Day 0 | Day 90 | | Day 0 | Day 90 | |
| 7C | 396 | 10.5 | 28.2 | 205 | 1 | 0.3 | 181 |
| 7CB | 340 | 1.8 | 15.9 | 189 | 1.1 | 0.2 | 177 |
| 7F | 357 | 5.7 | 24.8 | 190 | 0.8 | 0.3 | 180 |
| 20C | 334 | 12.9 | 25.1 | 201 | 1.2 | 0.4 | 184 |
| 20CB | 338 | 9 | 31.3 | 197 | 0.9 | 0.4 | 181 |
| 20F | 372 | 2.7 | 17.8 | 200 | 1 | 0.3 | 182 |
| 38C | 438 | 40.4 | 51.9 | 206 | 0.9 | 0.3 | 166 |
| 38CB | 453 | 39 | 54.7 | 203 | 0.9 | 0.2 | 171 |
| 38F | 351 | 10.6 | 26.5 | 186 | 0.7 | 0.2 | 177 |

### 5.3.3 Yield and nutrient uptake

The grain yield from the different treatments in the greenhouse trail is shown in Figure 11. Application of all amendments, except 7CB and 20F, at a rate of 20 t $ha^{-1}$ had a positive effect on the yield in comparison to no fertilization but only for 38C and 38CB the effect was significant. Supplementing the amendments with 60 kg N $ha^{-1}$ mineral fertilizer increased the yields. Except for 20F and 38F the yields were higher than the yield corresponding to mineral fertilizer with 60 kg N $ha^{-1}$. Among the amendments, the highest yields were measured for 38C, 38CB and 7C. As expected, the four treatments with mineral fertilizer illustrated a strong correlation between the amounts of mineral N and the yield. Figure 12 illustrates the high correlation between initially available N in the amendments and the yield.

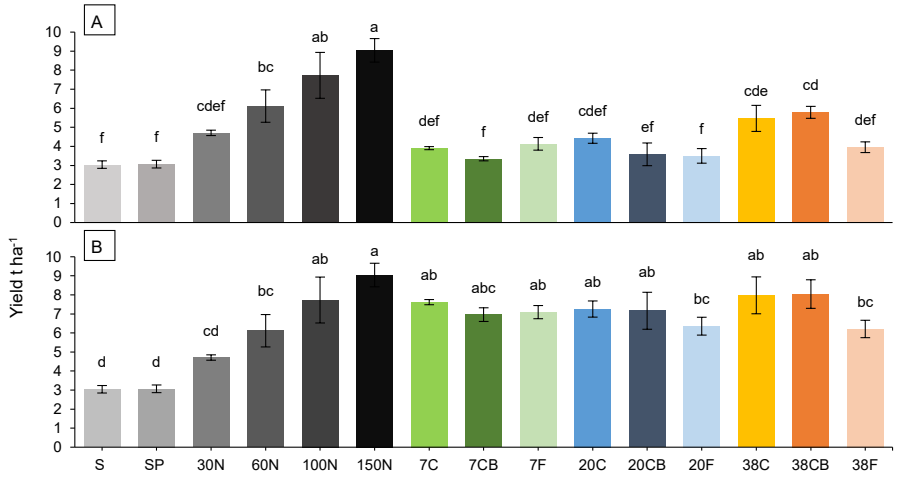



Figure 11. Barley yield from a greenhouse experiment (n=3, (SD)). Part A – only amendments. Part B – amendments with supplementary 60 kg N $ha^{-1}$. S and SP are soil and soil with peat controls. 30N, 60N, 100N and 150N are treatments with mineral fertilizer with the corresponding levels of applied kg N $ha^{-1}$. The amendments were applied at 20 t $ha^{-1}$. Figure 2 from Paper III.

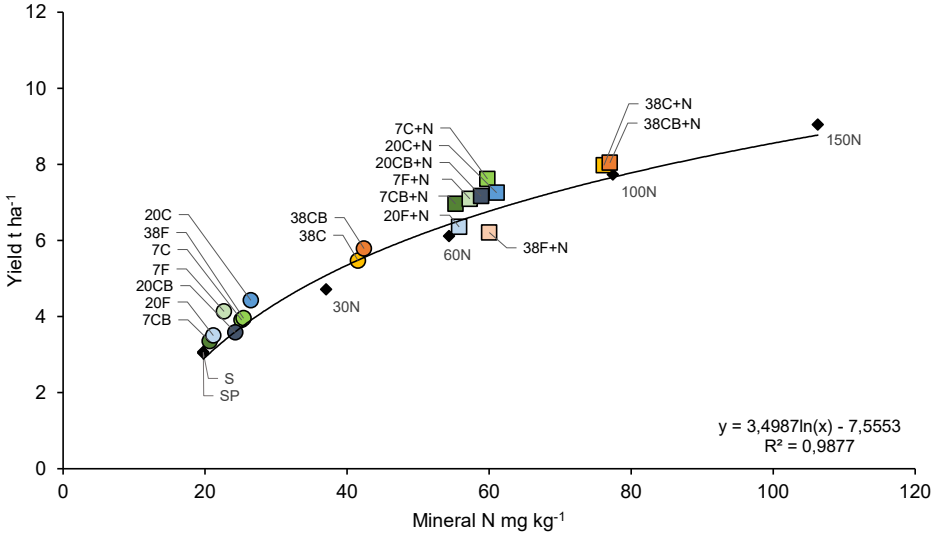



Figure 12. Relationship between mineral nitrogen and yield. The trend line is based only on the correlation of the mineral fertilizer control treatments with four levels of N kg $ha^{-1}$ – 30, 60, 100 and 150 and the no fertilizer controls – S and SP (soil and soil with peat). Figure 3 from Paper III.

# 6 Discussion

The main goal of the research was to improve the understanding of on-site treatment in dry toilets and the application of the resulting materials to agriculture. The experimental work compiled in this thesis compared composting and lactic acid fermentation followed by vermicomposting as suitable low-tech methods. A follow-up on the comparison used the resulting organic amendments to assess their potential to substitute mineral fertilizer. The discussion addresses the experimental results first (Paper II and Paper III), whereas the results of Paper I are addressed in the integrated discussion (6.4).

## 6.1 Comparison of treatments - Composting, composting with biochar and lactic acid fermentation (LAF) (Paper II)

### 6.1.1 Composting - microbial activity and physicochemical changes

The results from the comparison between composting, composting with biochar and lactic acid fermentation (Paper II) showed that the selected ambient temperatures had a significant effect on the composting process. As evident from the temperature dynamics and the respiration rates, composting at 38˚C supported a higher activity throughout the entire period, close to doubling the amount of respired C compared to composting at 20˚C. The rate of the process depends on the availability of easily degradable substrates, but also on maintaining optimal conditions as temperature, aeration moisture, etc. (Haug, 1993). It is likely that the higher temperature activated specific biota for a higher rate degradation, resulting in higher turnover of carbon and nitrogen which in turn facilitated the succeeding phases of degradation during the active phase of the composting (Wang et al., 2015; Zhang et al., 2011). At 7˚C, however, the activity was low and with time the material became more compacted, the airflow was restricted and accordingly the rate of degradation decreased. The reactors were small, without insulation, and the self-produced heat could not be retained to maintain the degradation/microbial activity. Therefore, the ambient temperatures had a strong influence on how active the composting was and accordingly on the resulting material. The material composted at 38˚C had undergone the greater transformation as indicated by the high amount of respired C and lower VS and C/N. Total C (as percentage of the

dried samples) decreased only for the composting without biochar treatments. Considered together with the differences in total C between the initial mixtures, it shows that the addition of biochar resulted in more recalcitrant carbon in the respective treatments.

### 6.1.2 Composting - fecal indicators and pharmaceuticals

The higher temperature and activity in the composting at 38˚C also resulted in the material with the lowest number of fecal indicators. Even though sanitizing temperatures of 55˚C were not achieved, temperatures high enough to affect the survival of pathogens were recorded for the reactors at 38˚C. In the core of the composting mass in those treatments, the temperature exceeded 45˚C in the first three days and only in some it reached temperatures above 50˚C. However, even without sanitizing temperatures, the composting process resulted in 4*6 $\log^{10}$ lower cell numbers of *E. coli* in the more active composts at 20˚C and 38˚C in comparison to the treatments at 7˚C and *E. coli* < 1000 MPN $g^{-1}$ DM in treatment 38˚C. During small-scale composting of fecal sludge, sanitizing temperatures are rarely achieved (Hill et al., 2013c; Niwagaba et al., 2009b) but the process can still be efficient for reducing the pathogenic load (Germer et al., 2010; Vinnerås, 2007).

The results of our study indicated that higher microbial activity and temperature in the compost resulted in more efficient removal of most of the investigated pharmaceutical compounds as evident from the clear effect of temperature on removal/degradation of caffeine, atorvastatin, losartan, diclofenac and warfarin. However, the composting was also more active at higher temperatures, thus it is not possible to discuss the effects of temperature and composting activity separately. Both thermal decomposition and microbial transformation are possible mechanisms for the observed reduction. Most of those compounds have been shown to be biodegradable. Caffeine has been identified as an easily degradable compound (de Wilt et al., 2018; Deblonde et al., 2011). In a comparison between mesophilic and thermophilic anaerobic digestion, Gros et al., (2020) demonstrated more efficient removal of atorvastatin in a thermophilic treatment. Pharmaceuticals that are recalcitrant to aerobic degradation, like carbamazepine, metoprolol and losartan, had removal below 90 %. Such slowly degrading compounds can thus still pose a risk to the environment. However, that can be mitigated by further treatment.

An assessment of the potential risk for human health is challenging, as other relevant pathogens were not investigated and the actual numbers in the fecal material were not measured. Enumeration of *E. coli* and enterococci is only indicative but, in this work, useful for relative comparison between the different treatment methods. Similarly, conclusions concerning environmental risks from spread of pharmaceuticals are difficult because the pattern and concentrations of the selected pharmaceutical compounds reflect only the regional consumption and are subject to variability within the matrix. The fecal matter and urine used in the study are not directly comparable with fresh excreta, and the initial concentrations were expected to primarily reflect compounds that partitioned to the solids, as those excreted with urine are more soluble and could have drained away.

### 6.1.3 Composting with biochar

In this study, the addition of biochar to the composting did not have a clear effect on the treatment or the resulting material. Addition of ~ vol. 5% biochar did not result in changes in the temperature profile or the dynamics of $CO_2$ evolution. There was no significant difference in the *E. coli* and enterococci between the compost with and without biochar. However, the pharmaceutical compounds carbamazepine and diclofenac had lower concentrations in the CB compared to the C treatment products. Some of the results indicate that greater amounts of biochar could have resulted in a more prominent effect. For instance, the cumulative respired C was lower for the composting amended with biochar at 7˚C and at 20˚C and carbamazepine and diclofenac were detected in lower concentrations. Carbamazepine is resistant to degradation and is mostly removed by sorption (de Wilt et al., 2018; Min et al., 2018) and biochar has been shown previously to be an efficient sorbent (Dalahmeh et al., 2018).

### 6.1.4 Lactic acid fermentation

The materials resulting from the treatments that were fermented with lactic acid did not indicate that the temperature had an effect, even though studies on the fermentation process found the process to be enhanced at higher temperatures within the range of 22-55˚C (Tang et al., 2016; Zhou et al., 2016). In comparison to composting, the LAF resulted in preservation of the organic matter, as indicated by the high VS, C/N and the retained $NH_4$-N. While the composting at the lower temperatures 7˚C and 20˚C showed similar physicochemical characteristics (VS, C/N), the LAF resulted in different

properties at the two lower temperatures, i.e., the retention of $NH_4$-N close to the level in the initial materials and the significantly lower numbers of *E. coli* at 20˚C in comparison to those detected in 7C and 7CB. LAF has been shown to efficiently reduce fecal indicator bacteria, while the organic matter and nutrients are retained (Anderson et al., 2015; Andreev et al., 2017; Odey et al., 2018b). However, LAF alone does not sufficiently stabilize and sanitize fecal matter, and further treatment is needed before application as soil conditioner or fertilizer (Andreev, 2017). Yet, the evaluation of LAF presented in this work is limited as the process was not assessed based on concentration of lactic acid or the presence of lactic acid bacteria. Even though there was no assessment on how successful LAF was, it is a reasonable depiction of how the method would be applied in real-life context.

Greater concentrations of pharmaceutical residues were detected after LAF in comparison to composting for most compounds except atorvastatin, losartan, and diclofenac. Those are anionic compounds with better sorption at low pH (atorvastatin pKa = 4.46; losartan pKa = 5.5; diclofenac pKa = 4.15 (PubChem, 2020)). A summary of the selected analytes with their molecular formula, structures, CAS numbers, supplier, and some physiochemical properties is given in Paper II, Table S1. Thus, removal of pharmaceutical compounds in LAF seems to be mostly due to sorption whereas the main mechanism in composting seems to be aerobic biodegradation.

## 6.2 Comparison of treatments - Composting and LAF followed by vermicomposting (Paper II)

Vermicomposting further stabilized the material from all treatments, as evident from the physicochemical characteristics (VS, tot. C, tot. N, C/N, and $NH_4$-N). It should be noted that the low pH values for 38C and 38CB could reflect chemical changes that started during the composting. At the end of the composting process the pH of the leachate for those treatments drops (Paper II, Figure 5) but due to the difference in the pH measuring method for the leachate and the solid samples, this change is not yet evident in the samples collected after composting (Table 3). One possible explanation is that it is a result of intensified nitrification, as the release of $H^+$ in the nitrification process acidifies the composting mix. Sánchez-Monedero et al. (2001) found a correlation between the concentration of nitrates and the pH. Supporting that

explanation are also the higher values for total nitrogen with low percent ammonium in the treatments at 38˚C (Table 3). However, nitrate was not measured in the experiment.

Worms are the drivers of the process, and any transformations are therefore dependent on how well they establish themselves in the material. The numbers of E. fetida per g DM (Table 5) showed higher densities in the treatments that were composted at 7˚C and 20˚C compared to the fermentation treatments, and lowest densities in general in the 38C and 38CB treatments. Possible explanations could be the lower pH after 38˚C, more stabilized organic material that has a lower food value for the worms and the high $NH_4$-N in the fermentation treatments. E. fetida prefer a pH range between 7 and 8 and even though they can tolerate lower pH, reproduction and growth are affected differently to survival (Edwards and Arancon, 2022).

The negative relationship between worm density and *E. coli* counts suggests that vermicomposting as post-treatment of fecal matter can reduce the load of fecal pathogens, particularly after the ineffective composting in the 7˚C composting treatments. The increase in the fecal indicators after vermicomposting found in 20C, 20F, 38C and 38F could be due to regrowth (Lalander et al., 2013b), re-introduction of *E. coli* through the worms (Prysor Williams et al., 2006), or a reflection of the high variability in the material and the extent to which the bacteria could be extracted from the matrix. It should be noted that the enumeration method did not differentiate between different development stages of E. fetida and therefore represents only a snapshot of the population in each treatment on the day of the collection. However, the comparison is comprehensive and supported by changes in physiochemical characteristics and *E. coli* MPN.

## 6.3 Fertilizer potential (Paper III)

### 6.3.1 Macro- and micronutrient content

The ranges of the concentrations of nutrients, measured in the nine vermicomposts derived from the excreta, correspond to the ranges reported for other organic amendments such as municipal waste compost, and composted or vermicomposted manures (Hargreaves et al., 2008; Lazicki et al., 2020; Rini et al., 2020). At a low to moderate application rate of 20 t $ha^{-1}$, the amendments will contribute to soil enrichment with all elements required for plant growth. The total concentration of

nutrients that would be applied with an application rate of 20 t $ha^{-1}$ are given in Paper III, Table S3. Except for N, there was little variation in the concentrations of the nutrients between the nine amendments. The small differences found are probably due to differences in organic matter mineralization, as shown by the respiration and VS in Paper II and corroborated by the lack of significant differences in the comparison per ash content (Paper III, Table S1).

Interpretation of absolute values for the nutrient concentrations is difficult as concentrations in excreta vary with dietary intake (Rose et al., 2015) and due to variations from additional substrates such as sanitary bark. In addition, even though application of the amendments will enrich the soil, the nutrient availability to plants will depend on changes of the organic matter quality during treatment and multiple factors in the soil – plant – environment (Havlin, 2014). The availability of the most important nutrients for plant growth, N, P, and K, is further investigated and discussed below.

### 6.3.2 N, P and K after application to soil

Even with considerable variability between treatments, the mineral N content is within the common range of 0 to 25% found for a variety of composts and vermicomposts (Ebertseder and Gutser, 2003; Lazicki et al., 2020). The variation found between the amendments in our experiment illustrates the effect of treatment methods on the availability of N. Higher retention of N during composting is linked to higher nitrification as that is dependent on the concentration of $NH_4$ (Tiquia, 2002). A higher rate of degradation in the 38˚C composting can be expected to result in greater ammonification and to increase the porosity of the composting matrix, both of which are factors facilitating nitrification. Vermicomposting transformed and stabilized the treatment products that were not actively composted under 7˚C and 20˚C or thoroughly fermented during the LAF. It reduced the concentrations of $NH_4$ but does not seem to have enhanced nitrification to the extent that would match the $NO_3$ content after active composting at 38˚C. Another explanation may be that the vermicomposting of those treatments resulted in greater losses.

In the vermicomposts, the prevalence of $NO_3$-N to $NH_4$-N indicates that the organic matter is relatively stable and slow mineralization can be expected in the short term (Bernal et al., 1998; Sánchez-Monedero et al., 2001). This was confirmed in the 90-day

incubation which showed that under the investigated conditions little release of mineral N can be expected for the first 60 days. C/N ratios can also predict N mineralization and the amendments range of 21 to 29 (Table 5) suggests that mineral N can be immobilized in the short term (Lazicki et al., 2020), but it is possible that the fertility of the soil and the already present $NO_3$ concealed that effect.

In addition to N, P and K are two major plant nutrients that are often added as fertilizers. While most of the N in composts is organically bound, P and K are generally more plant-available (Arienzo et al., 2009; Wei et al., 2015). The decline of the KCl-extractable fraction of P in the amendments during the incubation period may be explained by the capacity of the soil to absorb the labile P, which was also observed by Griffin et al., (2003) in manure applications. The plant availability of P will be controlled by the existing P pools in the soil, soil characteristics and the plant ability to extract P (Gagnon et al., 2012; Havlin, 2014). Most of the K is immediately available and the application rate can be considered to represent its availability (Arienzo et al., 2009). Therefore, an application of 20 t $ha^{-1}$ would result in a substantial K supply of between 166-184 kg $ha^{-1}$ (Table 7).

### 6.3.3 Yield and nutrient uptake

The comparison of the yields between the amendments clearly shows that the available N was the determining factor. It is a common limitation in using organic amendments that they have low initial availability of N as most of it is organically bound. Application rates based on the available N could achieve yields comparable to mineral fertilizer but will result in over-fertilization with other nutrients and burden the environment by increasing the risk of leaching, pollution, and toxicities (Rigby and Smith, 2014). An alternative strategy for higher yields can be supplementing the amendments with additional mineral N as it is shown by the yield increase in the pots with supplementary N and corroborated by the correlation between mineral N available per pot and the yield (Figure 12).

The concentrations of nutrients in the grain did not show a distribution pattern that can be linked to the availability of nutrients in the fertilizers or the amendments. The general trend was that higher concentrations of nutrients were measured in the grain from the pots without fertilizer (Paper III, Table 4). This trend can be explained by

limited growth compared to treatments with higher amounts of available nutrients. The latter results in a higher rate of plant growth and yield than the rate of uptake and is referred to as a dilution effect (Jarrell and Beverly, 1981). Therefore, the dilution effect in the treatments with higher yields can be overshadowing a potential variation in nutrient uptake between the amendments.

## 6.4 Integrated discussion

Dry toilets are common in rural areas, areas without infrastructure, and regions with water scarcity. Those with containment and treatment/safe disposal on-site are the least demanding in terms of infrastructure and investment, which makes them a more feasible option for those currently lacking sanitation, but also an opportunity to recycle the excreta into new products. Where the products are desired, they can be an incentive for improvements of the technology or how the treatment is managed. More efficient resource management in rural locations, where the lowest coverage with safely managed sanitation services exists today (WHO/UNICEF, 2021) and at the same time the greatest potential to increase agricultural outputs (Giller et al., 2021), represents a possibility to address multiple sustainability goals.

This thesis' focus was to investigate treatment options for mixed excreta (urine and feces) that are suitable for small scale on-site treatment and would transform the excreta to products that can be used to recycle the nutrients. Treatment in this work is understood as an intentional transformation of the dry toilet waste, and thus pit latrines are understood as sanitation systems comprised of a collection and on-site storage unit, followed by treatment and re-use/disposal off-site. On–site treatment methods are then identified as those methods suitable for in-latrine treatment or treatment close by at household level.

At that level, there is a limited number of processes that have been in use to transform the mixed excreta into different products (Paper I). The most common active intentional treatment process that is feasible at household level for excreta (mixed urine and feces) is composting (Orner and Mihelcic, 2018). Other options were identified in the concept of Terra Preta sanitation, namely LAF (Andreev et al., 2018), vermicomposting (Yadav et al., 2011) and sanitizing the material through alkalizing chemicals or the biocidal effect of high ammonia concentration (Anderson et al., 2015; Nordin et al., 2009b). In

the following sections, the processes of composting, lactic acid fermentation and vermicomposting are described (Paper I), and the results of the experimental comparison in terms of treatment efficiency, organic matter stabilization, potential human and environmental risks are discussed (Paper II) together with comparison of their nutrient content, and the fertilizer potential of their products (Paper III). The discussion extends to new developments in treatment methods and challenges in real-life context (Paper I).

### 6.4.1 Composting

In dry sanitation systems with on-site treatment, composting is the most common method to recirculate the nutrients in excreta (Strande and Brdjanovic, 2014). It is a controlled process of (predominantly) aerobic biological decomposition, in which the organic matter is mineralized and partially humified, resulting in more stable and humus-like organic material, compost. Self-contained composting toilets are commercially available, and backyard composting is a widely known and accepted practice also for other organic household wastes (Anand and Apul, 2014; Del Porto and Steinfeld, 1998). The high temperatures in the thermophilic phase of composting have been proven to eliminate pathogens and sanitize the material (Dumontet et al., 1999) but successful composting from large-scale and controlled industrial process is not easy to achieve in self-contained toilets or small-scale domestic composters. There is no evidence of thermophilic temperatures in many composting toilets, and the substrate does not heat up more than 10˚C above the ambient temperature (Nasri et al., 2019; Niwagaba et al., 2009b). Due to lack of insulation and the small volume of composting mass, there is no barrier for the process-produced heat to be lost to the environment. Therefore, the outcome of the process and thus the product is very variable and depends on the input substrates and how the process is managed.

The lab scale comparison under controlled conditions illustrated that the degradation process is limited at low ambient temperatures (7˚C). Even at optimal C/N, moisture, pH and under forced aeration, there was low microbial activity, and little indication that the material was degraded after 71 days. Furthermore, fecal indicator numbers were corresponding to numbers detected in fresh feces (Germer et al., 2010; Ogunyoku et al., 2016) and pharmaceutical residues were in higher concentrations in comparison to concentrations detected in the substrates composted at higher ambient temperatures

(20˚C and 38˚C). The low ambient temperatures in combination with no barrier, that can retain the process-produced heat, result in a net heat loss, which in turn slows microbial degradation (Nasri et al., 2019; Niwagaba et al., 2009b). On the other hand, higher ambient temperatures during the composting at 38˚C supported higher microbial activity and active decomposition. It also resulted in lower numbers of fecal indicators and lower concentrations of pharmaceutical residues.

During the active composting at 38˚C, more organic carbon was mineralized, and other elements were concentrated. The notable increase in both total N and $NO_3$-N in 38C and 38CB could be explained with increased nitrification. A higher rate of degradation in the 38˚C composting can be expected to result in greater ammonification and to increase the porosity of the composting matrix, both of which are factors facilitating nitrification (Tiquia, 2002). The results from the incubation and pot experiment showed that at an application rate of 20 t $ha^{-1}$, those amendments would contribute with approximately 40 kg N $ha^{-1}$ initially and the contribution will increase in time with mineralization. Thus, they result in a yield that is higher than that based on mineral fertilizer given at a rate of 30 kg N $ha^{-1}$. Composting at lower ambient temperatures, followed by vermicomposting, resulted in a significantly lower availability of N, and yields comparable or lower than those achieved with 30 kg N $ha^{-1}$ mineral fertilizer. Our research thus illustrated that composting at lower temperatures is limited, whereas higher ambient temperatures facilitated an active process and a product with a lower concentration of fecal indicators and pharmaceuticals but higher concentration of nutrients and most notably available nitrogen. Even a post-treatment of vermicomposting did not remove the differences in nitrogen caused by the initial composting step.

In colder environments different options can be considered to ensure higher temperatures, e.g., minimizing the heat loss with insulation (Björn Vinnerås et al., 2003), addition of easily-degradable organic matter to trigger fast decomposition and self-heating (Germer et al., 2010), or external heating. Passive solar heating could be a sustainable way to achieve higher temperatures (Kelova, 2015; Redlinger et al., 2001). Because composting is limited in cold environments and requires control over factors such as moisture, C/N ratio, and aeration, it is not suitable for every context. More

suitable alternatives for those contexts could be processes that are easier to manage, such as LAF or vermicomposting.

### 6.4.2 Alternatives to composting – LAF and vermicomposting

**Lactic acid fermentation** is an interesting low-tech treatment alternative as it has been shown to reduce pathogens and odors within a short time and without the need for on-going management (Andreev et al., 2018). It is widely used in food preservation but has emerged as an excreta treatment method only recently. During LAF of excreta, the buildup of lactic acid and the higher acidity are the mechanisms used to eliminate pathogens and inhibit the formation of volatile compounds (fatty acids). Successful LAF of fecal sludge has been shown to reduce fecal coliform numbers to below 3 log CFU $100ml^{-1}$ (CFU - Colony Forming Units) in 2-3 weeks (Odey et al., 2018b) and *E. coli* below detection after one week (Anderson et al., 2015).

In our experimental comparison with composting, LAF was not affected by the difference in ambient temperatures (7˚C, 20˚C, 38˚C) and resulted in a substrate that had retained the initial high $NH_4$ content, but showed higher numbers of fecal indicators and pharmaceutical residues compared to the active composting at 38˚C. However, in comparison with the unsuccessful composting at 7˚C, LAF resulted in significantly lower numbers of *E. coli*.

The organic amendments that resulted from LAF followed by vermicomposting did not differ significantly in their macro- and micronutrient content from the ones that resulted from the low temperature composting (followed by vermicomposting). The similarity was also in the lower availability of N and accordingly yield compared to the more active composting. As an alternative to composting, LAF can thus be used as a quick pretreatment for some reduction of the pathogenic load that requires very little commitment and reduces odors. Without additional treatment, the fermented substrate can be expected to be acidic with high concentrations of ammonium and labile organic carbon (Anderson et al., 2015; Andreev et al., 2018). Therefore, direct application to soil could have a negative effect due to potential ammonia emissions and microbial turnover in the soil. Furthermore, little is known about how the reduction in indicator organisms is related to a reduction in other relevant pathogens, e.g., Salmonella, Ascaris spp., or

viruses. The fermented substrate also requires additional treatment to be suitable for application as a fertilizer and amendment in agriculture (Andreev et al., 2018).

**Vermicomposting** is another low-tech alternative to composting. The worms transform the organic matter through their digestive system to vermicasts and facilitate the microbial decomposition by fragmenting, mixing, and increasing the porosity of the substrate. In comparison to composting, it requires less management because it does not require turning or aeration. But the worms are sensitive to ammonia and heat buildup (Buzie-Fru, 2010) and do not survive when applied directly to excreta (Yadav et al., 2010). It is therefore more common for treatment of feces in urine-separating toilets or to condition the material after composting.

In our study, the vermicomposting was used as a secondary treatment after composting or LAF. The earthworm activity resulted in improved mixing and aeration and facilitated further decomposition and stabilization of the material. Changes were more evident in the treatments where worms established themselves better - those that were initially composted at 7˚C or 20˚C. The most notable change was the reduction in the numbers of *E. coli* detected at the end of the limited composting at 7˚C and after the vermicomposting. Hill and Baldwin (2012) reported similar results, i.e., more stable material and fewer fecal indicators when comparing vermicomposting toilets to inefficiently managed composting toilets, the majority of which were operated at low ambient temperatures.

There was no evident trend on how the vermicomposting affected the concentration of N and other nutrients. Besides the apparent decline in $NH_4$-N because the material was more stable, and the concentration effect that can be expected due to biomass mineralization (as evident from lower VS), the organic amendments resulting from the vermicomposting had similar elemental composition, except for the higher N in the substrate that was initially composted at 38˚C. Therefore, in this research there is no clear indication of how or whether the vermicomposting affected the agricultural value of the amendments.

As an alternative to composting, the vermicomposting is less laborious, and provided that the material is palatable for the worms, it can stabilize the organic matter and reduce the pathogen load. It can be an alternative to composting especially when active

composting at high temperatures, and with regular turning, or aeration cannot be maintained. However, there is evidence that it does not eliminate pathogens such as A. *suum* (Hill et al., 2013a) and its effect on pharmaceutical residues is mostly unknown.

### 6.4.3 New developments in treatment methods

As pointed out in Chapter 5.1, there are multiple new developments such as hydrothermal carbonization, pyrolysis anaerobic digestion bioelectrochemical processes, and digestion by larvae in the case of black soldier fly larvae (BSFL) composting, which transform excreta into new resources. Most of the new treatment methods have shorter time-span in comparison to methods as composting and vermicomposting, and provide better elimination of pathogens. The output of those processes are resources, such as energy and solid fuels. An exception to that is pyrolysis with the application of the resulting chars as soil amendment, and BSFL composting in which the waste is converted to insect protein and fat. However, these new methods can be very energy and resource-demanding and at the same time too complex for a single latrine with on-site treatment in resource-restrained locations (Castro et al., 2014; Leicester et al., 2020; Mooijman et al., 2021). Currently, most of them are only lab- or pilot-tested, and their implementation could require changes in multiple dimensions of the current sanitation service regime (McConville et al., 2022).

### 6.4.4. Challenges in real-life context

Treating and recycling excreta from dry toilets on-site have to be considered within a complexity of external factors, including environmental (e.g., available resources, climate) and socioeconomic factors (e.g., knowledge, desire for the product, institutional support), which could affect the long-term sustainability of the sanitation practice. Therefore, a limited discussion on the different factors that can have an effect on the treatment process, product, and the long-term sustainability is presented below.

The physical environment could have a strong influence on the on-site treatment processes in dry toilets due to the relatively small size and mostly uncontrolled conditions as shown by the effect ambient temperature had on the composting process (Paper II). Biological transformations, such as composting, LAF, and vermicomposting of excreta, are limited outside optimal temperature ranges and moisture levels that ensure an environment which supports the organisms driving the process. During

chemical or thermal processes, the temperature is affecting the rate of the process but the efficiency of the treatment is dependent of the characteristics of the substrate (volume, moisture, total solids, etc.) (Andriessen et al., 2019). Furthermore, processes differ in time and space requirements – humification and stabilization of the organic matter takes months in aerobic degradation, whereas chemical and physical processes, or fermentation can be as short as minutes (disinfection with lime) to days and weeks (LAF) (Paper I). Exceptions are passive drying or storage with desiccation which take longer. Climate and seasonality, as well as the possibility for catastrophic events such as floods, droughts, earthquakes, should also be considered as they can lead to destruction, disruption, and release of untreated waste into the environment and the nearby water bodies.

The environmental conditions are a similarly important consideration for the fertilizer or amendments derived from excreta. Their agricultural value and impact on the environment depend on specific parameters, such as soil type, crops requirements, climate, quantity, and method of application. The experimental results of this work also showed how factors like the type of treatment, and temperature or climate can affect the nitrogen concentrations and accordingly the fertilizer potential of excreta-derived amendments (Paper III). In addition, as biological materials, their agricultural value could be affected by the storage time and method. These variations, in addition to differences in the initial inputs, may lead to variability in the amendments in terms of volume, nutrients, pH, salt content, and residual pathogens or contaminants such as heavy metals and pharmaceutical compounds.

Furthermore, the attitudes towards sanitation systems and practice are shaped by pre-existing knowledge and perceptions. Recycling excreta on-site or at domestic level require willingness, motivation, and commitment to maintain the treatment process but also to obtain and use the product. Pre-existing knowledge and perceptions play a role in shaping the attitudes towards sanitation systems and practice as shown in the work of Jensen et al.,( 2008), Roxburgh et al., (2020), and Simha et al., (2021).

Lack of regulations and recognition of the practice and the products by local and global institutions could be a barrier to resource recovery of excreta. According to (Mallory et al., 2020), establishing the circular economy in the context of sanitation requires

increased enforcement, policies and subsidies for fertilizers derived from human waste. Major barriers for the commercialization of human excreta-derived products are unclear regulations for their use and the absence of normative or legal regulations for such fertilizers within the European countries (Krause et al., 2021; Moya et al., 2019b). Guidelines for ensuring safety of the products could increase trust and enable market mechanisms, which could give an economic incentive for human excreta-derived products. Still, a challenge would remain to integrate those products in the users' routines, and their utilization would require that they are harvested reliably, and as needed – in quantity, quality, and in time.

# 7 Conclusions

Dry toilets with on-site treatment are an opportunity for sanitation with resource recovery that is detached from infrastructure and transport and could contribute to local recirculation of resources. However, the treatment methods and excreta-derived products must be considered within a complexity of external factors arising from environmental and socio-economic contexts. The key considerations identified in a literature review were 1) that environmental conditions have strong effect due to the smaller size and less controlled treatment;2) the treatment process and products vary in result of differences in input materials, management of the process and the local context; and 3) the products can be valuable resources locally but must be desired and their application regulated.

Low-tech options for on-site treatment, i.e., composting, lactic-acid fermentation and vermicomposting were evaluated under controlled laboratory conditions. The comparison between composting and LAF, followed by vermicomposting determined that: 1) Under controlled conditions, in small, uninsulated reactors, the ambient temperature (7˚C, 20˚C, 38˚C) had a significant effect on the composting process and the quality of the resulting material; 2) the active composting at 20˚C and 38˚C yielded more stabilized material with less *E. coli* and pharmaceuticals, but lactic acid fermentation was comparatively successful in reducing the number of *E. coli* at 7˚C; and 3) vermicomposting resulted in further maturation and stabilization of the material in all treatments, it was particularly beneficial in reducing *E. coli* numbers and transforming the substrates for the treatments that were previously composted at low ambient temperatures, i.e. 7˚C and 20˚C. The results highlight the limitations of composting at low ambient temperatures and how lactic acid fermentation and/or vermicomposting can be an alternative or an additional treatment in such instances. Therefore, depending on the local conditions, possibilities, and desired qualities of the end-product, different alternatives for resource recovery can be considered.

An evaluation of the fertilizer potential of the resulting organic amendments showed that all of them contributed to soil enrichment with essential plant nutrients. Upon application to fertile soil, the contribution with mineral nitrogen was low and the mineralization of organic nitrogen slow. In a greenhouse experiment with barley as a

test crop, an application rate of 20 t $ha^{-1}$ of the amendments resulted in yields which were higher than treatments without fertilizer but lower in comparison to conventional amounts of mineral fertilizer. Differences in the yields between the amendments were determined by the mineral nitrogen content. Active composting at high ambient temperatures (38˚C) resulted in more available nitrogen and accordingly higher yields. Higher application rates could increase the mineral nitrogen but would lead to excessive application of other nutrients, therefore supplementing the amendments with mineral nitrogen is a more suitable approach for balanced fertilization.

# 8 Future challenges and some remaining questions

Some of the challenges and questions that go beyond the scope of this work but should be considered in future research are listed below:

*How can biochar amounts, feedstock, or method of preparing impact the process and the quality of the resulting material?* It is possible that more biochar could alter the process dynamics and the effect might not be in the same direction at different temperatures. When comparing the composting with and without biochar, the $CO_2$ evolution indicated lower respiration in the 7˚C and 20˚C composting amended with biochar but higher at 38˚C (Figure 7). Differences can be expected also for the degradation and sorption of pharmaceutical residues and concerning the retention and availability of nutrients.

*What is the fate of pharmaceutical compounds during vermicomposting and after application to soil?* In this work the pharmaceutical residues were not quantified after the vermicomposting. While it could be speculated that more compounds would be degraded during the process, it is unclear how the different treatments would compare to each other and what would be the effect from differences in worm density.

*Research integrating traditional and novel practices and management of manures and organic wastes with sanitation and excreta management.* Because the waste in dry toilets is not diluted with water it can be more intuitive to perceive it as organic matter or organic waste. Therefore, it will fit well with the management of other organic wastes within a farm or a household such as manures, kitchen waste, or garden waste. For example, methods for treatment and application of animal manures can be incorporated in the treatment and application of human manure. Knowledge of soil management, suitable organic fertilizer application rates and methods would facilitate a more sustainable utilization of excreta-derived products.

*How will excreta-derived products impact the environment?* Beyond critical issues such as pathogen transmission and eutrophication, future research also needs to address other possible environmental impacts. Increase in soil application of excreta-derived products could result in positive effects on soil health due to buildup of organic matter and fertility but could have negative impacts on nutrient-poor ecosystems or flood-prone areas.

*How can excreta-derived products be integrated with the existing agricultural practices and routines at household, farm, or community level?* Local institutions, organizations and cooperatives can facilitate the application of excreta-derived products based on the local knowledge and understanding of climate, soils, hydrogeology, epidemiology, crops as well as the cultural and economic context. Regulations can be established by local agricultural or environmental institutions through restrictions, recommendations, and local enforcement.

Despite existing research needs, the recognition of the resources in our excreta, and the opportunity dry toilets with on-site treatment provide to recycle them, will contribute towards solving sustainability challenges within water, sanitation, and agriculture.

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# Paper I

# Methods for resource recovery in dry toilets with on-site treatment – a review

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**Abstract**

Treatments aiming at resource recovery in dry toilets create an opportunity to re-circulate the resources locally and thus limit the disposal as waste, which can endanger human health and contaminate the environment. Many studies have illustrated different technology options for resource recovery in dry toilets or designed improvements and amendments to enhance the treatment, but they still encounter challenges in real contexts. The aim of this work was to critically review the existing methods with focus on practical considerations and to highlight challenges that need to be addressed to implement them more successfully with long-lasting results. Hence, the methods currently used for on-site treatment, i.e., composting, vermicomposting, lactic-acid fermentation, and chemical disinfection are defined, and their application for treatment of excreta critically evaluated. In addition, new developments in treatment methods for on-site dry sanitation are outlined.

The review illustrates that treatment methods and excreta-derived products have to be considered within a complexity of external factors arising from particular environmental and socio-economic contexts. The key challenges identified were 1) the variability resulting from differences in input materials, management of the process and the local context; 2) environmental conditions have strong effect due to the smaller size and less controlled treatment; and 3) the products can be valuable resources locally but have to be desired and integrated in the user's daily life. Acknowledging such factors can help in decision making by identifying possible pitfalls and failures, and therefore contribute towards more sustainable and long-term solutions within a particular context.

**Keywords: dry toilet, on-site treatment, resource recovery, excreta, latrine**

## 1. Introduction

In 2020, more people rely on on-site sanitation technologies than sewer connections (WHO/UNICEF, 2021). Dry toilets with on-site treatment are non-sewered sanitation systems for collection or collection and treatment of human excreta. Dry toilets are common in rural areas, areas without infrastructure, and in regions with water scarcity. Those toilets are commonly associated with a simple, rudimentary type of latrine but goals like ensuring availability and sustainable management of water and sanitation for all (SDG 6, UN) and to eliminate waste, circulate materials and regenerate nature (Ellen MacArthur Foundation, 2022) require rethinking this association due to their sustainability. Dry toilets with containment and treatment/safe disposal on-site are the least demanding infrastructure and investment, which makes them a more feasible (i.e., easily available) option for those currently lacking sanitation. Moreover, water is a resource that is under increased pressure due to excessive use and changing climate, and the flush toilet is among the big consumers of drinking water at a household level

(Bradley, 2004). Diluting the excreta with water increases the burden of fecal contamination and eutrophication of water ecosystems due to planned and accidental discharges in the environment (Lapointe et al., 2015; Van Drecht et al., 2009). The volume of human excreta is only a small fraction of the average volume of black water, and separated excreta are therefore easier to manage, treat, store, and recycle. Nevertheless, human excreta also contain hazards such as pathogens and micro pollutants (Gros et al., 2020; Schönning et al., 2007), and therefore require treatment.

The existing technology options for treatment in dry toilets are pit latrines, or single or double vault latrines for collection with disposal; Arborloos and Fossa Alterna for collection with partial treatment; and composting and urine diversion dry toilets (UDDTs) for collection, on-site treatment, and resource recovery (Tilley et al., 2014). More recent methods for treatment of excreta include vermicomposting and lactic acid fermentation (LAF; e.g., Terra Preta sanitation), as well as disinfection with lime and urea (Buzie-Fru, 2010; Factura et al., 2010; Niwagaba et al., 2009a; Nordin et al., 2009a). Furthermore, increased attention to the resources in excreta has driven innovations in treatment options for resource recovery such as electrochemical systems, anaerobic digestion, black soldier fly larvae composting, and thermal treatments (Andriessen et al., 2019; Lalander et al., 2013a; Lansing et al., 2017; Leicester et al., 2020). However, household level treatment options for dry toilets are limited and there is insufficient understanding about how effective they are in a real-life context. Among the reasons are the variability of the input (excreta and possible additional amendments), the variety of designs and treatment methods that can be utilized, and what is the impact of different environmental conditions (Bhagwan et al., 2008; McKinley et al., 2012; Rose et al., 2015). Accordingly, the safety, quantity, and quality of the derived products will vary from one application of the sanitation technology to another. Research on the utilization of excreta-derived products is scarce and even though they are commonly used as a soil conditioner or fertilizer, little is known about how different treatments compare, the effects of suboptimal conditions, and what could determine or limit their use. These research gaps limit the confidence of institutions about the risks associated with the management of excreta, and the short- and long-term consequences from their utilization (McConville et al., 2017). Therefore, the objectives of this work were to critically review the existing methods for resource recovery in dry toilets with focus on practical considerations and to highlight challenges that need to be addressed to make them more successfully implemented with long lasting results.

## 2. Methods for on-site treatment

### 2.1. Composting

The composting process mimics the natural decomposition and transformation of organic matter and has been utilized since ancient times for recycling of organic wastes and to improve soil quality and fertility. It is a controlled process of (predominantly) aerobic biological decomposition, in which the organic matter is mineralized and partially humified, resulting in more stable and humus-like organic material, compost. During the process, the microbial populations and accordingly the degradation rate follows a trend with time of initial quick increase, superseded by a gradual decline (Epstein, 1997). Usually the quick increase constitutes a thermophilic phase with high temperatures that sanitize, and the gradual decline represents a maturation phase that further stabilizes and humifies the material.

### 2.1.1. Composting of excreta

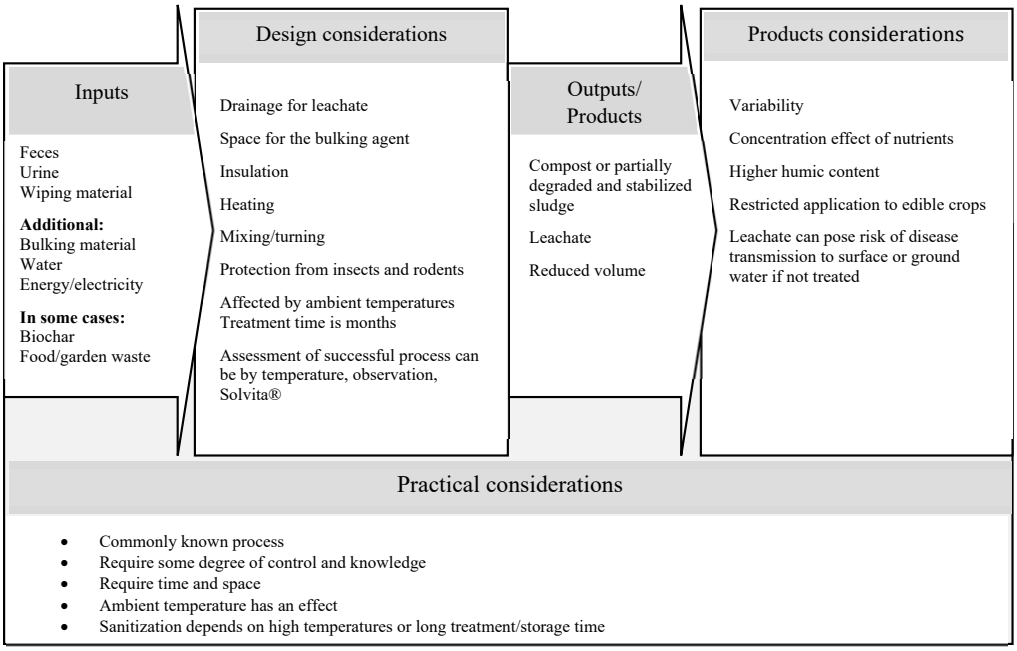
Human excreta are organic substrate which is suitable for composting but requires additional carbonaceous material to achieve active composting with self-heating. The process has been researched and applied to treat various wastewater streams such as sewage sludge (Mena et al., 2011), fecal sludge (Manga et al., 2021), dehydrated feces or human excreta (Nasri et al., 2019; Niwagaba et al., 2009b). In dry sanitation systems with small-scale on-site treatment, composting is the most common method to recycle human excreta (Strande and Brdjanovic, 2014). The waste can be collected and composted in a central facility to ensure good management or composted at household level, where it can be treated within the toilet system or in a separate composter. Self-contained composting toilets are commercially available, and backyard composting is a widely known and accepted practice also for other organic household wastes (Anand and Apul, 2014; Del Porto and Steinfeld, 1998).

The high temperatures during the thermophilic phase of composting have been proven to eliminate pathogens and sanitize the material (Dumontet et al., 1999). However, the successful composting from large scale and well controlled facilities is not easy to achieve in a self-contained toilet or small-scale domestic composters. Among the common problems are ensuring optimal conditions for decomposition with respect to moisture, C/N ratio, aeration, and heat losses. The moisture level of the composting mix should be within a range of 40 to 60% and can require regular adjustments. The C/N ratio of human excreta is below 10, whereas the optimal ratio for composting is within the range of 25 to 35 (Anand and Apul, 2014; Funamizu, 2018). The inflow of oxygen required for the process could be ensured by mixing or turning but has to be balanced against moisture and heat losses. Ambient temperatures also affect the process and the effect is more pronounced in uninsulated reactors and smaller volumes (Kelova et al., 2021a). When the composting process is not actively managed, it does not support active degradation and heat build-up. In addition, malodors and insects can create a nuisance. In many composting toilets there is no evidence of thermophilic temperatures and the substrate heat-up does not exceed 10˚C above ambient temperatures (Nasri et al., 2019; Niwagaba et al., 2009b).

Tracking the change in temperature during composting is thus an easy way to assess how successful the process is. In cases where the temperatures remain mesophilic, the transformation can be visually assessed, the material should be handled with protection and applied in a matter that would reduce exposure to and transmission of pathogens. Other methods for relatively easy assessment include the Solvita® Compost Maturity Test that measures the concentration of ammonium (Hill et al., 2013b) and a germination test that is used for evaluation of phytotoxicity (Zucconi et al., 1981).

Multiple design and management options can improve the treatment, some of the examples include choice and ratio of carbon-rich bulking materials (Hijikata et al., 2015; Niwagaba et al., 2009b), heating, insulation, drainage, turning, ventilation (Chen et al., 2020; Del Porto and Steinfeld, 1998; Niwagaba et al., 2009b), or co-composting with other organic waste streams (Germer et al., 2010). In addition, composting is often used in combination with other treatments such as LAF and vermicomposting (Andreev et al., 2018; Yadav et al., 2012). Figure 1 gives an overview over inputs, outputs and important considerations for composting of excreta and the use of their products.

Overall, composting is a suitable low-tech treatment with the benefit of transforming the excreta to a valuable product that can be utilized to contribute with nutrients and organic matter in soils and plant production. It reduces the waste volume and has been shown to eliminate pathogens and contaminants. However, the outcome of the process and thus the product is very variable and depends on the input substrates and how the process is managed.




References

Anand and Apul, 2014; Berger et al., 2011; Del Porto and Steinfeld, 1998; Epstein, 1997; Funamizu, 2018; Hijikata et al., 2015; McConville et al., 2020; Nasri et al., 2019; Niwagaba et al., 2009b; Vinnerås, 2007

Figure 1: Overview of composting of excreta, presented as inputs, outputs/products, and considerations concerning design, products and practicality of the method.

### 2.2. Lactic acid fermentation (LAF)

Lactic acid fermentation is a process in which bacteria (lactic acid bacteria) metabolize simple sugars for energy and produce lactic acid as a byproduct. The produced lactic acid accumulates, resulting in an increase of the acidity and its accumulation together with the acidity is inhibiting the growth of other bacteria (Battcock and Azam-Ali, 1998). Lactic acid fermentation is widely used in food preservation and production for a range of foods in various global regions, such as sauerkraut, kimchi, pickles, yogurts, milk-wheat mixes, sourdough bread, or miso.

### 2.2.1. LAF treatment of fecal sludge and excreta

Lactic acid fermentation has only been investigated as a treatment method for human excreta in the past 10 years. It is included as a pretreatment with sanitizing effect in the Terra Preta sanitation (De Gisi et al., 2014; Factura et al., 2010). In the Terra Preta sanitation concept, LAF can be used for separated urine and feces or for mixed excreta with addition of charcoal, and is followed by vermicomposting. In contrast to other methods of degradation, LAF minimizes odorous gases and volatile compounds and limits the carbon and nitrogen losses. Successful LAF of fecal sludge has been shown to reduce fecal coliforms to below 3 log10 CFU $100ml^{-1}$ (CFU: colony-forming unit) in 2-3 weeks (Odey et al., 2018a) and *Escherichia coli* below detection after one week (Anderson et al., 2015). However, the reduction of pathogens is dependent on maintaining the high acidity and build-up of lactic acid (Odey et al., 2018a), and the effect on a variety of pathogenic organisms is not yet sufficiently investigated. Lactic acid fermentation can be applied to both liquid and solid waste and is relatively independent of environmental conditions, but the product require further treatment before it can be re-used as a soil conditioner or disposed in a safe manner. Figure 2 gives an overview over inputs, outputs and important considerations for LAF treatment of fecal sludge and excreta, and the use of their products.

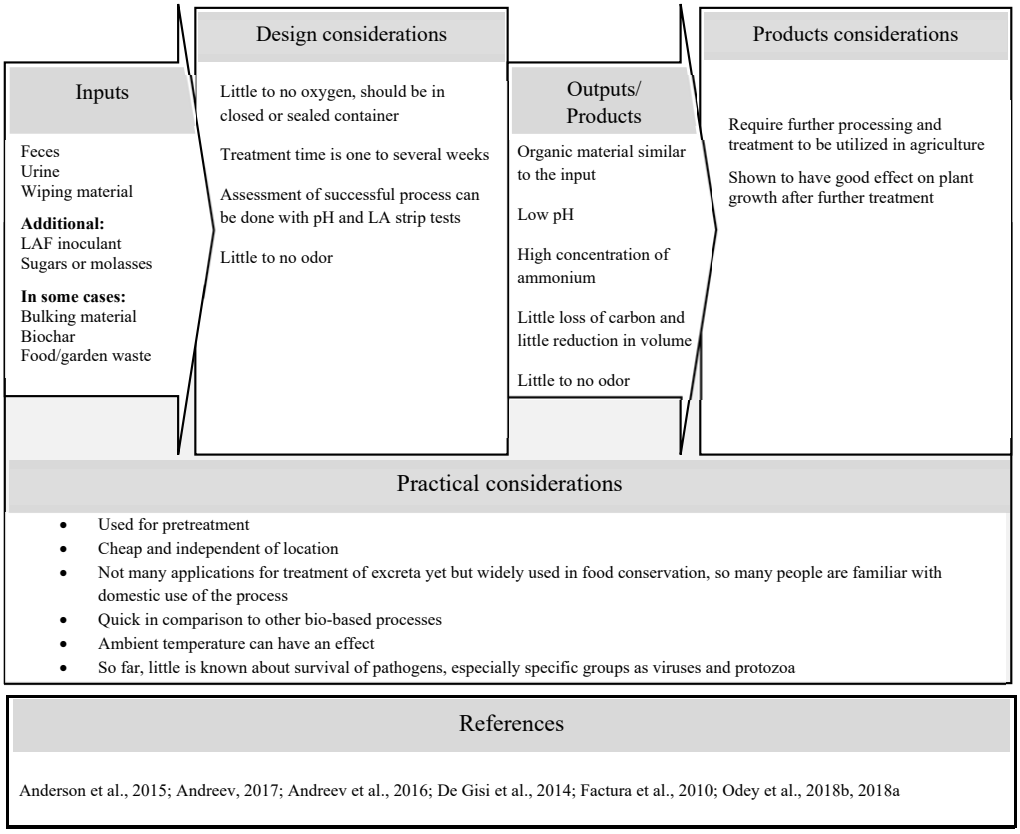



Figure 2: Overview of LAF treatment of fecal sludge and excreta, presented as inputs, outputs/products, and considerations concerning design, products and practicality of the method.

### 2.3. Vermicomposting

Vermicomposting is invertebrate driven decomposition of organic matter. It combines the microbial oxidation of the organic matter with its digestion by earthworms. The worms transform the organic matter through their digestive system to vermicasts and facilitate the microbial decomposition by fragmenting, mixing, and increasing the porosity of the substrate. Similar to composting, the process is affected by the substrate and the environmental conditions. Suitable organic substrates have a C/N ratio of around 30, a pH in the range of 5 to 8 and a relatively high moisture content of 50 to 70% (Lim et al., 2016). Toxic compounds and high salinity can negatively affect the worms. The optimal temperatures are in the range of 20 to 25˚C, but a wider range can be tolerated by the worms although their survival and reproduction is limited at lower temperatures or if the substrate self-heats to thermophilic temperatures (Dominguez and Edwards, 2004). Different worm species can be utilized but the most commonly used is *Eisenia fetida*, as they are abundant, resilient, with wide temperature tolerance and worldwide distribution (Dominguez and Edwards, 2010).

#### 2.3.1. Vermicomposting of excreta

As a treatment of sewage sludge and human excreta, vermicomposting is an emerging technology. It has been utilized directly for treatment of sewage sludge and fecal matter (Eastman et al., 2001) or following composting or anaerobic digestion, including lactic acid fermentation (Andreev et al., 2017; Lim et al., 2016; Yadav et al., 2013). A pre-treatment is often used, as it has been shown that worms do not survive when directly applied to excreta (Yadav et al., 2010). Carbonaceous bulking material is required to increase the C/N ratio, to facilitate microbial decomposition and to provide a more porous structure. Furthermore, build-up of ammonia and other volatile compounds can be toxic to the worms (Buzie-Fru, 2010) and pre-treatment and bulking material can reduce the toxicity and make the material more palatable for the worms. Because the urea in the urine quickly hydrolyses to ammonium and ammonia and thus may pose a problem, vermicomposting is more suitable as a direct treatment of feces in urine-diverting dry toilets.

The process can span from several weeks to several months, depending on substrate, environmental conditions, initial stocking density of the earthworms, as well as growth and reproduction. Those factors also affect the process in regard to biomass reduction, nutrients and pathogens. Loss of biomass is governed by the oxidation of the organic matter and as a consequence the nutrients, with the exception of N, increase in concentration (Kelova et al., 2021b; Yadav et al., 2012). Several studies have established reduction of fecal indicators (Hill and Baldwin, 2012; Lalander et al., 2013b; Yadav et al., 2010), however, Hill, Baldwin and Lalander, (2013) found that vermicomposting was not efficient in eliminating *Ascaris suum* ova. So far, there are very few studies on the fate of different pathogenic organisms in vermicompost and the mechanisms of elimination. Figure 3 gives an overview over inputs, outputs and important considerations for vermicomposting of excreta and the use of their products.

In comparison to composting, vermicomposting requires less management and can be assessed more easily. It does not require turning or mixing, and only the moisture level needs to be monitored. Good worm survival and reproduction can be visually established and the material's physical transformation to casts can be observed. However, there is no clear indication, whether the material is properly sanitized and that should be considered in handling and applications.

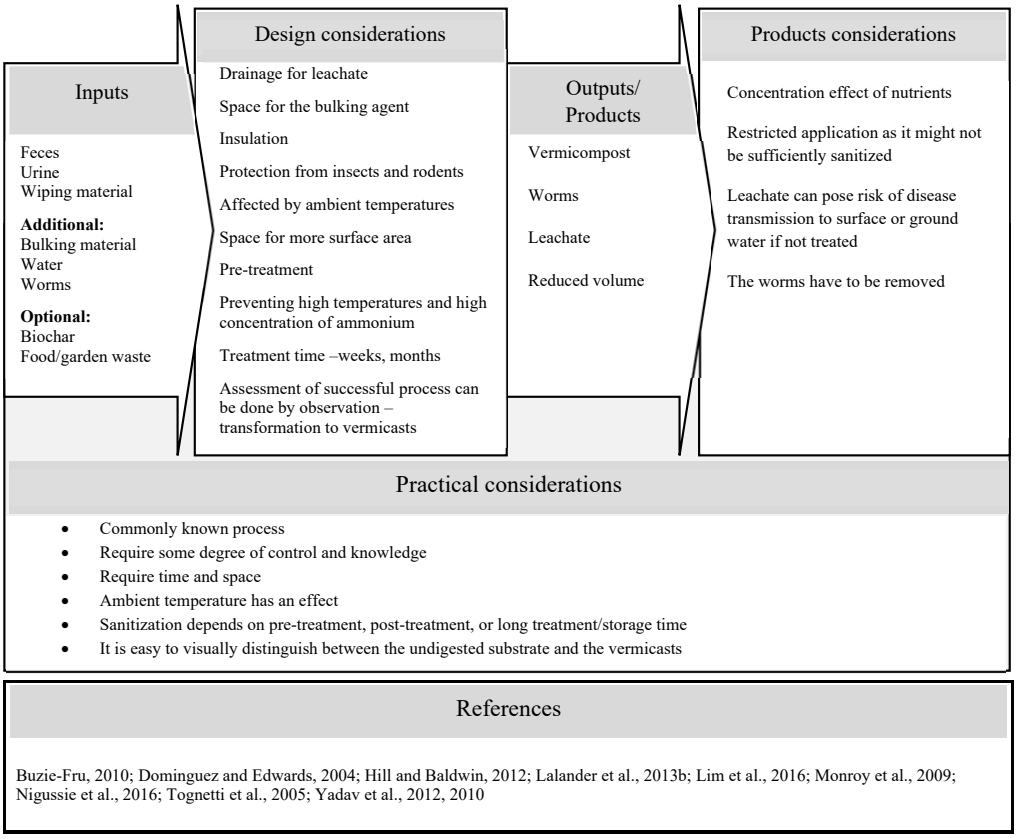



Figure 3: Overview of vermicomposting of excreta, presented as inputs, outputs/products, and considerations concerning design, products and practicality of the method.

### 2.4. Chemical disinfection

Chemical agents are used to create hostile environment for living organisms and eliminate the pathogenic organisms. Two methods used for disinfection that have been applied in on-site sanitation are disinfection through high pH – alkalization, and by ammonia from urine or additional urea. Alkalizing chemicals (bleach, KOH, NaOH) destroy the pathogens and living cells by damaging the cell structures. The basic environment destroys the hydrogen bonds holding together the DNA, hydrolyses lipids and alters the amino acids in the proteins causing denaturation (Maris, 1995). High concentration of free ammonia and high pH have an inhibitory and/or biocidal effects on microorganisms, possibly due to changes in internal pH balance and changes in extracellular polymeric substances (Liu et al., 2019). For both methods, the effect is usually quick and depends on the concentration and environmental conditions.

#### 2.4.1. Chemical disinfection of excreta

Both high pH and ammonia have been explored as disinfection methods for sewage sludge (Pecson et al., 2007), excreta or source separated feces (Hijikata et al., 2016; Nordin et al., 2009a; Ogunyoku et al., 2016). A traditional practice in the use of dry toilets is the addition of ash or lime after every use (Niwagaba et al., 2009a), which creates an alkaline environment. The

high alkalinity leads to inactivation of pathogenic bacteria and virus pathogens (Hijikata et al., 2016) but does not affect more resistant pathogens such as *Ascaris suum* ova (Senecal et al., 2020). High concentration of ammonia, however, is efficient in eliminating bacteria, viruses and protozoa including the *Ascaris* ova (Fidjeland et al., 2013). An increase in the ammonia concentration can be achieved with addition of urine or urea, which are then hydrolyzed by the enzyme urease, present in the excreta (Vinnerås et al., 2003). Both high temperature and alkalinity, i.e., a pH up to 10, as higher values would inhibit the enzyme activity, favor the formation of ammonia and accordingly disinfection (Fidjeland et al., 2013; Nordin et al., 2009a). As ammonia is volatile, it is important that the treatment is applied in a sealed container.

Both alkaline and ammonia sanitization can be achieved quickly but the time will depend on dose and environmental conditions (Anderson et al., 2015; Vinnerås, 2007). Addition of lime can achieve sanitization within an hour, whether urea can sanitize within a few days to couple of months (Anderson et al., 2015; Nordin et al., 2009b). The treatment products can be used as soil conditioner or fertilizer, but high alkalinity will require neutralization. The high ammonia concentration generates unpleasant odor but increases the fertilizer potential due to the additional nitrogen. Figure 4 gives an overview over inputs, outputs and important considerations of chemical disinfection of excreta and the use of their products.

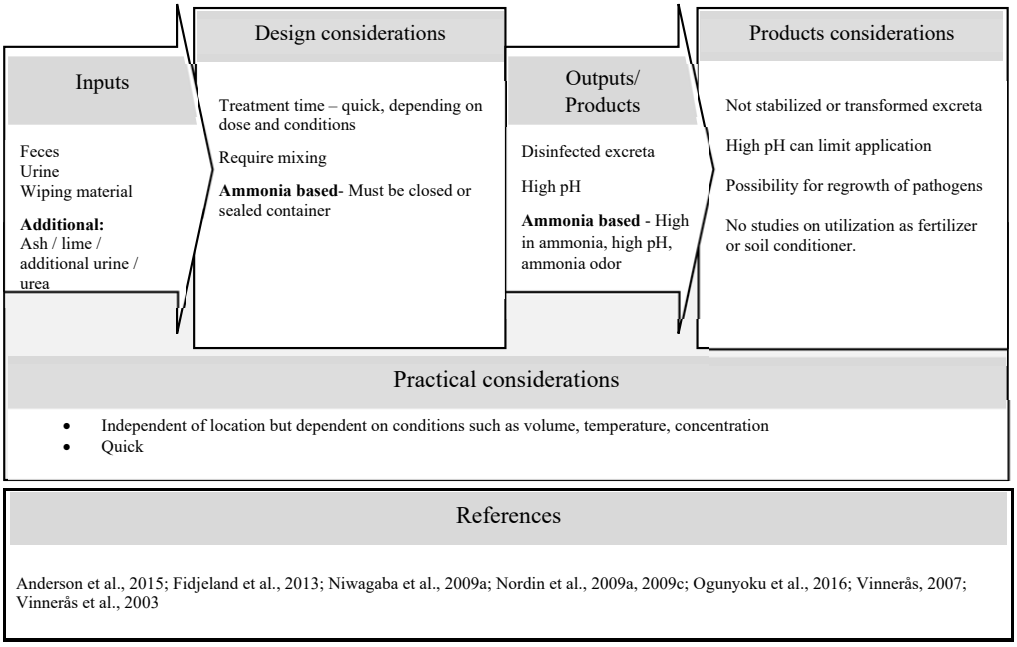



Figure 4: Overview of chemical disinfection of excreta, presented as inputs, outputs/products, and considerations concerning design, products and practicality of the method.

### 2.5. New developments in treatment methods

Recent developments in wastewater treatment technologies have inspired research on their application for fecal sludge and excreta and the implementation in single-toilet prototypes. The innovative treatment methods that can transform waste into new resources are briefly described and discussed in this section. They are generally divided into two categories: thermal and biological treatment methods.

### 2.5.1. Thermal treatment methods

Thermal treatment methods are used for production of solid fuels and chars from fecal sludge, excreta, and feces (Andriessen et al., 2019). The main focus is on transforming the biomass to solid fuels as the demand for the product and the acceptance are higher (Andriessen et al., 2019). Chars can also be utilized as a soil amendment in agriculture if the process is adjusted to conserve the nutrients (Bleuler et al., 2021).

Different technology options exist to transform the wet biomass (excreta) to solid fuels, i.e., as drying, pelletizing, hydrothermal carbonization and pyrolysis (Andriessen et al., 2019). The calorific value of the treatment product from feces or excreta is in the range of 17.2 to 28.1 MJ $kg^{-1}$, but varies depending on the substrate and treatment method (Afolabi et al., 2015; Andriessen et al., 2019; Wüst et al., 2019). Higher temperatures result in better sanitization but can lower the calorific value due to the higher ash content. In general, the treatment is quick and except for drying requires little space. Most of these technologies are better suited for centralized treatment and few are lab- or pilot – tested for in-toilet treatment or for a single household. Examples of in-toilet prototypes are the Sol-Char toilet, where feces are treated by pyrolysis (Ward et al., 2014) and the Nano Membrane Toilet where drying and combustion are used (Jurado et al., 2018). Hydrothermal carbonization with microwaves is also suggested as possible for in-toilet treatment (Afolabi et al., 2017; Mawioo et al., 2016).

### 2.5.2. Biological treatment methods

New research directions within biological treatments and resource recovery of excreta explore the options for biochemical energy production by anaerobic digestion or bioelectrochemical processes. **Anaerobic digestion** with production of biogas is a well-established method for wastewater treatment and wastes with high organics load. Black water, feces and excreta are also feasible substrates (Lansing et al., 2017; Regattieri et al., 2018). Anaerobic digestion is attractive due to the production of both energy and the remaining digestate that has a high nutrient content and requires less time than composting or vermicomposting. At small scale, the technology is more challenging because it requires time for inoculation, constant feeding of the reactor, and a relatively high level of expertise for maintenance and troubleshooting. In addition, the high concentration of ammonium when urine is not separated inhibits the anaerobic digestion (Zuo et al., 2021). However, single household or latrine systems have been proposed by Forbis-Stokes et al., (2016) and Regattieri et al., (2018) suggesting that this could be a feasible option in the future.

**Bioelectrochemical systems** include microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) and are interesting innovations that are researched for wastewater treatment. The organic matter is oxidized by electrochemically active microorganisms, which produce electric current flowing on an external circuit between an oxidizing anaerobic anode chamber and a reducing cathode chamber, aerobic in MFC and anaerobic in MEC (Leicester et al., 2020). From the two, MFCs are simpler and the electricity from the current is directly harnessed, whereas in MEC, an additional voltage and the anaerobic cathode facilitate hydrogen gas production. Microbial fuel cells have been tested for rural dry latrines (Castro et al., 2014; Kretzschmar et al., 2017; Perlow, 2012) but a shortcoming is the complexity of the technology and the low voltage produced with higher amount of solids (Kretzschmar et al., 2017; Perlow, 2012). Bioelectrochemical treatment of source-separated urine is the more feasible option in dry sanitation systems (Ieropoulos et al., 2013; Kuntke et al., 2014; Ledezma et al., 2015). The bioelectrochemical systems are currently at lab and pilot-scale and challenging to implement due to their complexity and cost (Leicester et al., 2020).

**Black soldier fly** (*Hermetia illucens*) **larvae (BSFL) composting** is a biological treatment that uses the larvae stage of the fly to digest organic material. The BSFL are efficient in converting the organic waste to protein and fat and have been researched for treatment of organic wastes, including fecal sludge and feces (Lalander et al., 2018, 2013a). The treatment is quicker than composting and the larvae are valuable as an animal feed. The BSFL composting is, however, more suitable for more centrally managed facilities because the management of the fly life cycle for production of eggs is complex and the residual organic matter needs additional treatment to ensure the elimination of *Ascaris suum* ova (Lalander et al., 2013a). Single latrine application has been documented within the biological urban sanitation project (BUSP) in Maputo, Mozambique but without successful implementation of the BSFL treatment (Mooijman et al., 2021).

### 2.6. Source-separation of urine and feces

Urine and feces can be collected and treated separately through dry urinals, specifically designed toilet bowls, pedestals, or slabs that divert urine. Separating the urine can be beneficial because it is low in organic solids and pathogens but has a higher fractions of the excreted essential nutrients N, P and K (Rose et al., 2015). The feces represent the smaller volume fraction of the excreta and, when separated, require less space for collection, storage, and treatment. Commonly, dry toilets with urine diversion are known as urine-diverting dry toilets (UDDTs). The term refers to the toilet user interface and is not associated with a specific treatment. In most cases, where UDDTs are described or implemented, the urine is diverted and stored (or infiltrated) and the feces are subjected to prolonged storage and dehydratation (Rieck et al., 2013).

In the faecal matter most pathogenic organisms are reduced and inactivated during prolonged storage (> 2 years) and desiccation, but a known exception are the *Ascaris* eggs (Schönning and Stenström, 2004; WHO, 2018). Following storage, the feces can be collected for additional on-site or off-site treatment, or utilized as a soil amendment. Separated feces can also be treated with the methods for excreta listed in this paper, but without the urine, the material has lower moisture and nutrient content, especially N, which would have an effect on its suitability as substrate for any biological treatment (e.g. C/N ratio, moisture, structure) and the final product's value as fertilizer or soil amendment. However, this could be an advantage for some of the thermal treatments, for example drying and pelletizing.

The separately collected urine of a healthy person is relatively free of pathogens and contains most of the excreted nutrients. Some enteric microorganisms are excreted with urine and cross-contamination with faecal matter can easily occur (Hoglund et al., 1998; Lahr et al., 2016). However, increase in alkalinity and ammonium as a result of the urea hydrolysis during storage have a disinfecting effect (Vinnerås et al., 2008) and WHO advises storage of six months as sufficient for sanitization before disposal or re-use (WHO, 2018). The essential nutrients N, P and K are mostly excreted through urine (90% N, 60-65% P and 73% K; Heinonen-Tanski and Van Wijk-Sijbesma, 2004; Rose et al., 2015). Therefore, urine is suitable as a liquid fertilizer, and several studies have reported high yields from fertilization with urine (Akpan-Idiok et al., 2012; Heinonen-Tanski and Van Wijk-Sijbesma, 2004; Karak and Bhattacharyya, 2011; Viskari et al., 2018).

The treatment of urine for recovery of one, several or all nutrients and other resources such as energy or hydrogen has recently gained attention. For most the goal is recovery of nutrients in solid state or highly concentrated liquid due to the benefits of lower volume, easier transport and better acceptance and marketability. Among the newly proposed and discussed treatments are: struvite precipitation to recover P as precipitate of Mg salts (Aguado et al., 2019; Tilley et al., 2008); alkaline dehydratation for preserving N and all nutrients as a solid fertilizer (Simha et al.,

2018); adsorption of nutrients to zeolites (Regmi and Boyer, 2021); nitrification-distillation and membrane filtration and distillation to concentration (Ray et al., 2020; Tun et al., 2016; Udert and Wächter, 2012; Volpin et al., 2020); electro-chemical treatment for nutrient recovery with electricity or hydrogen (Ieropoulos et al., 2013; Luther et al., 2015); air-stripping and acid scrubbing for N recovery (Wei et al., 2018); and combinations to achieve multi-nutrient recovery (De Paepe et al., 2018; Tao et al., 2019).

## 3. Discussion

Dry toilets with on-site treatment (or household level treatment) create an opportunity for treatment and resource recovery that is detached from infrastructure and transport and provides re-circulation of nutrients and resources. However, the current review of the possible treatments illustrates how different these systems are to the prevailing convention of "flush and forget", as they require knowledge, management, control, and multiple other considerations for both successful treatment and utilization of the product. Furthermore, decisions on treatment and recycling excreta from dry toilets on-site have to be considered within a complexity of external factors (e.g., available resources, space, knowledge, climate, desire for the product, regulations) that affect the long-term sustainability of the practice. Acknowledging such factors can help in decision making to avoid failures and implement them more successfully with-long lasting results.

### 3.1. Environmental factors

The physical environment has a strong influence on the on-site treatment processes in dry toilets due to the relatively small size and mostly uncontrolled conditions. Biological transformations of excreta by any type of composting (composting, vermicomposting, black soldier fly larvae composting), fermentation (lactic acid fermentation, anaerobic digestion) and bioelectrochemical systems are limited outside optimal temperature ranges and moisture levels. During chemical or thermal processes, the temperature is affecting the rate of the process, but the efficiency of the treatment is dependent of the characteristics of the substrate (volume, moisture, total solids, etc.). Processes differ in time and space requirements – humification and stabilization of the organic matter takes months in aerobic degradation, whereas chemical and physical processes, or fermentation can be as short as minutes (disinfection with lime) to days and weeks (LAF), except for passive drying or storage with desiccation. Climate and seasonality as well as the possibility for catastrophic events such as floods, droughts, earthquakes, should also be considered as they can lead to destruction, disruption, and release of untreated waste in the environment and the nearby water bodies.

The environmental context is a similarly important consideration for the products derived from excreta. The product from most of the discussed treatment processes is rich in plant nutrients and organic matter (compost, digestate, sanitized or LA-fermented excreta, dehydrated excreta); and can be used as a liquid (urine, concentrated effluent) or solid fertilizer (struvite). Organic fertilizers derived from excreta have been shown to be beneficial for soil health and fertility (Moya et al., 2019a; Sangare et al., 2015; Viskari et al., 2018) but research is scarce and little is known about how different treatments compare, the effects of suboptimal conditions, and what could determine or limit their impact. Their potential as fertilizers or amendments, and impact on the environment depends on specific parameters such as soil type, crops requirements, climate, quantity, and method of application. In addition, they will vary according to the type of treatment process, storage method and time, and how the process is affected by the environmental conditions. These variations, in addition to differences in the initial inputs, may lead to variability in the amendments in terms of volume, nutrients, pH, salt content, and residual pathogens or contaminants as heavy metals and pharmaceutical compounds. Other products such

as chars, fuels, or electricity are more consistent but can increase the design complexity, required knowledge and costs.

### 3.2. Social, cultural, and economic factors

Recycling excreta on-site or at domestic level require willingness, motivation, and commitment to maintain the treatment process but also to obtain and use the product. Pre-existing knowledge and perceptions play a role in shaping the attitudes towards sanitation systems and practice. In central Vietnam, previous experience and wide-spread practice of fertilization with excreta went together with positive perceptions of the practice but limited knowledge about risk for pathogen transmission and disregard of the regulations (Jensen et al., 2008). Among peri-urban farmers in Malawi, willingness to use human excreta derived fertilizers was determined by previous knowledge and experience (Roxburgh et al., 2020). In a multinational survey, Simha et al., 2021 found that perceptions of risks and benefits, and social norms are strongly associated with the willingness to consume foods grown with human urine.

Lack of regulations and recognition of the practice and the products by local and global institutions is a barrier to resource recovery of excreta. Currently, international guidelines on the reuse of sanitation products are provided by the World Health Organization with focus on pathogen transmission (WHO, 2018, 2006). According to Mallory et al. (2020), establishing the circular economy in the context of sanitation requires increased enforcement, policies and subsidies for fertilizers derived from human waste. Major barriers for the commercialization of human excreta-derived products are unclear regulations for their use and the absence of normative or legal regulations for such fertilizers within the European countries (Krause et al., 2021; Moya et al., 2019b). Guidelines for ensuring safety of the products could increase trust and enable market mechanisms, which could give an economic incentive for human excreta derived products. Still, a challenge would remain to integrate those products in the users' routines and their utilization would require that they are harvested reliably, and as needed – in quantity, quality, and in time.

## 4. Conclusions

In this study, literature on the processes used in dry sanitation systems to transform excreta on-site were critically reviewed with focus on the practical considerations. Dry toilets with on-site treatment are household level technology and as such have a small size, little controlled treatment conditions, and are therefore limited by the prevailing environmental conditions. The treatment process and the products differ in space and time due to the variability in the composition of excreta, additional input materials, how the treatment is managed, and the local context. The products can be valuable resources locally but must be desired and their application regulated. Better understanding how environmental conditions affect treatment, what can limit the effectiveness, and how that is reflected in the product and its utility is a step towards building the confidence of institutions, sanitation and agriculture practitioners, and users in excreta-derived products from dry toilets with on-site treatment.

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# Paper II

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RESEARCH IN ENVIRONMENTAL PLANNING AND MANAGEMENT

# Small-scale on-site treatment of fecal matter: comparison of treatments for resource recovery and sanitization

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**Abstract**
On-site small-scale sanitation is common in rural areas and areas without infrastructure, but the treatment of the collected fecal matter can be inefficient and is seldom directed to resource recovery. The aim of this study was to compare low-technology solutions such as composting and lactic acid fermentation (LAF) followed by vermicomposting in terms of treatment efficiency, potential human and environmental risks, and stabilization of the material for reuse in agriculture. A specific and novel focus of the study was the fate of native pharmaceutical compounds in the fecal matter. Composting, with and without the addition of biochar, was monitored by temperature and $CO_2$ production and compared with LAF. All treatments were run at three different ambient temperatures (7, 20, and 38°C) and followed by vermicomposting at room temperature. Materials resulting from composting and LAF were analyzed for fecal indicators, physicochemical characteristics, and residues of ten commonly used pharmaceuticals and compared to the initial substrate. Vermicomposting was used as secondary treatment and assessed by enumeration of *Escherichia coli*, worm density, and physicochemical characteristics. Composting at 38°C induced the highest microbial activity and resulted in better stability of the treated material, higher N content, lower numbers of fecal indicators, and less pharmaceutical compounds as compared to LAF. Even though analysis of pH after LAF suggested incomplete fermentation, *E. coli* cell numbers were significantly lower in all LAF treatments compared to composting at 7°C, and some of the anionic pharmaceutical compounds were detected in lower concentrations. The addition of approximately 5 vol % biochar to the composting did not yield significant differences in measured parameters. Vermicomposting further stabilized the material, and the treatments previously composted at 7°C and 20°C had the highest worm density. These results suggest that in small-scale decentralized sanitary facilities, the ambient temperatures can significantly influence the treatment and the options for safe reuse of the material.

**Keywords** Feces · Dry toilet · On-site sanitation · Composting toilet · Resource recovery · Pharmaceuticals · Human excreta

The original online version of this article was revised: The images of figures 6, 8 and 10 are correctly presented in this paper.

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## Introduction

Only 38% of the global population have access to an improved sanitation facility connected to centralized treatment (WHO and UNICEF 2017). Hence, the majority of the remaining 72% uses on-site sanitation systems. Such systems are common in areas without or only with minor municipal infrastructure and in rural, remote, and small settlements and are commonly dry sanitation systems (WHO and UNICEF 2017). They have low resource input – no water and no complex and expensive infrastructure and, depending on the system, use little to no electricity (Tilley et al. 2014). The fecal sludge in those systems consists of mostly urine and feces and therefore is concentrated in a small, undiluted volume with high concentration of macro- and micronutrients, as well as organic matter, which can be valuable inputs to the surrounding agroecosystems. However, the fecal sludge is also associated with hazards as pathogens and micropollutants, including pharmaceuticals and other chemicals of emerging concern (Schönning et al. 2007; Hester and Harrison 2016; de Oliveira et al. 2020; Gros et al. 2020).

Most on-site sanitation systems do not treat the fecal sludge to facilitate safe reuse (WHO and UNICEF 2017). Currently, the common practices are not considering treatment or resource recovery and rely on subsequent storage or disposal (Strande and Brdjanovic 2014; Tilley et al. 2014). Dry composting toilets are considered one of the best current options for on-site treatment in terms of resource recovery (Orner and Mihelcic 2018; McConville et al. 2020). However, composting is not always successful, and the resulting material is usually neither stabilized nor sanitized (Niwagaba et al. 2009; Hill et al. 2013). Few studies have addressed this important aspect by examining how to improve the treatment physically or chemically by, i.e., solar heating (Redlinger et al. 2001), different bulking materials (McKinley et al. 2012; Hashemi et al. 2019), and/or amendments such as biochar (Hijikata et al. 2015) or urea (Vinnerås 2007). Others have focused on modifying the treatment by vermicomposting (Yadav et al. 2011), lactic acid fermentation (LAF) (Andreev et al. 2018), or fly larvae composting (Lalander et al. 2013a).

Combinations of treatments are considered promising. Integrated composting-vermicomposting has been investigated for a variety of organic wastes (Lim et al. 2016) including fecal slurry (Yadav et al. 2012). The material is first sanitized by thermophilic composting and conditioned further with earthworms to improve the quality of the end product. Precomposting facilitates better conditioning because earthworms are vulnerable to thermophilic temperatures and toxic compounds in the organic wastes (Yadav et al. 2012). Another treatment combination for on-site sanitation is pre-treatment with LAF followed by thermophilic composting (Andreev et al. 2016) or vermicomposting (De Gisi et al. 2014). LAF is easy to manage and reduces quickly fecal pathogens, while the organic matter and nutrients are retained (Odey et al. 2018a). However, LAF alone does not sufficiently stabilize and sanitize fecal matter, and further treatment is needed before application as soil conditioner or fertilizer (Andreev et al. 2018). The combination of LAF and vermicomposting is part of the Terra Preta sanitation, which is inspired by ancient practices of organic waste management for soil fertility in the Amazon region (De Gisi et al. 2014). Central to the Terra Preta sanitation concept is the addition of carbonaceous pyrogenic material as biochar to retain nutrients and increase the product value for improving soil health and fertility. Biochar amendment in organic waste treatment has been shown to have benefits for agricultural application, with respect to retention of nutrients and pollution remediation (Wu et al. 2017). However, the efficiency for pollutant removal has not yet been assessed.

Biological transformations of fecal matter in on-site sanitation systems based on composting, vermicomposting, and LAF, even though considered as low-tech, can contribute to a cleaner local environment. If those practices are suitable in the local social and economic context, they have the potential to increase sustainability through recirculating nutrients and organic matter from excreta to agriculture and contribute to the currently propagated circular bioeconomy strategy. It is therefore important to explore different treatments in more detail and compare them directly with regard to the risks to human health and content of contaminants such as pharmaceutical residues and others, as well as assess their value for agricultural application.

Both composting and fermentation rely on biological processes, which are influenced by environmental conditions and management practices. Microbial transformations of the organic material are the foundation of these processes and are strongly influenced by, e.g., ambient temperatures. In small-scale systems, such as decentralized on-site sanitary facilities, the influence of temperature will be important. The fecal matter treatment in cold environments may be inhibited requiring different design considerations and management (Chen et al. 2020).

The aim of this study was to compare small-scale conventional composting with and without addition of biochar and to compare it to lactic acid fermentation (LAF) at three different ambient temperatures (7, 20, and 38°C). We further evaluated the use of vermicomposting at room temperature as a secondary treatment step. The composting process was investigated by measuring microbial activity and was compared to LAF by determining the changes in physicochemical characteristics and enumerating fecal indicators as well as quantifying selected pharmaceutical residues. After vermicomposting, the different treatments were evaluated with respect to changes in physicochemical characteristics, abundance of fecal indicators, and worm density.

## Materials and methods

In order to compare small-scale on-site sanitization strategies for fecal matter, a controlled laboratory experiment was carried out with three fecal matter mixtures, each run in three replicates at three different temperatures. All treatments were subjected to degradation (by composting or LAF) under controlled temperature for 71 days, followed by 15 days of composting and 77 days of vermicomposting at room temperature.

### Composting reactors and experimental setup

The experiment was conducted in tailor-made 16-L small-scale reactors. The reactor size was chosen, as a compromise between real-world size for on-site sanitation from a single toilet and the need for multiple replicates under controlled conditions. As suitable for the purpose, commercially available bokashi bins (0.38 × 0.33× 0.27 m) were used. The bins used for LAF were kept closed, whereas the reactors used for composting were modified by replacing the tap at the bottom with a tube connector. The connector was linked via 6-mm (inside diameter) tube to a liquid trap and a pump (Mini Diaphragm Vacuum Pump LABOPORT, model N86 KN.18, KNF, Freiburg, Germany) to pump air through the material (Fig. 1a). Further, two holes were drilled on the two top opposite short sides and connected with hoses (inside diameter 3 mm) to a gas analyzer (Fig. 1b). When the lid was closed, this created a closed circuit for the headspace air and allowed to determine rates of $CO_2$ production. Aeration and $CO_2$ measurements were operated sequentially.

Replicate reactors were placed in three climate-controlled rooms, maintaining ambient temperatures of 7, 20, and 38°C, respectively, for 71days. The temperatures were chosen to demonstrate a range of low, normal, and high ambient temperatures. The 7°C is representative of cold environments and 20°C of warm environments, and 38°C corresponds to additional heating, which increases the microbial activity and speeds up the composting (Sundberg et al. 2004; Eklind et al. 2007). At each temperature, there were three replicates of (1) composting mix from excreta with sanitary bark (Mix C); (2) composting mix with excreta, sanitary bark, and biochar (Mix CB); and (3) fermenting mix of excreta, sanitary bark, and biochar (Mix F) (Fig. 2). The compost reactors were dynamically incubated by sucking ambient air top-down through the substrate (negative aeration). For this, a vacuum pump was connected to the bottom of each of the six composting reactors (Fig. 1a) via equally long tubing to avoid pressure differences between the reactors. The pump was operated for 15 min followed by 30 min off. This aeration regime was chosen to avoid drying out of the substrate. The aeration regime was interrupted for measuring $CO_2$ production and leachate pH. LAF reactors were incubated statically without aeration and closed lid. The moisture in the material was maintained by periodically returning the leachate collected in the liquid traps back into the composting mix and by sprinkling with tap water. After 71 days, the material from each reactor was emptied into another container, thoroughly hand-mixed with gardening tools, and subsampled for analyses.

Before vermicomposting, the reactors were mixed and sampled and left open for 2 weeks to compost at room temperature without forced aeration to increase pH and remove some of the $NH_3$. Thereafter, 150 red wiggler worms (*Eisenia fetida)*, provided by industrial waste treatment and recycling facility Lindum, Drammen (Norway), were placed in each reactor. The reactors were kept moist and open and at room temperature (23°C). After 77 days, the material from each reactor was emptied in another container, the earthworms

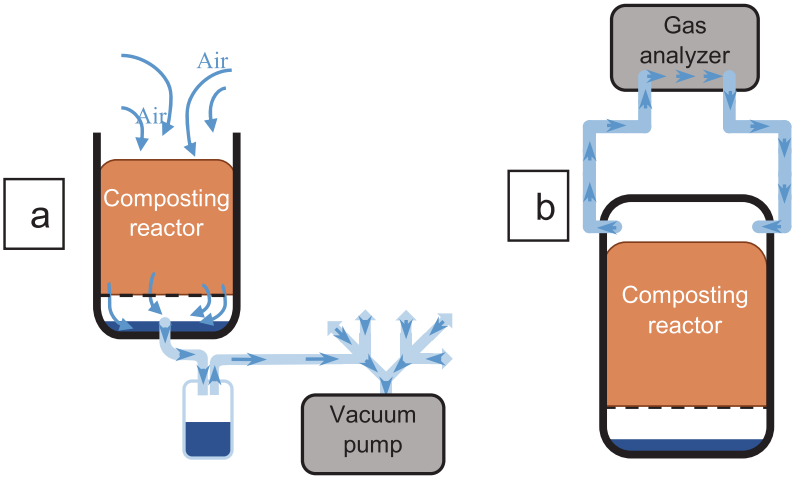



**Fig. 1** Schematic representation of the composting reactor setup. **a** When the pump is working. **b** When closed and connected to the $CO_2$ analyzer

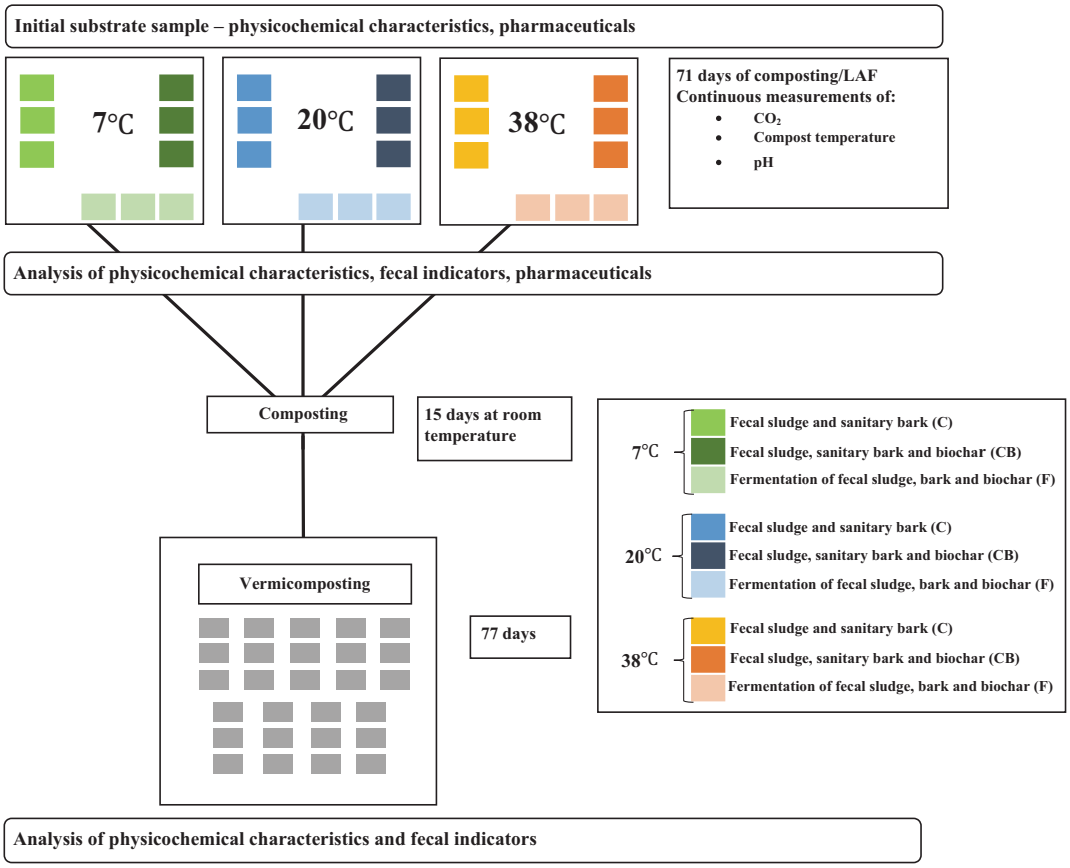



**Fig. 2** Overview of the experimental setup, treatments, and sampling timeline

were counted and removed, and the material was thoroughly mixed with gardening tools and sampled. The method of counting did neither differentiate between development stages of the earthworms nor include eggs.

**Table 1** Initial substrate composition presented as average wet weight per reactor

| | Fecal matter and sanitary bark (C) | Fecal matter, sanitary bark, and biochar (CB) | Fecal matter, sanitary bark, and biochar, for fermentation (F) |
|---|---|---|---|
| Fecal material (kg) | 3.2 | 3 | 3.2 |
| Sanitary bark (kg) | 1.7 | 1.6 | 1.4 |
| Compost inoculant (kg) | 0.13 | 0.13 | 0 |
| Urine (L) | 0.75 | 0.75 | 0.7 |
| Water (L) | 0.5 | 0.47 | 0.25 |
| Biochar (kg) | - | 0.28 | 0.3 |
| Lactic acid bacteria inoculant – sauerkraut juice (L) | - | - | 0.3 |
| Total (kg) | 6.3 | 6.2 | 6.25 |

## Initial mixtures and materials

The initial substrates and corresponding amounts per reactor are listed in Table 1. The fecal material used for the experiment was mixed with small amounts of toilet paper and residues from sanitary pads and wet wipes. It was acquired from one collection compartment servicing five dry toilets at Åstjern cabin complex, Bleiken, Norway. The fecal sludge was accumulated over 2 years with daily fresh inputs until the day of collection. Separately collected urine was added to the mixture for mimicking fresh input to a dry toilet. The urine was obtained from a nearby farm household, collected, and stored in closed containers prior to use in this experiment. Bark was used as a bulking material to adjust the C/N ratio (based on preliminary tests with the materials) and to improve the structure. The bark was from commercially available packs of sanitary bark (Nordic Garden, Steinsholt, Norway) consisting of finely cut (0–15 mm) coniferous bark. It is commonly marketed and used as a dry toilet amendment in Scandinavia. Compost from a preliminary trial with fecal matter, bark, and food waste was used as inoculant. In the biochar treatments (Mix CB and Mix F), biochar – collected from a Pyreg pilot pyrolysis plant (established as part of the Stockholm Biochar Project (2017), and using garden waste as a substrate for the process) – was added to the substrate mix. The amount was corresponding to approximately 5% of the volume of the corresponding mixture. For the lactic acid fermentation treatment (Mix F), the substrate was inoculated with sauerkraut juice, instead of compost and biochar added, as in the Terra Preta sanitation concept (De Gisi et al. 2014). Sauerkraut is a widely available LAF product in Europe and had already been reported as inoculant for fermentation of feces (Andreev et al. 2016, 2017). The sauerkraut juice was drained from a mix from homemade and commercially available sauerkraut. Each substrate was combined and mixed in a cement mixer (Atika, model Comet 130 S, Ahlen, Germany) in two batches of 25–30 L and then distributed to the nine reactors for each treatment (Table 1). An initial substrate sample was taken from each reactor for chemical analysis.

## Microbial activity in the composting treatments

The microbial activity during composting was followed by monitoring temperature continuously and by measuring respiration. Temperature was recorded with a HOBO Pendant Temperature Data Logger (Onset, Bourne, USA, 0.5°C accuracy). The loggers were buried in the center of the composting mix and remained there for the 71 days of treatment, logging at intervals of 10 min. Respiration was measured as $CO_2$ accumulation in the reactor headspace using a portable $CO_2$ infrared gas analyzer (EGM-5, PP-Systems, Amesbury, USA, dynamic range 0–100,000 ppm). Pump power and airflow rate were set to maximum, resulting in a circulating headspace airflow of ca. 0.5 L $min^{-1}$. To measure respiration, the gas analyzer was sequentially connected to each reactor while keeping the lock closed (Fig. 1b). To determine the respiration rate, $CO_2$ concentrations were recorded every 10 s for at least 6 min. The $CO_2$ measurements were carried out daily during the first 15 days of composting and every second day for the remainder of the 71-day treatment period. Preliminary composting trials with a similar reactor, substrate volume, and substrate mixture showed that the highest activity occurred within the first 5 to 10 days.

The $CO_2$ production rate was estimated from the increase of $CO_2$ concentration over time by linear regression of on average 200 s from the middle of the 6-min measurement period and expressed as mg $CO_2$-C $reactor^{-1}$ $day^{-1}$ using Eq. 1:

$$mgCO_2{-}C\ reactor^{-1} hour^{-1} = \frac{ppmCO_2 s^{-1} \times 10^{-6} \times V}{Vm \times M \times 3600 \times 1000} \quad (1)$$

where ppm $CO_2$ $s^{-1}$ is the change in $CO_2$ concentration, $V$ is the volume of the headspace ($L$), $V_m$ is the molar volume (L $mol^{-1}$) at each temperature, and $M$ is the molecular weight of C in $CO_2$ (12 g $mol^{-1}$).

Active degradation reduces the volume of the material with time. To account for the resulting increase in headspace volume, $V$ was estimated based on the difference between the level of the material in the reactor and the lid at Day 1 and Day 71. The composting period was divided into 3 periods based on observations and confirmed by temperature and $CO_2$ measurement: first, a period of intensive degradation – Day 1 to Day 5, then a period of active degradation – Day 6 to Day 20, and, finally, period of low activity – Days 21 to 71. The headspace volume was adjusted accordingly.

The total amount of C respired during composting was derived for each replicate by cumulating the average of each two adjacent measurements before averaging the values per treatment. The amount of cumulatively respired $CO_2$-C was expressed per kg initial $C$ in each reactor.

## Fecal indicators

The fecal indicators in the treatments were assessed by enumerating *Escherichia coli* and enterococci in composite samples taken in duplicates after the composting/fermentation and after the vermicomposting. The samples were stored at approximately 4°C and analyzed within 78 h of collection. A subsample of 10 g was diluted in 90-mL maximum recovery diluent (purchased from Sigma-Aldrich), and the mix was mechanically homogenized by a stomacher for 2 min. Preliminary samples from each treatment were used to determine the number of dilutions. They were further analyzed according to the method for the enumeration of *E. coli* by a defined substrate most probable number (MPN) technique

(APHA 2005) using Colilert 18 test kits (IDEXX Laboratories Inc., Westbrook, ME, USA). The cell numbers were determined according to the IDEXX Quanti-Tray/2000 MPN table and expressed per g of dry solid.

### Physicochemical characteristics

Samples were collected from each container after 71 days of composting and after 77 days of vermicomposting. The material from each reactor was emptied into a larger container and thoroughly mixed with gardening tools before sampling. Dry matter and moisture content were determined by drying the samples at 60°C for 48 h. Volatile solids (VSs) were determined by combustion of dry samples at 500°C for 3–4 h in a muffle furnace. Total C was determined in crushed samples by dry combustion (Nelson and Sommers 1982) at 1050°C using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA). Total N was measured on the same instrument according to the Dumas method (Bremner and Mulvaney 1982). Ammonium ($NH_4$-N) was measured by flow injection analysis (FIA, Tecator FIAstar 5010 Analyzer, Hillerød, Denmark) after extraction with 2 M KCl in both fresh and dry samples. The difference in the concentration of $NH_4$-N between fresh and dry samples was used to correct the tot N for the $NH_4$-N loss as $NH_3$ during drying. The pH was measured in leachate during the composting and at Day 77 of vermicomposting with a pH electrode (Orion™ ROSS Ultra, Thermo Fisher Scientific, Waltham, USA). pH was measured in solid samples with a wet sample to water volume ratio of 1:1.5. For the fermentation treatments, no leachate was collected because they were not subjected to aeration and did not require additional watering. Therefore, the pH was not monitored regularly but only measured twice during the first 10 days.

### Pharmaceuticals

For the quantification of targeted analytes in this study, a previously optimized analytical method was adopted with some modifications (Ali et al. 2019). The selection of the compounds (see Online Resource, Table S1) was based on their high rates of production and prescription in addition to their frequent detection in contaminated environmental samples in Norway.

Samples were prepared as described in Online Resource (S3) from initial mixtures and the products of composting/fermentation and analyzed with liquid chromatography-tandem mass spectrometry (see Online Resource, S4). The method performance characteristics are listed in Online Resource, Table S3 and described in S5.

### Statistics

Analysis of variance was used to compare the effects of the different treatments on the measured physicochemical characteristics. The assumptions were checked with Levene's test for homogeneity of variances and the Shapiro-Wilk test for normality. Two-way ANOVA was used when the assumptions were met. To evaluate differences of means per factor, the ANOVA was followed by Tukey's post hoc comparison of means ($p < 0.5$). The normality assumption was violated for concentrations of total N and $NH_4$-N after vermicomposting, and the data were log transformed. For VS, $NH_4$-N, and pH after composting and VS and total C after vermicomposting, the Kruskal-Wallis rank sum test was used for analysis. Differences between cumulative C-mass losses between the composting with and without biochar and initial and post-treatment concentrations of pharmaceutical compounds were evaluated with one-tail, unequal variance t-test. Statistical analyses were carried out using the R statistical package version 1.3.959 under the GNU public license (Boston, MA, USA).

## Results

### Effect of temperature on the composting process

Microbial activity during the 71 days of composting was monitored as temperature change and $CO_2$ production. Figure 3 shows the temperature profile for all treatments for the first 38 days of composting. The periodical fluctuations (positive in 7°C and negative in 38°C treatments) correspond to the time when the reactors were taken out of the climate-controlled room for weighing and pH measurements. In all treatments, the material was self-heated and maintained higher than ambient temperature during the first 6 to 10 days, but self-heating relative to ambient temperature was clearly larger at 38 than 7 and 20°C. The temperature profile shows clear differences between the treatments subjected to low, middle, and high ambient temperature; adding approx. 5% biochar to the mix had no effect on the released metabolic heat.

The $CO_2$ evolution rates for composting treatments are shown in Fig. 4 (top). Similarly to the temperature profile, highest $CO_2$ production was detected during the first 5 days before levelling gradually off and stabilizing after 30–40 days of composting. At 38°C, microbial activity was highest, and the $CO_2$ production rates remained above those of other temperature treatments throughout the entire period. Maximum observed respiration rates were 463–707 mg $CO_2$-C reactor$^{-1}$ h$^{-1}$ on Day 2 for the 38°C treatment, 254–422 mg $CO_2$-C reactor$^{-1}$ h$^{-1}$ on Day 3 for the 20°C treatment, and 100–146 mg $CO_2$-C reactor$^{-1}$ h$^{-1}$ on Day 2 for the 7°C treatment. The highest ambient temperature resulted in the highest $CO_2$

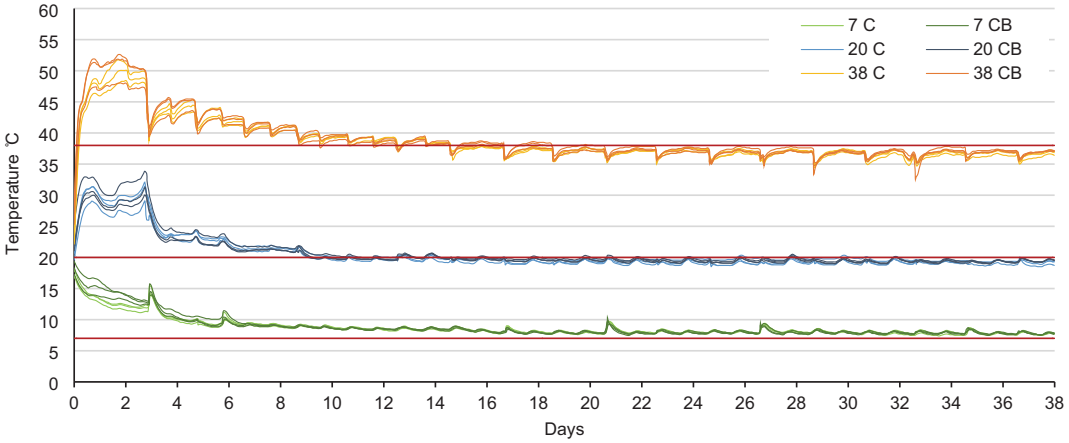



**Fig. 3** Temperature profile measured in the center of each reactor for the first 38 days of composting. The red lines represent the ambient temperature in the climate-controlled rooms – 7, 20, and 38°C. C composting, CB composting with addition of biochar

production. There was no indication that the addition of biochar affected rates or dynamics of $CO_2$ production.

The cumulative $CO_2$-C loss varied from 45.6 to 177.6 g C $kg^{-1}$ C across treatments. Ambient temperature had a strong effect on the relative amount of respired C, whereas the addition of biochar lowered respiration losses only insignificantly (Fig. 4 bottom). Two to three times more C was respired at 38°C than at 7°C after 69 days. The 38°C treatment also had the steepest initial increase in cumulative respiration, emitting half of the totally respired C within 9 days, whereas 12 (without biochar) and 11 days (with biochar) were needed at 20°C and 18 and 16 days at 7°C.

## Physicochemical characteristics

The physicochemical characteristics in the initial mixtures suggested that the materials were well-suited as a feedstock for active composting (Table 2). The C/N ratios in the initial mixtures corresponded to a ratio that facilitates active degradation (Epstein 1997) with little recalcitrant carbon materials as indicated by the high content of VS. The moisture content was at the higher limit of what is considered optimal for composting, 40–70% (Guo et al. 2012). The pH was alkaline.

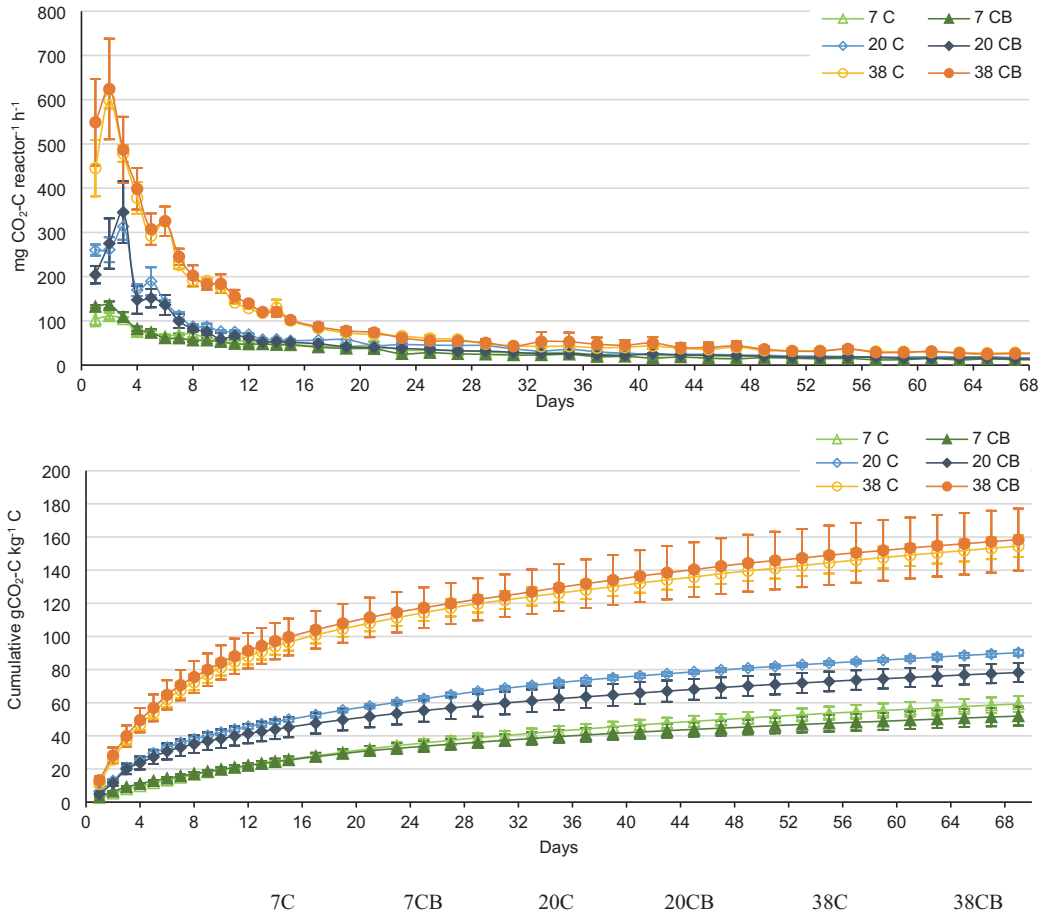
The 71 days of treatment affected physicochemical properties differently in fermentation and composting treatments. The fermentation treatment resulted in a significantly higher C/N and $NH_4$-N content, whereas no significant change was observed in the composting treatments. Clear differences between the treatments were observed in the 38°C composting treatments with lower C/N ratios, higher concentration of tot N, and lower VS. Furthermore, the pH in the leachate of 38 C and 38 CB decreased, which was not observed in the other treatments and indicates a chemical transition in the material that started around Day 57 (Fig. 5).

For the fermentation treatments, the pH was not followed continuously but was measured in leachate on Day 5 for 7 F as 4.5 and 20 F as 3.8 and on Day 9 as 4.3 for 7 F and 3.9 and 5.7 for two of the replicates in 20 F. The acidity in the leachate indicated successful inoculation with lactic acid bacteria and production of lactic acid in the first days. By contrast, the pH measured in the samples taken on day 71 indicated that the acidity was not maintained throughout the entire treatment period.

For the composting treatments, the changes of the pH in the leachate are plotted in Fig. 5. There was little change in the 7°C treatments. In the 20°C treatments, the pH decreased around Day 30 and returned back to neutral pH after about 20 days. This transient acidification was more pronounced in the compost without than with biochar. The pH in 38°C treatments was relatively stable throughout but started to decline during the last days of composting, i.e., after Day 57. The addition of biochar did not result in clear pH differences. However, in the 7°C and 20°C treatments, it resulted in slightly higher pH, whereas at 38°C, it resulted in lower pH values at the end.

## Fecal indicators

*E. coli* was detected in all treatments within the range of 90.6 MPN $g^{-1}$ DM to the upper limit of detection >8.5 $\times 10^8$ (Fig. 6). The smallest MPN values of *E. coli* were detected in the 38 C treatment. *E. coli* was most abundant in 7 C and 7 CB, at the upper limit of detection and 4–6 $\log_{10}$ units higher than in the other treatments. Interestingly, at 7°C, the MPN *E. coli* in the fermentation was 5 $\log_{10}$ units lower than in the composting treatments.




| | 7C | 7CB | 20C | 20CB | 38C | 38CB |
|---|---|---|---|---|---|---|
| *Total $CO_2$-C g $kg^{-1}$ C* | 59.26 ± 4.81 | 51.94 ± 5.68 | 90.2 ± 1.74 | 78.21 ± 5.74 | 154.52 ± 6.58 | 158.42 ± 18.73 |

**Fig. 4** $CO_2$ emission rates (top) and mean cumulative $CO_2$ emission (bottom) ($n$=3, ± SD) during composting at three different ambient temperatures (7, 20, and 38°C). C composting, CB composting with addition of biochar. Cumulative $CO_2$ emission estimation is based on periodic $CO_2$ production measurements in the headspace and adjusted for initial total C. The average values with standard deviations for three replicates for total cumulative $CO_2$-C g $kg^{-1}$ C are given below in the table

Enterococci were detected in high numbers in all treatments (Fig. 6). The concentration varied with temperature with higher numbers at 7°C and 20°C and comparatively lower numbers at 38°C. The values for the 7°C and 20°C treatments were in the range of $6.4\times10^4$–$1.9\times10^7$ MPN enterococci, whereas the treatments at 38°C had lower numbers in the range of $3.0\times10^4$–$1.3\times10^6$.

For both *E. coli* and enterococci, the higher temperature treatments resulted in lower numbers. There was no clear effect of adding biochar on the abundance of fecal indicators. The fermentation treatments showed variable results depending on temperatures and had significantly lower numbers of *E. coli* after LAF at 7°C compared to composting at the same temperature.

## Fate of pharmaceutical compounds

The pharmaceutical compounds were selected based on their relatively high prescription rates in Norway, and their pattern and concentrations reflect the regional consumption and are

**Table 2** Physicochemical characteristics for the initial mixtures and after 71 days of composting/fermentation. The data are means ($n$=3) and standard deviation. Capital "T" indicates means that are significantly different due to effect of temperature and capital "M" due to mixtures. *Note:* pH values measured in samples

| | | Moisture % | VS % | pH | Tot. C % | Tot. N % | C/N | $NH_4$-N mg $g^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Initial | Mix C | 68 ± 0.5 | 89 ± 0.3 | 8.4 | 48 ± 0.25 | 1.83 ± 0.00 | 26 ± 0.1 | 2.802 |
| | Mix CB | 68 ± 0.1 | 89 ± 0.4 | 8.5 | 49.5 ± 0.85 | 1.93 ± 0.04 | 26 ± 0.1 | 3.435 |
| | Mix F | 69 ± 1.5 | 89 ± 0.2 | 8.2 | 50.3 ± 0.57 | 1.87 ± 0.07 | 27 ± 1.3 | 4.61 |
| After composting/fermentation | 7 C | 70 ± 1.8 | 88 ± 0.2 | 7.2 | 47.6 ± 0.24$^{M}$ | 1.78 ± 0.15 | 27 ± 2.5 | 0.862 ± 0.031 |
| | 7 CB | 72 ± 0.7 | 89 ± 0.3 | 7.2 | 49.6 ± 0.58 | 1.83 ± 0.02 | 27 ± 0.1 | 0.791 ± 0.050 |
| | 7 F | 66 ± 0.4 | 89 ± 0.1 | 6.8 | 51.6 ± 0.58 | 1.83 ± 0.08$^{M}$ | 28 ± 1.1$^{M}$ | 3.905 ± 0.051 |
| | 20 C | 71 ± 0.6 | 89 ± 2.6 | 7.1 | 47.6 ± 0.33$^{M}$ | 1.76 ± 0.06 | 27 ± 1.1 | 0.101 ± 0.063 |
| | 20 CB | 70 ± 0.6 | 86 ± 1.0 | 7.2 | 49.4 ± 0.60 | 1.82 ± 0.06 | 27 ± 0.7 | 0.093 ± 0.022 |
| | 20 F | 67 ± 0.1 | 89 ± 0.5 | 6.8 | 49.9 ± 0.42 | 1.81 ± 0.03$^{M}$ | 28 ± 0.7$^{M}$ | 4.118 ± 0.292 |
| | 38 C | 69 ± 0.4 | 85 ± 0.7 | 7.3 | 46.8 ± 0.10$^{M}$ | 2.28 ± 0.05$^{T}$ | 21 ± 0.5$^{T}$ | 0.867 ± 0.145 |
| | 38 CB | 69 ± 1.3 | 79 ± 3.6 | 7 | 49.7 ± 1.44 | 2.19 ± 0.04$^{T}$ | 23 ± 1.1$^{T}$ | 0.455 ± 0.166 |
| | 38 F | 67 ± 0.8 | 89 ± 0.8 | 7.5 | 49.9 ± 0.78 | 1.7 ± 0.04$^{T,M}$ | 29 ± 1.0$^{T,M}$ | 4.236 ± 0.042 |
| | | | K-W test | K-W test | | | | K-W test |
| Temperature | | ns | . | ns | ns | *** | *** | ns |
| Mix | | *** | *** | ns | *** | *** | ** | *** |
| Temperature × mix | | * | / | / | ns | *** | *** | / |

Significance codes: ***0.001, **0.01, *0.05 , . 0.1 *ns* not significant

subject to the variability within the matrix. The fecal matter and urine used in the study are not directly comparable with fresh excreta, and the initial concentrations were expected to primarily reflect compounds that partitioned to the solids, as those excreted with urine are more soluble and could have drained away. The initial concentrations were measured in the mixtures for direct comparison of concentrations before and after treatment.

The results for ibuprofen, sulfamethoxazole, and diclofenac must be regarded as semi-quantitative due to

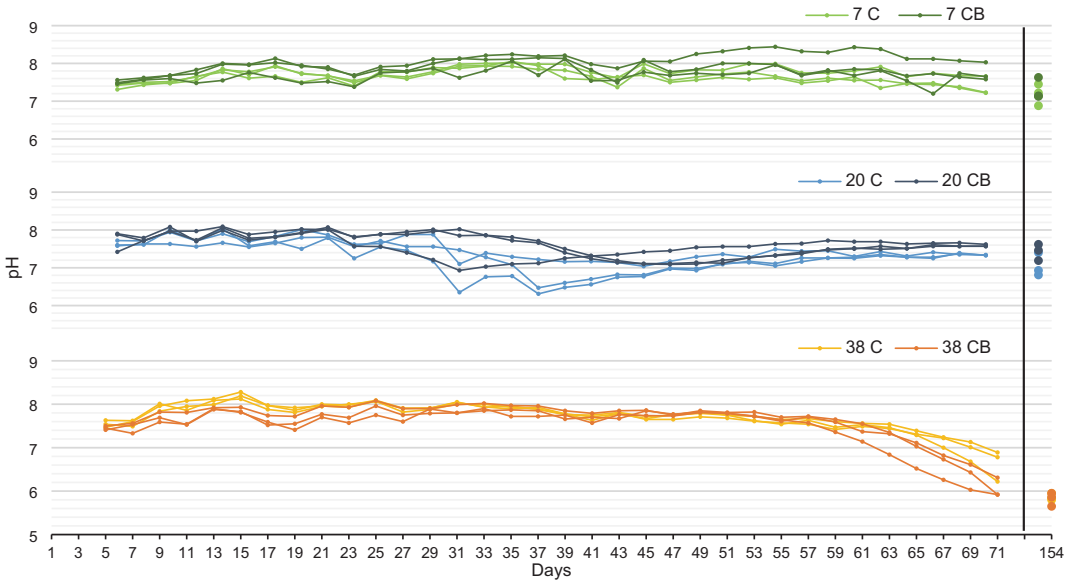



**Fig. 5** Leachate pH throughout 71 days of composting at 7, 20, and 38°C and on the final day of vermicomposting – Day 154. C composting material without biochar, CB composting with addition of biochar

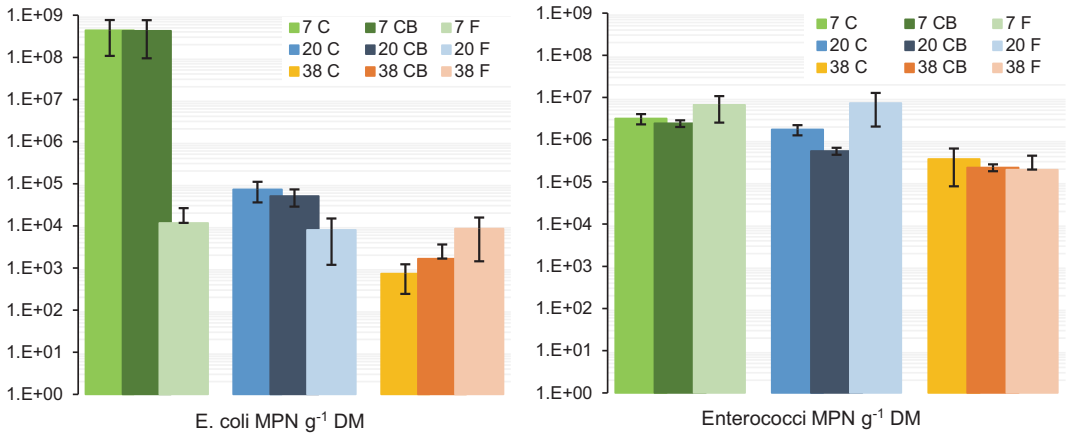



**Fig. 6** Enumeration of the indicator organisms *E. coli* (left) and enterococci (right) in samples after composting/fermentation at 7, 20, and 38°C ambient temperature. C composting, CB composting with addition of biochar. The error bars represent standard deviation

unreliable recoveries (see Online Resource, S5). The highest initial concentration among the detected pharmaceutical compounds was ibuprofen with a range of 8113–16551 μg $kg^{-1}$. Ibuprofen was not detected in any of the composting products, but in the fermentation products, it was in the range of 93–212 μg $kg^{-1}$. Sulfamethoxazole was detected in 7 out of 9 initial samples within the range of 3.5–11.7 μg $kg^{-1}$ and after treatment only in 5 out of 27 samples in a range of 0.2–21.1 μg $kg^{-1}$. Where detected in the products, amounts were lower than the initial values, with one exception where the concentration was 21.1 μg $kg^{-1}$ (one of the 20 BC samples). Diclofenac showed interesting pattern and therefore is discussed alongside the other compounds.

Figure 7 shows the detected concentrations for the other eight compounds, both in the initial and post-treatment samples. Caffeine was the compound with the second highest initial concentration with a range of 1351 to 2389 μg $kg^{-1}$, whereas the concentrations of warfarin were the lowest with values of 0.012 to 0.034 μg $kg^{-1}$ in initial samples. For caffeine, atorvastatin, losartan, diclofenac, and warfarin, the post-treatment concentrations were strongly negatively related to temperature, indicating that the increase in temperature and/or more active composting facilitated their removal.

Larger effects were observed when comparing the composting and LAF. For caffeine, carbamazepine, metoprolol, acetaminophen, and warfarin, there was a clear trend indicating more efficient removal during composting compared with fermentation. For caffeine, the results indicate higher removal in 38 C and 38 CB treatments and lower in the fermentation treatments. Carbamazepine showed low reduction in all treatments, and the highest concentrations were detected in the fermentation products. Post-treatment concentrations for metoprolol, acetaminophen, and warfarin showed a clear difference between composting and fermentation with larger reduction during composting. By contrast, atorvastatin, losartan, and diclofenac were detected in lower concentrations in the fermentation products compared to the composting products.

For most compounds, there was no clear effect of adding biochar, except for carbamazepine and diclofenac, for which the detected concentrations in the CB treatments were lower than those in C. For carbamazepine, the lowest detected concentrations were in the CB treatment.

The removal within the different treatments is shown in Fig. 8 but should be interpreted with caution due to the high variation in the concentrations detected between the replicates. Statistically significant reduction in concentrations between initial and after treatment was found only in some treatments for caffeine, metoprolol, losartan, and atorvastatin (Fig. 8, with *). Diclofenac and warfarin are not plotted as they were detected in higher concentrations after treatment with some exceptions for the treatments at 38°C. Also, losartan was detected in 7 CB and 20 CB at higher average concentrations. Likewise, carbamazepine and metoprolol concentrations increased in the fermentation treatment products. This can be explained by cleaving back of conjugates or by change in efficiency of extraction due to changes in the chemical conditions and degradation of particles to which they may have been adsorbed to initially (Leclercq et al. 2009; Jewell et al. 2014).

## Vermicomposting

Vermicomposting further stabilized and conditioned the composing/fermentation products. The resulting material was visually similar to conventional vermicompost with no unpleasant odors. The stabilization was also evident from

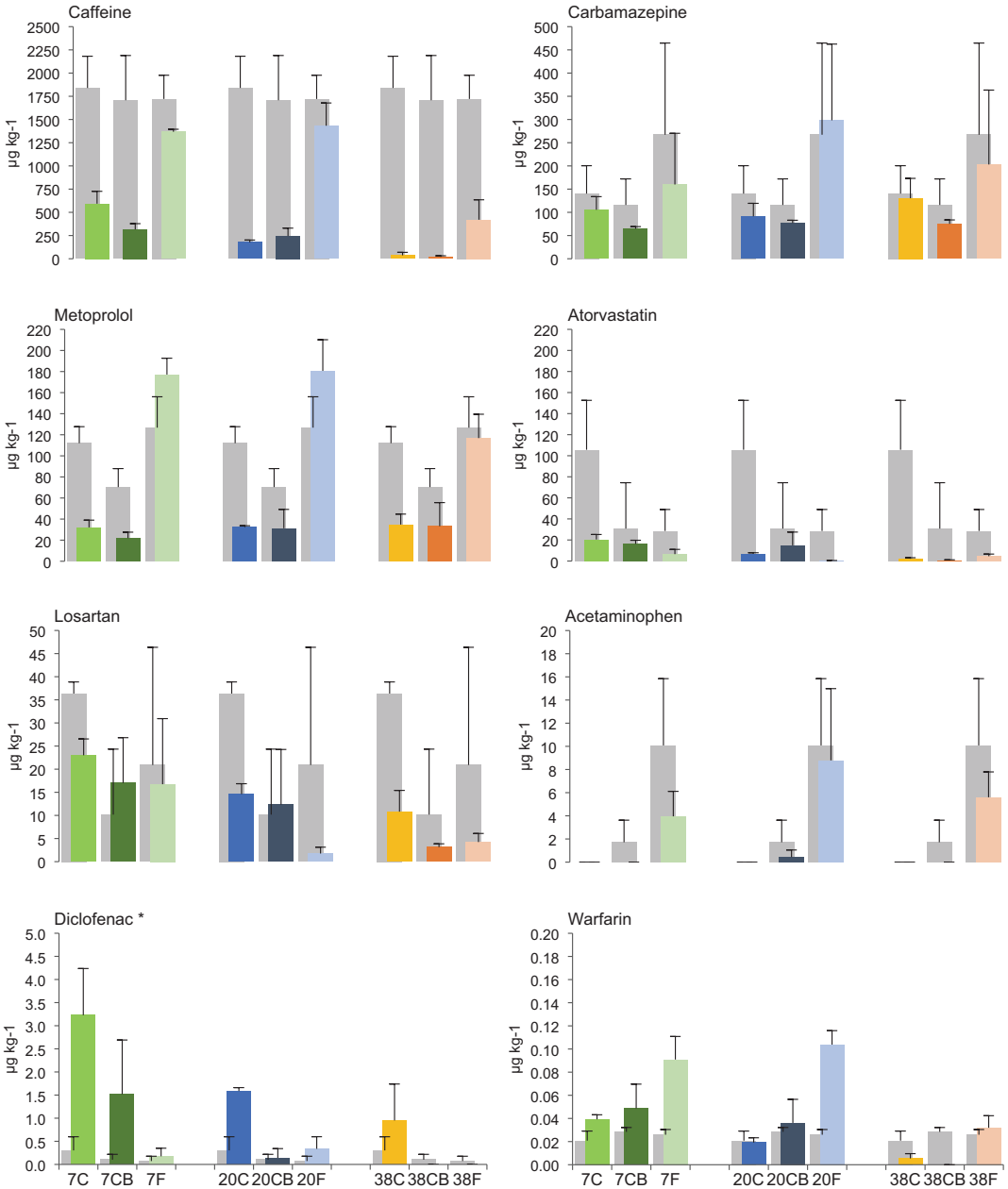



**Fig. 7** Comparison of caffeine, carbamazepine, metoprolol, atorvastatin, losartan, acetaminophen, diclofenac, and warfarin concentrations in the initial mixtures (gray columns) and after 71 days of composting/fermentation at 7, 20, and 38°C (colored columns). *The results for diclofenac are semi-quantitative. C composting, CB composting with addition of biochar. The error bars represent standard deviation

the significantly lower VS% and $NH_4$-N content (Table 3) than before vermicomposting.

The physicochemical parameters after vermicomposting differed between the treatments, but followed similar patterns

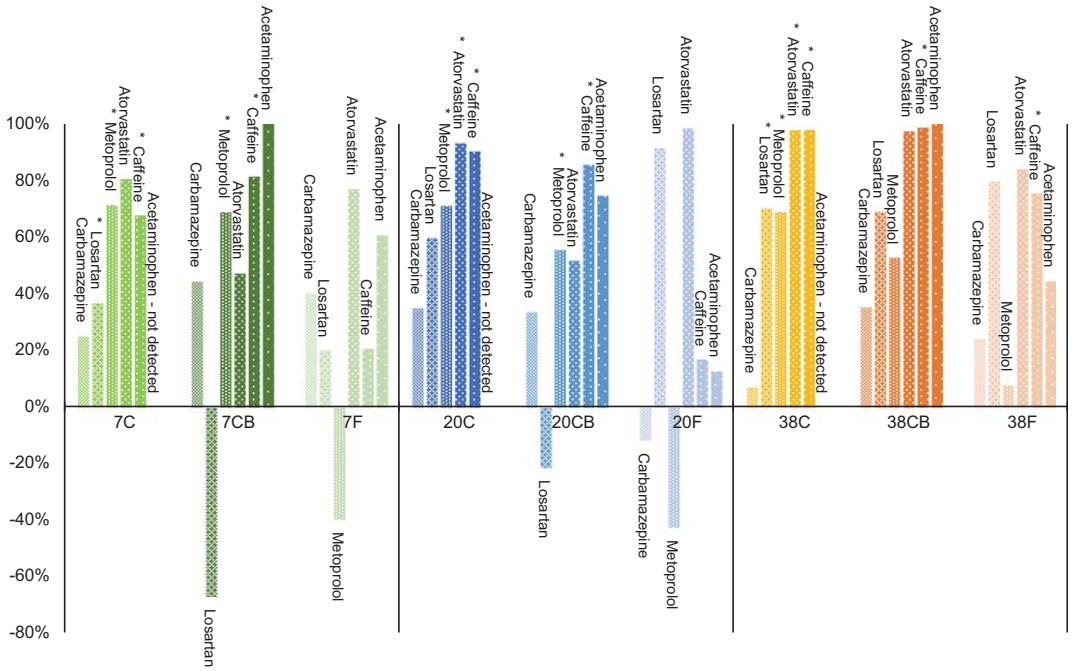



**Fig. 8** Removal of carbamazepine, losartan, metoprolol, atorvastatin, caffeine, and acetaminophen (in % of initial) after 71 days of composting/fermentation at 7, 20, and 38°C. The asterisk indicates the cases in which T-test showed statistically significant removal at $p>0.05$. C composting, CB composting with addition of biochar, F lactic acid fermentation

as those seen after composting/fermentation. The $NH_4$-N concentration was still significantly higher in the previously fermented vermicompost than in the previously composted treatments, but on a lower level. Only the highest temperature

**Table 3** Mean physicochemical characteristics ($n$=3, ±SD) for treatments after vermicomposting. The treatment factor refers to differences before and after vermicomposting. Capital "T" indicates means that are significantly different to the rest due to effect of temperature and capital "M" due to mixtures. *Note:* pH values measured in samples

| | Moisture % | VS % | pH | Tot. C % | Tot. N % | C/N | $NH_4$-N mg $g^{-1}$ |
|---|---|---|---|---|---|---|---|
| 7 C | 69 ± 0.6 | 87 ± 1.2 | 7.3 | 46.6 ± 0.51 | 1.98 ± 0.39 | 25 ± 3.2 | 0.026 ± 0.005 |
| 7 CB | 71 ± 0.1 | 85 ± 0.9 | 7.4 | 48.7 ± 0.58 | 1.7 ± 0.01 | 29 ± 0.6 | 0.024 ± 0.004 |
| 7 F | 70 ± 0.5 | 87 ± 0.6 | 7.8 | 48.3 ± 0.99 | $1.78 \pm 0.07^{M}$ | $27 \pm 1.5^{M}$ | $0.033 \pm 0.002^{M}$ |
| 20 C | 70 ± 0.6 | 87 ± 1.1 | 7.3 | 46.6 ± 0.33 | 1.67 ± 0.01 | 28 ± 0.1 | $0.02 \pm 0.003^{T}$ |
| 20 CB | 70 ± 0.2 | 87 ± 0.7 | 7.4 | 48.3 ± 0.40 | 1.69 ± 0.08 | 29 ± 1.3 | $0.02 \pm 0.002^{T}$ |
| 20 F | 70 ± 0.7 | 83 ± 2.0 | 7.5 | 48.5 ± 0.27 | $1.86 \pm 0.10^{M}$ | $26 \pm 1.3^{M}$ | $0.04 \pm 0.007^{T,M}$ |
| 38 C | 68 ± 1.0 | 86 ± 0.3 | 6.3 | 45.9 ± 0.50 | $2.19 \pm 0.09^{T}$ | $21 \pm 1.0^{T}$ | 0.018 ± 0.001 |
| 38 CB | 68 ± 0.7 | 86 ± 0.5 | 6.4 | 47.9 ± 0.31 | $2.27 \pm 0.08^{T}$ | $21 \pm 0.9^{T}$ | 0.015 ± 0.003 |
| 38 F | 70 ± 0.6 | 85 ± 1.0 | 7.4 | 49.9 ± 1.43 | $1.75 \pm 0.02^{T,M}$ | $28 \pm 1.1^{T,M}$ | $0.039 \pm 0.008^{M}$ |
| | | K-W test | | K-W test | | | |
| Treatment | ns | *** | ns | ** | ** | *** | *** |
| Temperature | * | ns | * | ns | *** | ** | ** |
| Mix | ** | ns | ns | *** | *** | *** | *** |
| Temperature × mix | ns | / | ** | / | *** | *** | . |

Significance codes: ***0.001, **0.01, *0.05 , . 0.1; *ns* not significant

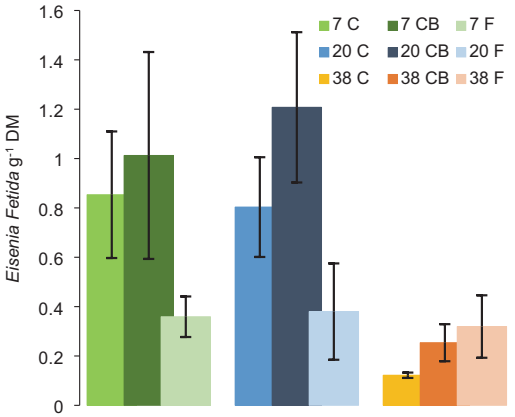



**Fig. 9** Mean density of *Eisenia fetida* ($n$=3, ± SD) after vermicomposting. The treatments were previously composted/fermented at 7, 20, and 38°C. C, composting, CB composting with addition of biochar

treatment (38 C and 38 CB) sustained a high total N content and low pH, even after vermicomposting.

Figure 9 shows the density of *E. fetida* in the different treatments after 77 days of vermicomposting. The worms propagated in all treatments but varied in density within the range of 0.11–1.63 worms $g^{-1}$ DM. Higher densities were detected in materials previously composted at 7°C and 20°C, whereas the lowest density is found in the 38°C material. In the CB treatments, there was a trend for higher average numbers of worms compared to the C treatments though not statistically significant.

The *E. coli* cell numbers in the samples from the vermicomposting are plotted in Fig. 10. Compared to the composting/fermentation step, *E. coli* counts were reduced

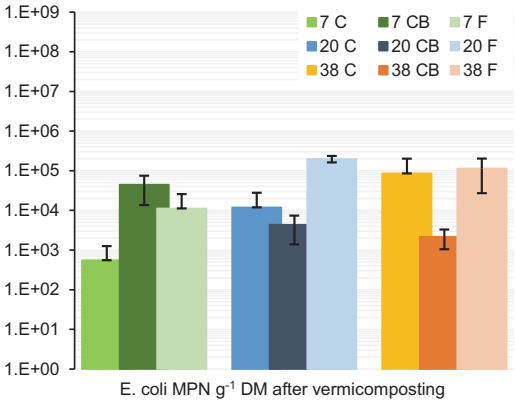



**Fig. 10** Enumeration of the indicator organisms *E. coli* after vermicomposting. The treatments were previously composted/fermented at 7, 20, and 38°C. C composting, CB composting with addition of biochar. The error bars represent standard deviation values

by 4–5 $\log_{10}$ during the vermicomposting for the 7 C and 7 CB treatments. However, in 20 C, 20 F, 38 C, and 38 F, higher cell numbers were detected after the vermicomposting indicating possible regrowth or contamination from the worms.

## Discussion

### Composting at different ambient temperatures

#### Microbial activity in the composting process

As evident from the temperature dynamics and the respiration rates, composting at 38°C supported a higher activity throughout the entire period, close to doubling the amount of respired C compared to composting at 20°C. The rate of the process depends on the availability of easily degradable substrates but also on maintaining optimal conditions such as temperature and aeration moisture (Haug 1993). It is likely that the higher temperature enhanced degradation by specific biota at a higher rate resulting in more available carbon for further degradation (Zhang et al. 2011; Wang et al. 2015), whereas at 7°C, the activity was low, and with time, the material became more compacted, the airflow was restricted, and accordingly the rate of degradation decreased. The lack of intensive degradation together with compaction, high moisture saturation, and no turning resulted in a sludge-like and water-logged material, which was not stabilized as indicated by the similar VS and C/N ratio in the substrate before and after the period of composting.

The temperature profile within the composting matrix is indicative of the process rate as a measure of the released heat but at the same time subject to the dynamics between heat production and heat loss to the environment. The conductivity of the material and the amount of composting substrate influence the temperature dynamics and can heighten temperatures or result in greater heat loss and affect the development of the process. Low ambient temperatures and lack of insulation result in higher net heat loss, which in turn slows microbial degradation (Niwagaba et al. 2009; Nasri et al. 2019). On the other hand, too high ambient temperatures can inhibit microbial activity as shown by Beck-Friis et al. (2001) for composting with external heating of 55°C.

As expected, the ambient temperature in these small-scale compost reactors without insulation had a significant effect on the composting dynamics, and an increase in the ambient temperature in the examined range between 7°C and 38°C resulted in more active and faster degradation and self-heating in the compost.

### Physicochemical characteristics

The change in the physicochemical characteristics between the initial mixtures and the materials after treatment suggests that those exposed to 38°C had undergone most transformation. 38 C and 38 CB were characterized by lowest VS and C/N ratios and highest N contents, indicating the material was more degraded. In the 38°C composts, the increase in total nitrogen was likely due to a concentration effect caused by the weight loss associated with the mineralization of organic matter (Sánchez-Monedero et al. 2001; Guo et al. 2012).

In all composting treatments, the concentration of $NH_4$-N was reduced; however, the changes in total nitrogen indicated that most of the $NH_4$-N was immobilized or nitrified, rather than lost through ammonia volatilization. During composting, nitrogen transformations are affected by temperature, pH, feedstock, and aeration (Sánchez-Monedero et al. 2001; Sundh and Rönn 2002). Several factors may have contributed to the low N losses in this study: the relatively low temperatures of composting, suboptimal aeration levels, and returning the leachate back into the composting mix.

Measured pH dynamics indicated biochemical changes in the middle of the composting period for treatments at 20°C and at the end for those at 38°C and little to no change for those at 7°C. The commonly observed trend in pH during composting is an initial drop due to the formation of organic acids, which is then followed by an increase and stabilization at neutral pH (Epstein 1997). In this study, the quick initial drop due to organic acid formation could have happened before Day 5, before the first measurements of pH. An interesting phenomenon is the drop in leachate pH at the end of the composting at 38°C. One possible explanation is that it is a result of intensified nitrification, as the release of $H^+$ in the nitrification process acidifies the composting mix. The study of Sánchez-Monedero et al. (2001) found a correlation between the concentration of nitrates and the pH. Supporting that explanation are also the higher values for total nitrogen with low percent ammonium in the treatments at 38°C.

### Fecal indicators

Different ambient temperatures resulted in different post-composting numbers of indigenous *E. coli* and enterococci with higher numbers for the treatments at 7°C and lower for those at 38°C. In the treatments at 7°C, the MPN of *E. coli* was considerably higher than at the other temperatures and corresponded to what has been observed in fresh feces (Germer et al. 2010; Ogunyoku et al. 2016). They were therefore considered to represent the original levels of fecal indicators, to which the other treatments were compared to.

Inactivation of pathogens during composting is related to temperature and microbial activity in the compost and is due to heat inactivation and competition with the microflora promoted by the composting process (Haug 1993). Reduction of pathogens is commonly characterized by a time-temperature relationship and is measured by $\log_{10}$ reduction or a specified abundance limit of an indicator microorganism. Common references are sanitizing temperatures of 55°C for a few days (Schönning and Stenström 2004) or the limit of 1000 MPN *E. coli* $g^{-1}$ DM in the final material (Jayathilake et al. 2019). In this study, sanitizing temperatures above 55°C were not reached in any of the treatments. Temperatures high enough to affect the survival of pathogens were recorded only for the reactors at 38°C. In the core of the composting mass in those treatments, the temperature exceeded 45°C in the first three days, and only in some, it reached temperatures above 50°C. However, even without sanitizing temperatures, the composting process resulted in 4×6 $\log_{10}$ lower cell numbers of *E. coli* in the more active composts at 20°C and 38°C in comparison to the treatments at 7°C and *E. coli* < 1000 MPN $g^{-1}$ DM in treatment 38°C. Enterococci have a high survival rate in composts (Vinnerås 2007) and were less affected by the temperature during the composting.

During small-scale composting of fecal sludge, sanitizing temperatures are rarely achieved (Niwagaba et al. 2009; Hill et al. 2013), but the process can still be efficient for reducing the pathogenic load (Vinnerås 2007; Germer et al. 2010). The method by which the compost is applied can introduce further log reductions due to application to soil, fertilization of crops that are not to be consumed raw, or fertilization of food crops with eatable parts that are not in contact with the soil (World Health Organization (WHO) 2006; Schönning et al. 2007).

### Fate of pharmaceutical compounds

Higher concentrations of pharmaceuticals can be expected in fecal sludge from source-separated sanitation compared to conventional wastewater (Butkovskyi et al. 2015; Gros et al. 2020). Ibuprofen and caffeine were the compounds detected at highest concentrations, which reflects their high consumption in Norway. Caffeine is in some cases considered a concern to the environment due to its high concentrations (Deblonde et al. 2011; Verlicchi and Zambello 2015). The initial concentrations of carbamazepine were also relatively high compared to what has been reported for wastewater and sludge (Martín et al. 2015; Verlicchi and Zambello 2015); nevertheless, Gros et al. (2020) reported higher concentrations in fecal sludge solids. Carbamazepine is a persistent, neutral compound that partitions to solids (Butkovskyi et al. 2015; Min et al. 2018; de Wilt et al. 2018), which can explain a possible accumulation over time in fecal solids.

The results of our study indicate a positive correlation between temperature and the removal/degradation of caffeine, atorvastatin, losartan, diclofenac, and warfarin. The concentrations in the treatment products were lower at a higher

temperature. However, as shown, the composting was also more active at higher temperatures; thus, it is not possible to discuss the effects of temperature and composting activity separately. Both thermal decomposition and microbial transformation are possible mechanisms for the observed reduction. Most of those compounds have been shown to be biodegradable. Caffeine has been identified as an easily degradable compound (Deblonde et al. 2011; de Wilt et al. 2018). In comparison between mesophilic and thermophilic anaerobic digestion, Gros et al. (2020) demonstrated more efficient removal of atorvastatin in a thermophilic treatment.

### Effect of the biochar

Addition of biochar had no clear effect on the measured endpoints in any of the temperature treatments. Addition of ~ vol. 5% biochar did not result in changes in the temperature profile or the dynamics of $CO_2$ evolution. At 7°C and 20°C, though, the CB treatments had lower cumulative $CO_2$ emissions and higher pH in comparison to the C treatments. There was no significant difference in the *E. coli* and enterococci between the compost with and without biochar. However, the pharmaceutical compounds carbamazepine and diclofenac had lower concentrations in the CB compared to the C treatment products. Carbamazepine is resistant to degradation and is mostly removed by sorption (Min et al. 2018; de Wilt et al. 2018), and biochar has been shown previously to be an efficient sorbent (Dalahmeh et al. 2018). Both carbamazepine and diclofenac are considered as high risk for the environment (Butkovskyi et al. 2016), suggesting that biochar addition in fecal matter subjected to composting can be used to mitigate the environmental effect of these compounds.

In a study on poultry litter composting, Steiner et al. (2010) found that 5% biochar had little to no effect, whereas addition of 20% biochar resulted in faster decomposition and lower nitrogen losses. Therefore, it would be interesting to investigate additions of biochar larger than 5%, particularly for its role for retaining nutrients and pharmaceuticals. However, larger amounts of biochar can result in alkaline pH, especially when composting organics with high initial pH, and thus inhibit composting through negative effects on the microorganisms (Khan et al. 2020). Different feedstocks, process conditions and amounts of biochar will give different results (Wu et al. 2017; Khan et al. 2020).

### Lactic acid fermentation

Sufficient production of lactic acid and sustained acidity are key factors for the LAF process and elimination of pathogens (Odey et al. 2018b). In this study, lactic acid was not measured, and it was not possible to monitor the pH as little or no leachate was produced. The few pH measurements in leachate during the first days indicated acidification, but the pH measured in samples from Day 71 revealed that acidity was not maintained. It is therefore difficult to judge how successful was the LAF process.

### LAF at different ambient temperatures

Comparison of physicochemical properties did not show significant effect of temperature on the LAF process. This was also indirectly confirmed by the results of the vermicomposting. Both the presence of fecal indicators and the density of worms after the vermicomposting did not differ significantly between LAF treatments conducted at different temperatures. There are no studies so far investigating LAF of fecal matter under different temperatures, but the existing literature suggests that higher temperatures (20–55°C) enhance the fermentation (Tang et al. 2016; Zhou et al. 2016).

### Physicochemical characteristics

LAF products are typically characterized by low pH, high content of organic acids, and low decomposition (Andreev et al. 2017). The higher C/N ratio and high content of $NH_4$-N in 7 F, 20 F, and 38 F corroborate this principal difference to the composting process. Studies have shown that LAF retains nutrients and organic carbon (Andreev et al. 2018). In the present study, even though post-treatment $NH_4$-N was high, total nitrogen was similar to the composting treatments; therefore, this experiment does not confirm higher retention of nitrogen in LAF compared to composting. A probable explanation is a high retention of N in the composting treatments because the leachate was returned to the mix, as well as lower temperatures and therefore a lower activity in the compost as shown by the respiration data.

### Fecal indicators

The MPNs of *E. coli* and enterococci suggest that the material was not properly sanitized (*E. coli* > 1000 MPN $g^{-1}$ DM). Interestingly though, in 7 F, the *E. coli* numbers were approximately 5 $\log_{10}$ lower than in 7 C and 7 CB. LAF has been shown to efficiently reduce fecal indicator bacteria (Anderson et al. 2015; Andreev et al. 2017; Odey et al. 2018b). However, it has not been extensively researched whether LAF has a specific effect on fecal pathogens, nor whether the reduction in the indicator organisms is related to a reduction in other relevant pathogens like Salmonella, Ascaris sp., and viruses.

### Fate of pharmaceutical compounds

To the best of our knowledge, there are no studies that have investigated LAF of fecal matter or wastewater in regard to its effect on pharmaceutical compounds. LAF is mostly utilized for food preservation and as such can be expected to have

minimal effect on degradation of organic compounds. Our study confirmed degradation by LAF for most of the detected compounds, but post-treatment concentration of caffeine, ibuprofen, acetaminophen, metoprolol, and warfarin clearly suggested more efficient removal by composting than LAF. Caffeine and acetaminophen are easily degradable compounds (de Graaff et al. 2011; Deblonde et al. 2011; de Wilt et al. 2018). Ibuprofen has been shown to have high biodegradability in aerobic treatments and low in anaerobic treatments (de Graaff et al. 2011; Butkovskyi et al. 2016; Min et al. 2018; de Wilt et al. 2018). Metoprolol has been shown to be recalcitrant under anaerobic conditions and with better removal in aerobic compared to anaerobic conditions (de Graaff et al. 2011; de Wilt et al. 2018). The concentrations of carbamazepine varied between replicates but on average showed the same trend. In contrast, atorvastatin, losartan, and diclofenac were detected in lower concentrations in the fermentation products compared to the composting products. Those are anionic compounds with better sorption at low pH (atorvastatin pKa = 4.46, losartan pKa = 5.5, diclofenac pKa = 4.15 (PubChem 2020), Online Resource Table S1). Thus, removal of pharmaceutical compounds in LAF seems to be mostly due to sorption, whereas the main mechanism in composting seems to be aerobic biodegradation.

## Vermicomposting

Worms from the species *E. fetida* were introduced to the treatment mixtures after the first composting/fermentation step and multiplied in numbers in all treatments over a period of 77 days. The earthworms were successfully established in the 7°C and 20°C treatments but thrived less in the 38 C and all LAF treatments. Possible explanations could be the lower pH after 38°C, more stabilized organic material that has a lower food value for the worms, and the high $NH_4$-N in the fermentation treatments (Edwards et al. 2010). It should be noted that the enumeration method did not differentiate between different development stages of *E. fetida* and therefore represents only a snapshot of the population in each treatment on the day of the collection. However, the comparison is comprehensive and supported by changes in physiochemical characteristics and *E. coli* MPN.

Vermicomposting further stabilized the material from all treatments, as evidenced by further reduction in VS and $NH_4$ content. In that respect, the process significantly altered the 7 C and 7 CB treatments. The treatments at 7°C underwent less active composting, and the substrate was sludge-like after the first composting step. Earthworm activity during vermicomposting resulted in improved mixing and aeration and facilitated further decomposition and stabilization.

The composting treatments 7 C, 7 CB, and 20 C, 20 CB, which had the highest density of *E. fetida*, had the lowest numbers of *E. coli*, whereas the treatments with lower densities showed higher *E. coli* counts in comparison to after the composting. This negative relationship between worm density and *E. coli* counts suggests that vermicomposting as post-treatment of fecal matter can reduce the load of fecal pathogens, particularly after ineffective composting. An increase in the fecal indicators after vermicomposting could be due to regrowth of bacteria or contamination from the worms (Lalander et al. 2013b). Vermicomposting has been shown to reduce pathogens from dry sanitation systems (Hill and Baldwin 2012; Yadav et al. 2012; Lalander et al. 2013b). However, the studies so far have focused on indicator organisms, and there are knowledge gaps with regard to effects on variety of pathogens and correlations to vermicomposting parameters such as feedstock, worm density, and temperature.

## Practical significance

This study showed that ambient temperature has a significant effect on compost quality and removal of pathogens during on-site small-scale composting of fecal matter. In colder environments, this should be considered, as low temperature inhibits biological processes. Different options can be considered to ensure higher temperatures, such as heat preserving insulation (Vinnerås et al. 2003), addition of easily degradable substrate to trigger fast decomposition, and self-heating (Germer et al. 2010) or external heating. Insulation is an easy optimization, but it depends on a well-maintained composting process and self-heating of the substrate. Easily degradable substrates can come from domestic food waste but are subject to availability and of variable composition. External heating can be energy demanding, and in areas with high solar irradiance, passive solar heating could be a sustainable way to achieve higher temperatures (Redlinger et al. 2001; Kelova 2015).

Maintaining active composting can be limited by the local context due to environmental, economic, or cultural constraints. Composting activity is sensitive to moisture content, aeration, and bulking materials, and the control of these variables requires some level of expertise. Therefore, depending on the context, small-scale composting might not be the most suitable solution for on-site management of fecal sludge. By contrast, LAF does not require maintenance, can be operated in a shorter time period, and results in a reduction of *E. coli* comparable to composting at 20°C. It has therefore been considered a suitable option in an emergency context (Anderson et al. 2015). However, its product requires further treatment before application to agriculture. Vermicomposting is another option. In our study, the activity of the earthworms transformed and stabilized the material where composting was ineffective. Hill and Baldwin (2012) reported that

vermicomposting toilets can produce more stable material with fewer fecal indicators in comparison to inefficiently managed composting toilets, the majority of which were operated at low ambient temperatures.

Recirculating the nutrients and organic matter from fecal matter back to the soil for crop and food production requires better understanding of the fate of pharmaceutical compounds that are released to the environment. We found that higher microbial activity and temperature in the compost resulted in more efficient removal of most of the investigated compounds. The main mechanism of removal of pharmaceuticals, therefore, is probably biodegradation under aerobic conditions. By contrast, LAF had minimal effect on the concentrations of most of the investigated pharmaceuticals, except for atorvastatin, losartan, and diclofenac, and sorption is assumed as a removal mechanism. Slowly degrading compounds such as carbamazepine, diclofenac, metoprolol, and losartan can still pose a risk to the environment; however, that can be mitigated by further treatment. Overall, combinations of LAF and composting for removal of pharmaceuticals would be an interesting enquiry due to the different effects these treatments have in combination, which might result in overall larger reduction.

## Conclusions

Our investigation compared composting of fecal matter with lactic acid fermentation under three different temperatures. Ambient temperature had a significant effect on the composting process and the quality of the resulting material. At 7°C, composting was less active, which resulted in limited transformation and material with high numbers of fecal indicators and pharmaceuticals. At 20°C, composting was more active, and the outcome was a more stabilized material with lower numbers of fecal indicators and more efficient reduction in concentrations of a variety of pharmaceutical compounds. At 38°C, the composting process resulted in the most stabilized and sanitized material. The addition of ~ vol. 5% biochar to the composting did not yield significant differences in the measured parameters. While the active composting at 20°C and 38°C yielded more stabilized material with less *E. coli* and pharmaceuticals, lactic acid fermentation was comparatively successful in reducing the number of *E. coli* at 7°C. The lactic acid fermentation, however, was not assessed with respect to lactic acid production and retained acidity, which limited the comparison with composting. The secondary treatment with vermicomposting resulted in further maturation and stabilization of the material in all treatments, and it was particularly beneficial in reducing *E. coli* numbers and transforming the substrates for the treatments that were previously composted at lower temperatures, i.e., 7°C and 20°C.

The results of our investigation highlight the limitations of composting at low temperature and how other treatments as lactic acid fermentation or vermicomposting can be a valuable alternative, particularly when composting is not successful. Therefore, depending on the local conditions, possibilities, and desired qualities of the end product, different alternatives for resource recovery can be considered. Sustainable utilization of the resources from on-site sanitation treatment of human excreta will also depend on expanding the knowledge on the nutrient values in these treatment products and how they can be best utilized in the local agroecosystems.

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**Author contribution** Conceptualization: MK, AA, SE, PD, RK, and PJ. Methodology: MK, AA, SE, and PD. Formal analyses and investigation: MK and AA. Writing – original draft preparation: MK and AA. Writing – review & editing: AA, SE, PD, RK, and PJ. Funding acquisition: SE and PJ. Supervision: SE, RK, and PJ. All authors read and approved the final manuscript.

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**Data and materials availability** The datasets used and analyzed during the current study are stored in the institutional repository at the Norwegian University of Life Sciences (NMBU), Faculty of Environmental Sciences and Natural Resource Management and so are not publicly available. Data are however available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate** Not applicable

**Consent for publication** Not applicable

**Competing interests** The authors declare no competing interests.

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# Small scale on-site treatment of fecal matter: comparison of treatments for resource recovery and sanitization

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**Electronic Supplementary Material**

**Analysis of Pharmaceuticals: Method description**

**S1. Chemicals and solvents**

The acetonitrile ($CH_3CN$) and methanol (MeOH) used in solid phase extraction (SPE) and during chromatography were HPLC grade purchased from VWR (West Chester, PA, USA). Reagent grade CAN formic acid ($CH_2O_2$), and ammonia solution ($NH_4OH$) were purchased from Sigma-Aldrich. The water used was Grade 1 purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

**S2. Selection of Analytes**

The selection of the compounds in this study (see Table S1) was based on their high rates of production and prescription in addition to their frequent detection in contaminated environmental samples in Norway.

**S3. Sample Preparation**

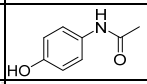
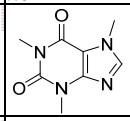
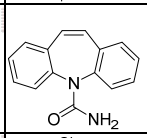
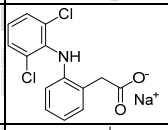
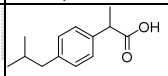
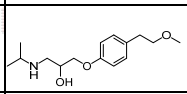
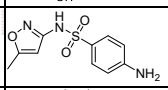
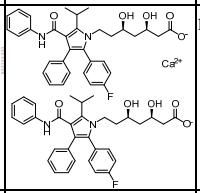
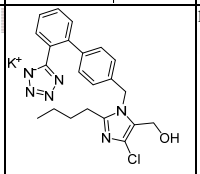
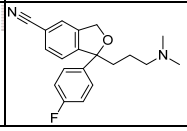
In brief, an aliquot of 5.0 g sample from the initial mixtures and the products of composting/fermentation (wet weight, ww) was spiked with 10 µL of a mixture of internal standards (ISTDs) of 10 µg $mL^{-1}$ concentration followed by 10 mL of extraction solution A (MeOH: $CH_3CN$: $H_2O$ with 0.2% formic acid; 40:30:30). Subsequently, the mixture was mechanically shaken for 5 min and placed in an ultrasonic bath for 10 min and then centrifuged for 5 min at 3000 rpm using IKA Vibrax VXR vibrator (Janke & Kunkel, Staufen, Germany). After transferring the supernatant, the extraction was repeated using solution B (MeOH: $CH_3CN$: $H_2O$ 0.1% NaEDTA and 0.2% $NH_4OH$;

40: 30:30). The combined supernatants were diluted with 30 mL of Milli-Q water and directly concentrated by 500 mg Oasis HLB cartridges (Waters, Milford, MA, USA) which were conditioned with 6 mL of acetonitrile, followed by 6 mL of Milli-Q water. The cartridges were washed with 3 mL of 5% MeOH in water and dried under vacuum. Analytes were eluted with 6 mL volumes of MeOH into a glass tube and dried under a gentle stream of nitrogen at 37°C using a Reacti-Therm III evaporating unit (Thermo Fisher Scientific Inc., Rockford, USA). Ten microliters of DEET-d10 (10 $\mu g\ mL^{-1}$) were added as a recovery standard and after adding 990 µL of 20 % $CH_3CN$ in water, the sample was then vortexed and subsequently filtered through a 0.2 µm microcentrifuge filter (Spin-X, Costar, Corning Inc., Corning, NY, USA). The resulting sample was transferred to polypropylene vials for immediate quantitative LC–MS/MS analysis.

**S4. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)**

The analysis was conducted using an Agilent 1200 series HPLC (Agilent Technologies, Waldbronn, Germany) coupled to an Agilent 6490 (Agilent Technologies, Santa Clara, CA, USA) triple quadrupole mass spectrometer with an Agilent Jet Stream electrospray ion source (ESI). The column used for chromatographic separations was a Zorbax Eclipse plus C18 RRHD (2.1 x 100 mm, 1.8 µm) (Agilent, Palo Alto, USA) with a respective Guard Cartridge (4 µm x 3.0 mm ID) (Zorbax, Agilent, Palo Alto, USA). The mobile phase flow rate was 300 µL/min, the column oven temperature was 35°C, and the injection volume was 10 µL. The chromatographic separation was performed using binary gradient mobile phases, consisting of water with 0.1% formic acid (A) and 100% $CH_3CN$ (B). The initial mobile phase composition was 100% (A). B was linearly increased to 100% in 8.0 min and held for 7 min. Initial mobile phase conditions were restored over 1.0 min, and the column was allowed to equilibrate for 3 min, a total run time of 20 min. The parameters of ESI were as follows: gas temperature of 200°C, gas flow of $14 L min^{-1}$, nebulizer 20 psi, sheath gas heater 250°C, sheath gas flow 11 $10L\ min^{-1}$, and capillary voltage 3000 V. The ions were monitored in multiple reaction monitoring (MRM) and are listed in Table S2. Agilent MassHunter software (Version B.07.00 /Build 7.0.457.0, 2008) was used for instrument control, method validation and quantification.

***Table S1 Summary of the selected analytes with their molecular formula, structures, CAS numbers, supplier, and some physiochemical properties[1].***

| Compound (Abbreviation) | Description | Mol. formula | Structure[2] | CAS Number | Supplier | LogP* | LogD (pH 7.4)* | $LogK_{OC}$ (pH 7.4)* |
|---|---|---|---|---|---|---|---|---|
| Acetaminophen (ACE) | nonsteroidal anti-inflammatory | $C_8H_9NO_2$ |  | 103-90-2 | Sigma Aldrich, Oslo, Norway | 0.34 | 0.40 | 1.6 |
| Caffeine (CAF) | Psychostimulants | $C_8H_{10}N_4O_2$ |  | 58-08-2 | Sigma Aldrich, Oslo, Norway | -0.13 | 0.28 | 1.5 |
| Carbamazepine (CAR) | anticonvulsant | $C_{15}H_{12}N_2O$ |  | 298-46-4 | Sigma Aldrich, Oslo, Norway | 2.67 | 2.28 | 2.6 |
| Diclofenac sodium salt (DCF) | nonsteroidal anti-inflammatory | $C_{14}H_{10}Cl_2NNaO_2$ |  | 15307-79-6 | Sigma Aldrich, Oslo, Norway | 4.06 | 1.37 | 5.0 |
| Ibuprofen (IBP) | nonsteroidal anti-inflammatory | $C_{13}H_{18}O_2$ |  | 15687-27-1 | Sigma Aldrich, Oslo, Norway | 3.75 | 0.45 | 0.29 |
| Metoprolol (MTP) | β-blocker | $C_{15}H_{25}NO_3$ |  | 37350-58-6 | Sigma Aldrich, Oslo, Norway | 1.79 | -0.25 | 0.28 |
| Sulfamethoxazole (SMX) | antibiotic | $C_{10}H_{11}N_3O_3S$ |  | 723-46-6 | Sigma Aldrich, Oslo, Norway | 0.89 | -0.56 | 0.52 |
| Atorvastatin calcium salt trihydrate (ATO) | antilipidemic | $C_{66}H_{68}CaF_2N_4O_{10}$ |  | 134523-03-8 | Toronto Research Chemicals, Toronto, Canada | 4.13 | 1.25 | 0.64 |
| Losartan potassium (LOS) | Anti-hypertensive | $C_{22}H_{22}ClKN_6O$ |  | 124750-99-8 | Sigma Aldrich, Oslo, Norway | 3.56 | 1.29 | 1.17 |
| Citalopram | Antidepressant | $C_{20}H_{21}FN_2O$ |  | 59729-33-8 | Sigma Aldrich, Oslo, Norway | 2.51 | 1.27 | 1.10 |

1 Predicted data is calculated with ACD/Labs Percepta Platform − PhysChem Module, Toronto, CA. (http://www.chemspider.com/Chemical Structure.18219.html)

2 Structures were prepared using ChemDraw Professional (PerkinElmer Informatics, In. version 15.0.9.106, Boston, Massachusetts, USA)

*Table S2 MRM Parameters of the selected analytes and their internal standards*

| Cpd Name | RT | Prec Ion | Prod Ion1 | Prod Ion 2 | Prod Ion 3 | Frag (V) | CE (V) 1 | CE (V) 2 | CE (V) 3 | Polarity |
|---|---|---|---|---|---|---|---|---|---|---|
| Losartan | 5.0 | 423.1 | 377 | 207 | 191.9 | 380 | 13 | 24 | 41 | + |
| Sulfamethoxazole-$^{13}C_6$ | 6.2 | 260 | 161.1 | 114 | 97.9 | 380 | 13 | 24 | 28 | + |
| Sulfamethoxazole | 6.3 | 254 | 156 | 108 | 92 | 380 | 11 | 22 | 27 | + |
| Carbamazepine-$d_{10}$ | 6.9 | 247.1 | 204 | 202 | | 380 | 21 | 39 | | + |
| Carbamazepine | 6.1 | 237 | 193.9 | 178.9 | | 380 | 18 | 39 | | + |
| Caffeine $^{13}C_3$ | 4.0 | 198.1 | 140.1 | 112.1 | | 380 | 18 | 25 | | + |
| Caffeine | 4.1 | 195.1 | 138.1 | 110.1 | | 380 | 18 | 24 | | + |
| Warfarin | 6.0 | 307 | 249.9 | 160.9 | | 380 | 20 | 15 | | - |
| Diclofenac | 7.9 | 296 | 249.9 | 214.9 | 213.8 | 380 | 9 | 17 | 35 | + |
| Diclofenac-$^{13}C_6$ | 7.9 | 316 | 272.1 | | | | | | | - |
| Metoprolol | 5.2 | 268.1 | 116.1 | 73.9 | | 380 | 16 | 21 | | + |
| Citalopram | 6.4 | 325.2 | 262 | 109 | | 380 | 15 | 35 | | + |
| Ibuprofen | 16 | 559.5 | 292 | 250 | | 380 | 35 | 45 | | + |
| Acetaminophen | 2.5 | 152.2 | 110 | 65 | | 380 | 15 | 35 | | + |
| Atorvastatin | 6.2 | 205 | 161.1 | | | 380 | 1 | | | - |

**S5. Method Validation**

The method performance characteristics which are listed in Table S3 were determined as follows. Due to the lack of pharmaceuticals free fecal sludge samples, cow manure mixed with bark was used in the method validation. The average percent recovery with relative standard deviation (RSD) was calculated form six replicates samples fortified with a mixture of target compounds and their respective ISTDs at 200 ng $g^{-1}$. Instrument limit of detection (ILOD) and Instrument limit of quantification (LOQ) were determined as the concentration of the pharmaceutical dissolved in 20% $CH_3CN$ in water that gave a signal/noise ratio (S/N=3) of 3 and 10, respectively. The method detection limit (MDL), was determined as the concentration the pharmaceutical prepared in samples that gave S/N=3. Matrix match and solvent matched calibration curves for targeted analytes were prepared using 10 concentration levels in the rang (0.200- 2000 ng $mL^{-1}$). The recovery percentages of ISTDs (sulfamethoxazole-(phenyl-$^{13}C_6$), caffeine-$^{13}C_3$, carbamazepine-$D_{10}$, diclofenac-(acetophenyl ring-$^{13}C_6$)) were calculated based on their calibration curves over five concentration levels (10, 20, 50, 80, and 100 ng $mL^{-1}$) applying $^2H_{10}$-DEET at a concentration of 100 ng $mL^{-1}$ as a recovery standard. In order to determine the influence of matrix on method performance, matrix effect (ME%) was estimated using the equation depicted below, where Ss and Sm are the slope of the solvent matched calibration and matrix matched curves respectively. Positive and negative ME% values indicate signal enhancement and ion suppression by the matrix respectively.

$$ME\% = \left[\left(\frac{Sm}{Ss}\right) - 1\right] \times 100$$

The performance of extraction efficiency of the optimized method was evaluated in terms of Acetaminophen caffeine, carbamazepine, metoprolol, losartan, atorvastatin, warfarin showed satisfactory average recoveries ranged from 38 -124%, and the majority of their RSDs were below 20%; however, a few compounds demonstrated unacceptable recoveries, including diclofenac, ibuprofen, sulfamethoxazole, citalopram. Therefore, the concentration obtained for these compounds are considered semi-quantitative data. It has been widely reported that pharmaceutical compounds analyzed in environmental samples suffer from significant matrix effects resulting in either ionization suppression or enhancement, when analyzed by LC-ESI-MS/MS. In the current study, isotopically labeled internal standards were used to compensate for potential losses during the sample preparation and differences in ionization of the analytes between different samples. All selected compounds experienced significant ionization suppression. However, as matched isotopically labeled ISTDs were not available for each target compound, matrix matched calibration is adopted to account for these matrix effects. The average absolute sample specific recovery % of carbamazepine-$D_{10}$ was found to be 47.0±15%.

**S6. Removal**

Removal was calculated based on the equation:

Removal % = $(C_0\text{-}C)/C_0$

Where $C_0$ is the initial concentration of the compound and C is the actual concentration of the compound.

***Table S3 Summary of the method performance characteristics; Instrument limits of detection (LOD) and quantification (LOQ), method detection limit (MDL), and the percent recoveries ± relative standard deviations (RSD).***

| Compound | LOD (ng $mL^{-1}$) | LOQ (ng $mL^{-1}$) | MDL (ng $g^{-1}$) | Matrix Effect | (Recovery± RSD, n=6) % |
|---|---|---|---|---|---|
| Acetaminophen | 0.028 | 0.093 | 0.171 | -91 | 38.4±9 |
| Caffeine | 0.025 | 0.082 | 0.341 | -89 | 62.9±10 |
| Carbamazepine | 0.002 | 0.006 | 0.007 | -95 | 119±8 |
| Diclofenac | 0.306 | 1.020 | 1.020 | -75 | 195±5 |
| Ibuprofen | 10.34 | 34.4 | 13.6 | -82 | - |
| Metoprolol | 0.005 | 0.016 | 0.424 | -81 | 112+9 |
| Sulfamethoxazole | 0.007 | 0.024 | 2.41 | -97 | - |
| Citalopram | 3.48 | 11.6 | 18.7 | -50 | - |
| Losartan | 0.247 | 0.823 | 0.823 | - | 107±46 |
| Atorvastatin | 0.064 | 0.215 | 0.215 | - | 119±37 |
| Warfarin | 0.004 | 0.012 | 0.012 | - | 124±6 |

# Paper III


Composted / fermented
at 7, 20 or 38˚C
Vermicomposted
Soil amendment
N
P
Mineralization
NH4-N
NO3-N
Mineralization
KCL exctractable P

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Full length article

# Human excreta as a resource in agriculture – Evaluating the fertilizer potential of different composting and fermentation-derived products

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ABSTRACT

Acknowledging the resources in human excreta provides new options to address future challenges for fertilizer availability and nutrient management in a sustainable way. In dry on-site sanitation systems, the high nutrient concentration and low volume of the waste provides an opportunity for low-tech recycling, while the product can be a valuable input to the local agro-ecological system. However, successful recycling requires balanced fertilization with a low environmental impact. The aim of this study was therefore, to evaluate and compare the nutritional content and fertilizer potential of different organic amendments derived from human excreta. Nine amendments were obtained previously by treating human excreta from a dry toilet through composting or fermentation at 7°C, 20°C or 38°C, followed by a secondary vermicomposting. We evaluated the fertilizer potential of the amendments by conducting a soil incubation study to determine nitrogen and phosphorus mineralization, and by a pot experiment with barley. For all amendments, the nitrogen mineralization was slow and the available nitrogen determined the yield. The treatment method had a significant effect on the availability of nitrogen and the amendments obtained by active composting at 38°C had significantly higher levels of total and mineral nitrogen than those composted/fermented at 7°C and 20°C. These amendments also led to the highest barley yields, which were equivalent to a mineral nitrogen fertilization of 30 to 60 kg N $ha^{-1}$. These findings contribute towards better agronomic value quantification of products from on-site dry sanitation systems and the effect of treatment method on the availability of nitrogen.

## 1. Introduction

The recycling of human excreta closes the loop between agriculture and sanitation and addresses future challenges for fertilizer availability and better nutrient management. Only a small part of the nutrient content of the food is retained by the organism of an adult and the nitrogen and phosphorus excretion is at or near 100% of the intake (Jönsson et al., 2004; Rose et al., 2015). Returning these nutrients back to the soil and food production is important for nutrient cycling and soil health. Currently, only a small part is recycled to land application, while the majority is disposed of in a linear way, as a waste driven to landfills or as an effluent discharged to water bodies (Harder et al., 2019; Trimmer et al., 2017; Xu et al., 2020). As a result, the nutrients and the organic matter create a burden to the receiving environments, while they are lost to soils and food production. The increasing demand for food production, coupled with the limitations of the production and supply of mineral fertilizers, drives a demand towards more sustainable and circular alternatives.

Dry toilets with on-site treatment present an opportunity for non-complex and low-tech nutrient recycling through established practices for biological transformations of organic matter, such as composting, vermicomposting and lactic acid fermentation (LAF). In dry toilet systems, the waste is collected without dilution with flush water, contains mostly urine and feces, and is characterized by high nutrient concentration in low volume, as well as low contamination with heavy metals, industrial or household chemicals (Butkovskyi et al., 2017). Therefore, if the treatment and the application method adequately address the risks from pathogens and pharmaceuticals for human and environmental health, the transformed organic matter can be a valuable input to the local agroecological system.

Transformative biological processes and organic matter degradation affect the concentrations and availability of nutrients. During such processes, the organic matter composition is altered through the metabolism of the microorganisms. Composting is an active aerobic degradation method with substantial mineralization of carbon and nitrogen that yields stable organic material with higher humic content (Epstein,

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1997). In contrast, LAF is a preserving process with partial degradation (Battcock and Azam-Ali, 1998). The agronomic value of the resulting products can be expected to differ, and an important determinant is the fate of the nitrogen (N). The turnover of N is different in the two processes. Composting is characterised by high losses of N as $NH_3$ and an end product with a low concentration of $NH_4$ in relation to $NO_3$. Lactic acid fermentation, on the other hand, preserves the N and the end product has a higher $NH_4$ concentration that is not further nitrified to $NO_3$ under anaerobic conditions (Andreev et al., 2017). Lactic acid fermentation products require further treatment to become suitable for an agricultural application, and the process is often combined with composting or vermicomposting (Andreev et al., 2018). During both processes, part of the $NH_4$ is also volatilized to $NH_3$ and lost. The fate of N during vermicomposting is not that clear, with some studies showing better retention compared to composting (Nigussie et al., 2016), while others do not find significant differences (Yadav et al., 2012). For the elements that are not a main part of the microbial metabolic respiration, aerobic organic matter degradation typically results in a concentration effect due to the C mineralization of organic matter, with marginal losses through volatile compounds or leaching (Bernal et al., 2009; Eneji et al., 2001).

Human excreta have been utilized extensively in the past to maintain soil fertility in rural regions (Ferguson, 2014; Rockefeller, 1998). Their potential as fertilizer has been well identified, but the research effort has been focused on macronutrient content and yields of products from individual sanitation systems (Andreev, 2017; Harder et al., 2019; Sangare et al., 2015). However, a more detailed examination of the fate of both macro- and micronutrients during different treatments and applications is needed for balanced fertilization with a low environmental impact. Therefore, this study aimed to evaluate the fertilizer potential of different products derived from human excreta processed under different conditions by 1) quantifying the content of macro- and micronutrients in the products, and upon application in soil, 2) examining the continued mineralization of N and changes in easily available P over time, as well as 3) determining plant yield and uptake of nutrients for a test crop under controlled conditions.

## 2. Methodology

### 2.1. Human excreta-derived organic amendments

Nine different human excreta-derived amendments were prepared from fecal matter (feces and urine) obtained from one collection compartment servicing five dry toilets at Åstjern cabin complex, Bleiken, Norway. The fecal matter was mixed with small amounts of toilet paper and residues from sanitary pads and wet wipes and had accumulated over 2 years with fresh daily inputs until the day of collection. The fecal matter, together with additional separately collected urine, was mixed in three batches with 1) sanitary bark, 2) sanitary bark and biochar, and 3) sanitary bark, biochar, and lactic acid inoculant. For the first batch, 3.2 kg (wet weight) fecal matter, 1.7 kg sanitary bark, 0.75 L urine, 0.5 L water, and 0.13 kg compost inoculant were mixed and composted. For the second batch, 3.0 kg (wet weight) fecal matter, 1.6 kg sanitary bark, 0.75 L urine, 0.5 L water, 0.28 kg biochar and 0.13 kg compost inoculant were mixed and composted. For the third batch, 3.2 kg (wet weight) fecal matter, 1.4 kg sanitary bark, 0.70 L urine, 0.25 L water, 0.30 kg biochar, and 0.3 L lactic acid bacteria inoculant (sauerkraut juice) were mixed and fermented. The first two mixtures were composted and the third was fermented at three different ambient temperatures, i.e., 7, 20 and 38°C for 71 days. After that, all treatments were composted at room temperature for 15 days and then vermicomposted at room temperature for 77 days. Detailed description of the substrates and processes can be found in Kelova et al., 2021. The products of the different treatments are labelled as 7C, 7CB, 7F, 20C, 20CB, 20F, 38C, 38CB, 38F, where the number corresponds to the ambient temperature of composting/fermentation and the letters to the mixture, i.e., C for composting with sanitary bark, CB for composting with sanitary bark and biochar, and F for the lactic acid fermentation (LAF) with sanitary bark, biochar, and lactic acid inoculant. They are referred to throughout the text as organic amendments. Selected characteristics of the different human excreta-derived organic amendments are listed in Table 1. After vermicomposting, the materials were stored at 4°C.

**Table 1**
Mean physicochemical characteristics ($n = 3$) after vermicomposting (adjusted from (Kelova et al., 2021).

| | Moisture % | pH | Total C % | Total N % | C/N | $NH_4$–N mg $g^{-1}$ | Kjeldahl-N g $kg^{-1}$($n = 2$) |
|---|---|---|---|---|---|---|---|
| 7C | 69 | 7.1 | 46.6 | 1.98 | 25.4 | 0.026 | 16.9 |
| 7CB | 71 | 7.2 | 48.7 | 1.7 | 28.6 | 0.024 | 15.9 |
| 7F | 70 | 7.8 | 48.3 | 1.78 | 27.2 | 0.033 | 17.2 |
| 20C | 70 | 7 | 46.6 | 1.67 | 27.9 | 0.02 | 16.1 |
| 20CB | 70 | 7.4 | 48.3 | 1.69 | 28.6 | 0.02 | 17.6 |
| 20F | 70 | 7.5 | 48.5 | 1.86 | 26.2 | 0.04 | 16.1 |
| 38C | 68 | 5.9 | 45.9 | 2.19 | 21 | 0.018 | 19.4 |
| 38CB | 68 | 5.8 | 47.9 | 2.27 | 21.2 | 0.015 | 18.2 |
| 38F | 70 | 7.4 | 49.9 | 1.75 | 28.5 | 0.039 | 18.1 |

### 2.2. Macro- and micronutrient concentrations in the amendments

Dried (48 h, 60°C) and ground samples from the initial mixtures before treatment and from each treatment product were analysed in triplicates for total concentrations of P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B, Ni, as well as Na. The elemental concentrations in the extracts were determined by inductively coupled plasma mass spectrometry (Agilent ICP-MS 8800 TripleQ, Santa Clara, USA) after ultraclave digestion with concentrated, double-distilled $HNO_3$. Total N was determined by dry combustion (Nelson and Sommers, 1982) at 1050°C using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA), according to the Dumas method (Bremner et al., 1982). Ammonium ($NH_4$–N) was measured by flow injection analysis (FIA, Tecator FIAstar 5010 Analyzer, Hillerød, Denmark) after extracting both fresh and dry samples with 2 M KCl. The difference in the $NH_4$–N concentration between fresh and dry samples was used to correct the total N for the loss of $NH_3$ during drying. In addition, duplicate samples from each treatment product were analysed for total N by the Kjeldahl method.

### 2.3. Changes in nitrogen and phosphorus availability of the amendments in soil

A soil incubation experiment was performed to investigate nitrogen mineralization from the different organic amendments. The incubation was carried out under aerobic conditions at 15°C for 90 days. The soil was pre-incubated at 50% of its water holding capacity (WHC) and at 15°C for 10 days. For the incubation, the equivalent of 10 g dry soil was weighed into 50 ml plastic tubes with holes in the lids to allow for gas exchange. Air-dried, finely ground samples from each human excreta-derived organic amendment were weighed and added to the soil at a rate equivalent to 150 mg Kjeldahl-N $kg^{-1}$ soil (DW). Soil without an amendment was used as control. The soil and amendment mixtures were adjusted to 60% WHC and incubated in the dark at 15°C. The moisture content was adjusted twice a week according to weight loss. Three tubes were sampled destructively at Days 0, 1, 3, 7, 14, 29, 42, 60, and 90, respectively. Concentrations of N and P were measured in 2 M KCl extracts of the samples. The extracts were prepared by adding 25 ml 2 M KCl, shaking for 30 min, and filtration with 125 mm Blue ribbon paper filters. The extracts were stored at 4°C and analysed for $NO_3$–N and $NH_4$–N by flow injection analysis (FIA, Tecator FIAstar 5010 Analyzer, Hillerød, Denmark). The concentration of P in the 2 M KCl extracts was determined with the molybdenum blue method according to Murphy and Riley (1962) measured spectrophotometrically at 882 nm (Agilent Cary 60, Santa Clara, USA).

### 2.4. Greenhouse experiment

A plant growth experiment was carried out in a greenhouse with two-row spring barley (*Hordeum vulgare*) of the variety Salome as a test crop. The plants were grown at a temperature range of 15 to 23°C. Artificial lighting with a minimum light intensity of 200 μmol $m^2$ $s^{-1}$ was used for a period of 16 h per day. During hours with sufficiently intensive sunlight, the artificial lighting was off.

For the experiment, 3 L pots were filled with 2.6 kg soil (dry weight) and adjusted to 60% WHC. Altogether, 24 treatments with three replicates each were prepared. Two were without any fertilization, four were with mineral fertilizer covering a range of N levels, nine with only amendments and an additional nine with amendments supplemented by mineral N. The unfertilized controls were only soil (S) and soil with peat (SP), where the peat was matching the amount of the added organic amendments (100 g wet weight). The peat was natural, brown sphagnum peat, low in nutrients, and with a pH of 4.0 measured in deionized water. The mineral fertilizer treatments also included peat (100 g wet weight). The mineral fertilizer was added per pot as 0.075 g P (as $Ca(H_2PO_4)_2$), 0.3 g K (as $K_2SO_4$), 0.031 g Mg (as $MgSO_4$), and N (as $Ca(NO_3)_2$) corresponding to 30, 60, 100 and 150 kg N $ha^{-1}$ (30 N, 60 N, 100 N, and 150 N), which amounted to 0.045, 0.09, 0.15 and 0.225 g N, respectively. To assess the fertilizer potential of the nine organic amendments, 100 g wet weight of each amendment were added to two sets of nine treatments; one set was fertilized only with an amendment and one set was fertilized with an amendment and additional mineral N fertilizer corresponding to 60 kg $ha^{-1}$. As the moisture level for the different amendments was similar and approximated 70% (Table 1), the 100 g wet weight was considered to correspond to an application of 20 t (DW) $ha^{-1}$. The application rate of the fertilizers and amendments, as well as yield, were converted to area based on the assumption that one hectare has 2 000 000 L topsoil.

12 barley seeds were sown per pot, which after germination were thinned to eight plants. The plants were grown for 3,5 months until maturation, and then harvested by cutting 2 – 3 cm above the soil. The collected aboveground biomass was weighed and dried at 60°C for 48 h. The plants were threshed, and the grains counted and weighed.

### 2.5. Plant and soil analysis

Dried and ground grain samples from each pot were analysed for P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B, Ni, as well as Na. The samples were digested by concentrated $HNO_3$ under pressure using an Ultraclave high performance microwave reactor (Milestone, Shelton, USA). The elemental concentration in the extracts was determined by inductively coupled plasma mass spectrometry (Agilent ICP-MS 8800 TripleQ, Santa Clara, USA). The total N in the grain was determined by dry combustion (Nelson and Sommers, 1982) at 1050°C using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA) according to the Dumas method (Bremner et al., 1982).

The soil used for laboratory incubations and the greenhouse experiment was a loam (20% clay, 37% silt, 38% sand) collected from the top layer (0 - 20 cm) of an agricultural field in south-eastern Norway. The organic matter content of the soil was 4.5% (SD = 0.08) and the total N content was 1963 mg $kg^{-1}$ (SD = 73). The soil pH was 6.1, measured in deionized water with a soil to water ratio of 1:2.5 (v/v).

### 2.6. Calculations and statistical analysis

The mineral nitrogen released from the amendments during the incubation was estimated based on Eq. (1):

$$minN_{amendment}\ mg\ kg^{-1} = (minN_{measured}\ (mg) - minN_{soil}\ (mg)) * 100 \quad (1)$$

Where $minN_{measured}$ is the measured concentration of $NH_4$–$N$ + $NO_3$–N mg in KCl extracts from the amended treatments, and the $minN_{soil}$ is the measured concentration of $NH_4$–$N$ + $NO_3$–N mg in KCl extracts from the soil control treatment. 100 is a conversion factor from 10 g sample to concentration per kg soil.

The percentage of mineral N as a fraction of the total N in the amendments was estimated based on Eq. (2):

$$Mineral\ N\ \% = \frac{minN\ mg\ kg^{-1}}{Total\ N\ mg\ kg^{-1}} * 100 \quad (2)$$

The estimation of the available mineral N in each treatment in the greenhouse experiment was based on Eqs. (3) and (4).

$$minN\ mg\ kg^{-1} = \frac{minN_{soil}\ mg\ kg^{-1} + minN_{added}}{Dry\ weight_{\ soil}\ (kg)} \quad (3)$$

For the amendments:

$$minN_{added}\ mg\ kg^{-1} = \frac{(minN_{measured}\ (mg) - minN_{\ soil}\ (mg))}{Dry\ weight_{\ amendment}\ (g)} * \%\ DM_{amendment} \quad (4)$$

Analysis of variance was used to compare the variation between the different treatments. The assumptions were checked with Levene's test for homogeneity of variances and the Shapiro-Wilk test for normality. Two-way ANOVA was used when the assumptions were met. To evaluate differences of means per factor, the ANOVA was followed by Tukey's post hoc comparison of means ($p < 0.05$). When the normality assumption was violated, the Kruskal-Wallis rank sum test was used for analysis. Statistical analyses were carried out using the R statistical package Version 1.3.959 under the GNU public license (Boston, MA, USA).

## 3. Results and discussion

### 3.1. Macro- and micronutrient concentrations

The concentrations of nutrients per dry matter for the amendments are listed in Table 2. The concentrations for most nutrients after treatment were higher than in the initial mixtures. The accumulation during treatment is a result of a concentration effect following from the mineralization of the organic matter. The variations in the concentrations of nutrients due to a different treatment method were low, except for N, which was detected in notably higher concentrations in the 38C and 38CB. The higher organic matter mineralization due to the more active composting is a probable explanation (Kelova et al., 2021). To distinguish which differences were due to the variations in organic matter mineralization, the treatment products/amendments were also compared per ash content (Table S1). The comparison per ash content only indicated significant variation in the cases of total N and Na.

The nutrient concentrations in the initial mixtures are a result of the different materials and their constituents. The macro- and micronutrients mainly come from the excreta – both feces and urine, and will depend on the dietary intake (Rose et al., 2015). In addition, some of the nutrients come from the coniferous bark which was used as a bulking material. The concentrations are within the reported ranges for a variety of other organic amendments such as municipal waste compost, and composted or vermicomposted manures (Hargreaves et al., 2008; Lazicki et al., 2020; Rini et al., 2020). The total concentrations of macro- and micronutrients suggest that at an application rate of 20 t $ha^{-1}$ or even lower (Table S2), the amendments will contribute to soil enrichment with all elements required for plant growth. The plant availability of the nutrients, however, will depend on the changes of the organic matter quality during treatment and multiple factors from the soil – plant – environment interaction (Havlin, 2014). The variation in the concentration between the different treatment products/amendments reflects the differences in the mineralization of biomass during the treatment processes as shown by the comparison per ash content.

**Table 2**
Macro- and micronutrient concentrations in initial mixtures ($n$ = 2, (SD)) before treatment and in the resulting amendments ($n$ = 3, (SD)).

| | N g kg$^{-1}$ | P g kg$^{-1}$ | K g kg$^{-1}$ | Ca g kg$^{-1}$ | Mg g kg$^{-1}$ | S g kg$^{-1}$ | Fe g kg$^{-1}$ | Mn g kg$^{-1}$ | Zn mg kg$^{-1}$ | Cu mg kg$^{-1}$ | B mg kg$^{-1}$ | Mo mg kg$^{-1}$ | Ni mg kg$^{-1}$ | Na g kg$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 18.3 (0) | 10.5 (0.5) | 9.2 (0) | 17.5 (0.5) | 4.8 (0) | 3.0 (0) | 0.78 (0.05) | 0.53 (0.01) | 230 (10) | 21.0 (1.0) | 22 (0) | 1.25 (0.05) | 3.2 (0.05) | 6.7 (0.1) |
| CB | 19.3 (0.4) | 9.2 (0.2) | 9.3 (0.4) | 16.5 (0.5) | 4.3 (0) | 2.8 (0.1) | 1.12 (0.28) | 0.51 (0.01) | 235 (5) | 21.0 (1.0) | 22.5 (0.5) | 1.20 (0) | 3.3 (0.1) | 6.0 (0.45) |
| F | 18.7 (0.7) | 9.0 (1) | 8.9 (0.4) | 16.0 (2) | 4.2 (0.5) | 2.7 (0.3) | 1.10 (0.21) | 0.47 (0.01) | 255 (5) | 20.5 (1.5) | 22.5 (2.5) | 1.15 (0.15) | 3.1 (0.1) | 6.7 (1) |
| 7C | 19.8 (3.9) | 10.3 (0.5) | 9.0 (0.3)$^{A}$ | 19.8 (0.5) | 4.8 (0.2) | 3.4 (0.1)$^{a}$ | 1.35 (0.12) | 0.60 (0.01)$^{a}$ | 295 (8.2) | 26.0 (0.7)$^{B}$ | 24.3 (0.6) | 1.28 (0.05) | 3.5 (0.36) | 5.7 (0.19)$^{Aba}$ |
| 7CB | 17.0 (0.1) | 9.5 (0.5) | 8.8 (0.3)$^{A}$ | 20.7 (0.8) | 4.6 (0.1) | 3.1 (0.1)$^{ab}$ | 1.62 (0.05) | 0.56 (0.02)$^{b}$ | 273 (8.5) | 26.2 (0.8)$^{B}$ | 24.3 (0.5) | 1.27 (0.12) | 3.7 (0.32) | 4.9 (0.31)$^{Abb}$ |
| 7F | 17.8 (0.7) | 9.5 (0.7) | 9.0 (0.1)$^{A}$ | 20.7 (0.6) | 4.8 (0.3) | 3.1 (0.2)$^{b}$ | 2.32 (0.96) | 0.55 (0.01)$^{c}$ | 315 (16.3) | 26.5 (1.1)$^{B}$ | 25.2 (1.0) | 1.35 (0.11) | 3.7 (0.58) | 5.7 (0.30)$^{Aba}$ |
| 20C | 16.7 (0.1) | 10.1 (0.3) | 9.2 (0.1)$^{A}$ | 20.0 (0.4) | 4.7 (0.1) | 3.5 (0.1)$^{a}$ | 1.32 (0.02) | 0.59 (0.01)$^{a}$ | 293 (8.5) | 26.5 (0.0)$^{AB}$ | 24.7 (0.6) | 1.28 (0.06) | 3.1 (0.12) | 5.8 (0.15)$^{Aa}$ |
| 20CB | 16.9 (0.8) | 9.9 (0.5) | 9.1 (0.4)$^{A}$ | 20.8 (0.6) | 4.8 (0.2) | 3.3 (0.1)$^{ab}$ | 1.50 (0.11) | 0.54 (0.02)$^{b}$ | 287 (4.7) | 27.3 (0.8)$^{AB}$ | 25.3 (1.0) | 1.30 (0.04) | 3.4 (0.17) | 5.2 (0.40)$^{Ab}$ |
| 20F | 18.6 (1.0) | 10.0 (0.7) | 9.1 (0.2)$^{A}$ | 21.0 (1.1) | 4.9 (0.1) | 3.3 (0.2)$^{b}$ | 1.65 (0.22) | 0.52 (0.00)$^{c}$ | 303 (13.1) | 27.5 (1.9)$^{AB}$ | 25.5 (0.4) | 1.42 (0.08) | 3.5 (0.23) | 6.0 (0.09)$^{Aa}$ |
| 38C | 21.9 (0.9) | 10.3 (1.0) | 8.3 (0.3)$^{B}$ | 20.8 (1.3) | 4.5 (0.2) | 3.6 (0.2)$^{a}$ | 1.50 (0.14) | 0.60 (0.02)$^{a}$ | 287 (13.1) | 28.5 (2.5)$^{A}$ | 24.8 (0.9) | 1.32 (0.12) | 3.4 (0.06) | 5.2 (0.23)$^{Ba}$ |
| 38CB | 22.7 (0.8) | 10.2 (0.6) | 8.6 (0.2)$^{B}$ | 22.0 (0.4) | 4.8 (0.1) | 3.4 (0.1)$^{ab}$ | 1.87 (0.23) | 0.59 (0.02)$^{b}$ | 310 (14.7) | 30.2 (0.5)$^{A}$ | 26.0 (0.4) | 1.30 (0.15) | 3.9 (0.14) | 4.8 (0.30)$^{Bb}$ |
| 38F | 17.5 (0.2) | 9.3 (0.6) | 8.9 (0.2)$^{B}$ | 19.8 (0.5) | 4.7 (0.3) | 3.1 (0.2)$^{b}$ | 1.55 (0.18) | 0.53 (0.02)$^{c}$ | 298 (13.1) | 28.5 (3.3)$^{A}$ | 24.3 (1.2) | 1.27 (0.13) | 3.4 (0.13) | 5.7 (0.29)$^{Ba}$ |
| | K-W test | | | | | | K-W test | | | | | | | |
| Temperature | * | Ns | ns | ns | ns | ns | ns | . | ns | * | ns | ns | ns | * |
| Mix | ns | Ns | ** | ns | ns | * | * | *** | . | ns | ns | ns | ns | *** |
| Temperature *Mix | / | Ns | ns | ns | ns | ns | / | . | * | ns | ns | ns | ns | ns |

Significance codes:
'***' 0.001, '**' 0.01, '*' 0.05, '.' 0.1 ns = not significant.
Uppercase letters indicate means that are significantly different from the rest due to the effect of temperature and lowercase due to mixtures.

### 3.2. N and P availability after application to soil

#### 3.2.1. N availability and mineralization

Ammonium was detected in very low concentrations in all treatments throughout the incubation period, with concentrations ranging from below the detection limit to 1.13 mg kg$^{-1}$ (Table S3). The concentrations of $NO_3$–N in the amended soils were in the range of 20 to 52.5 mg kg$^{-1}$, of which the soil-derived $NO_3$–N amounted to 54 to 97%. The changes in mineral nitrogen throughout the period showed small variations for the first 60 days of incubation, but the release of N increased between Day 60 and Day 90 (Fig. 1, part A). At the end of the incubation period (Day 90), the mineral N contribution from the amendments was highest in the 38C (20 mg kg$^{-1}$) and 38CB (22.5 mg kg$^{-1}$) treatments, whereas in the other treatments, the mineral N contribution was in the range of 7.5 to 15 mg kg$^{-1}$. However, relative to the initial concentration of mineral nitrogen, the net increase was higher in treatments that were composted/fermented at 7 and 20°C than those at 38°C. Mineral N in the amendments was estimated from the soil and amendment mix in the incubation study (Eq. (4)), therefore the quantification is not exact but still useful for evaluating relative differences.

The mineral N in the amendments amounted to 0.3 to 9.5% of the initial total N (Table 3). During the 90-day incubation, the percentage of mineral N increased by 3 to 7% in all treatments. 38C and 38CB stand out with higher total N but also with a greater proportion of mineral N. The rest of the amendments had similar concentrations of total N but differed significantly in regard to what percentage of the total N is mineral. Assuming an application rate of 20 t ha$^{-1}$, the mineral N in the amendments can substitute between 2 and 40 kg N ha$^{-1}$ from mineral N fertilizer at the time of application. Provided similar conditions as in the mineralization study, that contribution could increase to 16 to 55 kg N ha$^{-1}$ in the next three months (Table 3).

The low $NH_4$–N content together with a limited increase in $NO_3$–N suggest relatively stable organic matter and therefore slow mineralization in the short term (Bernal et al., 1998; Sánchez-Monedero et al., 2001). Both composting and vermicomposting are aerobic processes during which the nitrogen present in the urine and feces is transformed and partially mineralized to nitrates. During degradation, parts of the organically bound N are quickly hydrolysed to $NH_4$, and under aerobic conditions, the $NH_4$ is nitrified to $NO_3$.

The results indicate little release of mineral N, i.e. a low mineralization rate for the first 60 days, followed by a noticeable net release in the third month. Composts are in general amendments with slow N release, but the short-term mineralization is less predictable (Amlinger et al., 2003). In this study, the amendments' C/N ratios of 21 to 29 (Table 1) suggest that mineralized N could be immobilized by microorganisms in the short term (Lazicki et al., 2020), but it is possible that the fertility of the soil and the already present $NO_3$–N concealed that effect. While the application amount used in the incubation study, i.e. corresponding to 360 kg N ha$^{-1}$ estimated according to Kjeldahl-N, resulted in net mineralization for all treatments, the results can therefore not be directly extrapolated to different soils and environmental conditions.

Even with considerable variability between treatments, the mineral N content is within the common range of 0 to 25% found for a variety of composts and vermicomposts (Ebertseder and Gutser, 2003; Lazicki et al., 2020). The variation found between the amendments in our experiment illustrates the effect of treatment methods on the availability of N. The amount of N mineralized from amendments during a growing period will be accessible to plants and can substitute mineral fertilizer. At an application rate of 20 t ha$^{-1}$, the initially available N will be insufficient in comparison to commonly used amounts of mineral fertilizers. For example, cereal crops are often fertilized with 100 to 150 kg plant-available N ha$^{-1}$. Due to the low availability and slow mineralization of N, the amendments would have to be applied at a higher rate or supplemented with mineral N for optimal yields.

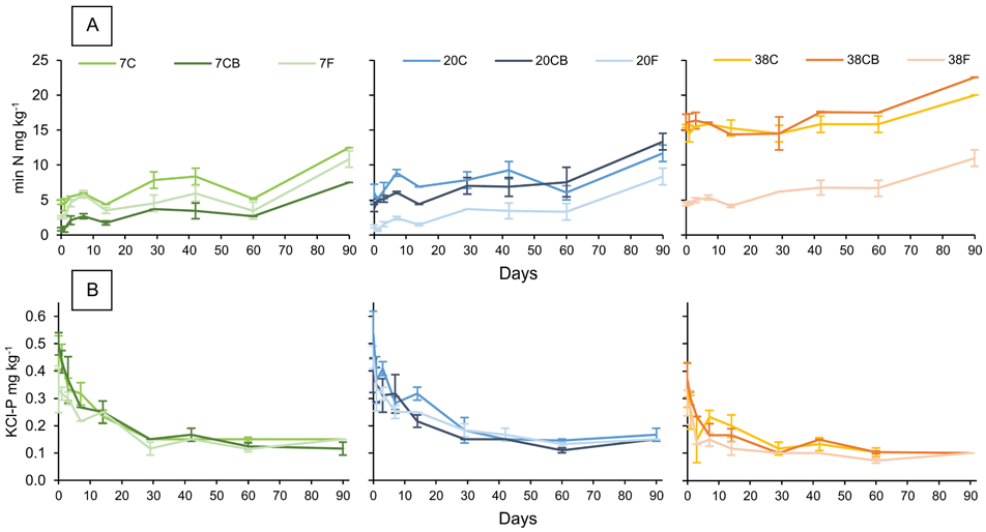


**Fig. 1.** Changes in KCl-extractable mineral N (Part A) and P (Part B) attributed to the different amendments applied to the soil at 150 mg Kjeldahl-N $kg^{-1}$ during 90 days of incubation at 15°C ($n = 3$, (SD)). The amendments were previously composted/fermented at 7, 20 and 38°C. C is for composting; CB is for composting with addition of biochar and F is for lactic-acid fermentation.

**Table 3**
Available N in the amendments before incubation and after 90 days of incubation at 15°C, presented as percentage of total N ($n = 3$, (SD)) and the corresponding amount of mineral N in kg $ha^{-1}$ estimated for an application rate of 20 t $ha^{-1}$.

| Treatment | Total N g $kg^{-1}$ | % mineral N from total N | % mineral N from total N | Total N kg $ha^{-1}$ | Mineral N kg $ha^{-1}$ | Mineral N kg $ha^{-1}$ |
|---|---|---|---|---|---|---|
| | | Day 0 | Day 90 | | Day 0 | Day 90 |
| 7C | 19.8 (3.9) | 2.7 (0.2) | 7.1 (0.0) | 396 | 10.5 | 28.2 |
| 7CB | 17.0 (0.1) | 0.5 (0.1) | 4.7 (0.0) | 340 | 1.8 | 15.9 |
| 7F | 17.8 (0.7) | 1.6 (0.1) | 7.0 (0.8) | 357 | 5.7 | 24.8 |
| 20C | 16.7 (0.1) | 3.9 (0.8) | 7.5 (0.8) | 334 | 12.9 | 25.1 |
| 20CB | 16.9 (0.8) | 2.7 (0.4) | 9.3 (0.8) | 338 | 9.0 | 31.3 |
| 20F | 18.6 (1.0) | 0.7 (0.1) | 4.8 (0.7) | 372 | 2.7 | 17.8 |
| 38C | 21.9 (0.9) | 9.2 (0.1) | 11.8 (0.0) | 438 | 40.4 | 51.9 |
| 38CB | 22.7 (0.8) | 8.6 (0.6) | 12.1 (0.0) | 453 | 39.0 | 54.7 |
| 38F | 17.5 (0.2) | 3.0 (0.2) | 7.5 (0.8) | 351 | 10.6 | 26.5 |

Total N adjusted from (Kelova et al., 2021).

### 3.2.2. P availability

During the incubation, the KCl-extractable P contribution from the amendments was within the range of 0.05 to 0.6 mg $kg^{-1}$. For all treatments, the KCl-P was decreasing during the first 30 days and then stabilized at around 0.1 to 0.15 mg $kg^{-1}$ above what was measured in the control soil (Fig 1, part B). There were no significant differences between the treatments indicating that the soil capacity to adsorb the P was determining the trend in KCl-P concentration.

In acidic soils like the loamy soil used in this experiment, amorphous aluminum (Al) and iron (Fe) (hydr)oxides are important for P sorption, whilst phosphorus status, pH, and the content of organic matter are also factors that influence the soil's capacity to bind and release P (Borling et al., 2004, 2001). The decline of the KCl-extractable fraction of P in the amendments during the incubation period may be explained by the capacity of the soil to absorb the labile P, which was also observed by Griffin et al. (2003) in manure applications. The labile P fraction represents only 0.3 to 0.5% of the total phosphorus added with the amendments, which suggests that most of the added P would not be lost through leaching. However, an equilibrium seems to be established between released P by mineralization and adsorption of P to soil components shown by a flattening of the curves in Fig. 1 after about 30 days.

## 3.3. Plant growth

### 3.3.1. Yield

In the greenhouse trial, the application of 20 t $ha^{-1}$ of the amendments resulted in barley yields in the range of 2.7 to 6.4 t $ha^{-1}$ (Fig. 2-A). The average yield was higher than in the unfertilized controls, but the Tukey comparison of means indicated that only the yields of 38C and 38CB were significantly higher in comparison to the controls without fertilizer. A significant increase in yield was observed when additional mineral N (60 kg N $ha^{-1}$) was added (Fig. 2- B). Those yields were in the range of 5.6 to 8.8 t $ha^{-1}$. While all treatments had higher yields than the control of 60 kg N $ha^{-1}$ in absolute values, the differences were not statistically significant. However, except for 20F and 38F, the yield levels of the organic amendments were comparable to mineral fertilization of 100 kg N $ha^{-1}$ or more. As expected, the four treatments with mineral fertilizer illustrated a strong correlation between the amount of N and the yield. The amendments with the highest amounts of mineral

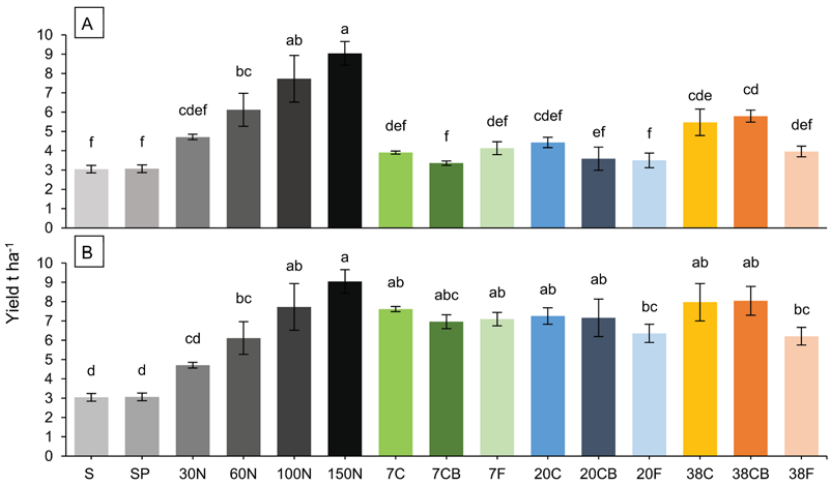


**Fig. 2.** Barley yield from a greenhouse experiment ($n$ = 3, (SD)). Part A – only amendments. Part B – amendments with supplementary 60 kg N ha$^{-1}$. S and SP are soil and soil with peat controls. 30 N, 60 N, 100 N, and 150 N are treatments with mineral fertilizer with the corresponding levels of applied kg N ha$^{-1}$. The amendments were applied at 20 t ha$^{-1}$. They were previously composted/fermented at 7, 20 and 38°C. C is for composting; CB is for composting with addition of biochar and F is for lactic-acid fermentation.

N, i.e. 38C and 38CB, also resulted in the highest yields in comparison to the other amendments.

The results show that the variation between treatments is highly correlated with initial mineral N. Fig. 3 illustrates the relationship between the yield and an estimate of the mineral N available at planting. The amendments had low variation in total N and other nutrients and a slow N mineralization rate, therefore the initial concentration of mineral N appears to be a determining factor for the yield. Furthermore, supplementary mineral N increased the yield significantly for all treatments and confirmed that N was the main limiting factor for plant growth.

The higher yields from the pots amended with 38C and 38CB correspond to the higher availability of N (Fig. 3). The availability of N in the amendments was mainly determined by the initial composting/fermentation conditions and not the following vermicomposting, suggesting that the initial conditions have a great effect on the fertilization value. Therefore, the active composting supported by high ambient temperatures resulted in greater fertilizer value due to the higher content of mineral N.

The majority of N in organic amendments such as composts is organically bound with only a small proportion that is directly plant-available. Higher application rates can increase the mineral N input but this may result in excessive concentrations of other nutrients and lead to a higher risk of leaching, pollution, and toxicities (Rigby and Smith, 2014). In our study, the application rate was 20 t ha$^{-1}$, which is low to moderate, and yet that resulted in surplus amounts for all nutrients (Table S2). The content of P and some trace metals such as Cu, Zn, Ni needs to be considered for the application rate as they can lead to negative environmental impacts, and often the application rate of organic amendments is regulated based on their concentrations (Westerman and Bicudo 2005).

### 3.3.2. *Nutrient uptake*

The concentrations of macro- and micronutrients in the barley grain are given in Table 4 together with soil pH at harvest. The soil pH was in the range of 6.6 to 6.8 in the soil and the amended treatments, and slightly lower (6.2 to 6.5) in the treatments with peat. The N

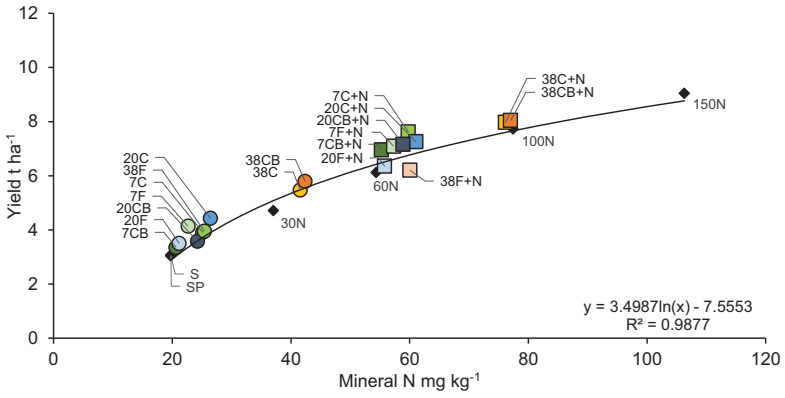


**Fig. 3.** Relationship between mineral nitrogen and yield. The trend line is based only on the correlation of the mineral fertilizer control treatments with four levels of applied N kg ha$^{-1}$ – 30, 60, 100, and 150 and the no fertilizer controls – S and SP (soil and soil with peat). The amendments were previously composted/fermented at 7, 20 and 38°C. C is for composting.

**Table 4**
Concentrations of macro- and micronutrients in the barley grain ($n = 3$, (SD)). +N indicates the amendments with supplementary 60 kg N ha$^{-1}$.

| | soil pH | N g kg$^{-1}$ | P g kg$^{-1}$ | K g kg$^{-1}$ | Ca g kg$^{-1}$ | Mg g kg$^{-1}$ | S g kg$^{-1}$ | Fe mg kg$^{-1}$ | Mn mg kg$^{-1}$ | Zn mg kg$^{-1}$ | Cu mg kg$^{-1}$ | B* mg kg$^{-1}$ | Mo mg kg$^{-1}$ | Ni mg kg$^{-1}$ | Na mg kg$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil | 6.7 | 11.8 (0.4) | 4.6 (0.1) | 7.4 (0.2) | 0.35 (0.03) | 1.40 (0.00) | 0.95 (0.05) | 79.0 (13.4) | 11.7 (0.5) | 40.0 (25.6) | 7.10 (0.29) | 0.84 (0.04) | 0.57 (0.02) | 1.10 (0.71) | 453 (41) |
| Soil + peat | 6.4 | 11.2 (0.8) | 4.3 (0.2) | 6.5 (0.2) | 0.31 (0.02) | 1.27 (0.05) | 0.93 (0.03) | 59.3 (6.6) | 11.7 (0.5) | 25.0 (1.4) | 7.23 (0.31) | 0.74 (0.05) | 0.54 (0.01) | 0.58 (0.09) | 257 (33) |
| PKMg+30 N | 6.2 | 10.8 (0.2) | 4.0 (0.2) | 6.5 (0.1) | 0.30 (0.01) | 1.27 (0.05) | 0.87 (0.02) | 47.7 (2.1) | 11.3 (0.5) | 21.3 (0.9) | 6.47 (0.05) | 0.57 (0.05) | 0.52 (0.01) | 0.51 (0.02) | 153 (5) |
| PKMg+60 N | 6.3 | 11.2 (0.7) | 3.7 (0.2) | 6.4 (0.2) | 0.30 (0.01) | 1.20 (0.00) | 0.87 (0.03) | 47.7 (1.2) | 12.0 (0.0) | 21.0 (1.4) | 6.17 (0.50) | < LOQ | 0.59 (0.10) | 0.41 (0.03) | 180 (22) |
| PKMg+100 N | 6.4 | 11.2 (0.4) | 3.1 (0.0) | 6.1 (0.2) | 0.30 (0.00) | 1.13 (0.05) | 0.86 (0.02) | 41.0 (0.0) | 13.0 (0.8) | 20.3 (0.5) | 6.10 (0.36) | 0.52 (0.02) | 0.56 (0.07) | 0.34 (0.04) | 203 (56) |
| PKMg+150 N | 6.5 | 12.5 (0.5) | 3.0 (0.0) | 6.2 (0.3) | 0.26 (0.02) | 1.10 (0.00) | 0.93 (0.02) | 39.0 (0.8) | 12.3 (1.2) | 20.0 (0.8) | 6.20 (0.29) | < LOQ | 0.62 (0.08) | 0.28 (0.01) | 203 (29) |
| 7C | 6.6 | 10.66 (0.5) | 4.30 (0.2) | 6.77 (0.5) | 0.29 (0.01) $^{A}$ | 1.30 (0.08) | 0.86 (0.02) | 51.0 (10.2) | 10.3 (0.5) | 21.7 (0.5) | 5.90 (0.29) $^{b}$ | 0.44 (0.04) | 0.73 (0.19) | 1.48 (1.64) | 327 (116) |
| 7CB | 6.6 | 10.33 (0.1) | 4.13 (0.1) | 6.37 (0.2) | 0.30 (0.02) $^{A}$ | 1.23 (0.05) | 0.87 (0.02) | 46.3 (1.2) | 10.1 (0.7) | 22.7 (0.5) | 6.00 (0.08) $^{ab}$ | < LOQ | 0.56 (0.01) | 0.41 (0.01) | 257 (39) |
| 7F | 6.7 | 10.61 (0.4) | 4.07 (0.2) | 6.30 (0.1) | 0.29 (0.01) $^{A}$ | 1.20 (0.00) | 0.86 (0.05) | 38.7 (1.7) | 10.3 (0.5) | 21.7 (1.2) | 6.17 (0.21) $^{a}$ | < LOQ | 0.57 (0.01) | 0.38 (0.05) | 230 (24) |
| 20C | 6.6 | 10.19 (0.2) | 3.93 (0.4) | 6.40 (0.5) | 0.27 (0.00) $^{B}$ | 1.17 (0.17) | 0.82 (0.02) | 42.7 (3.9) | 10.6 (0.6) | 21.0 (1.6) | 5.87 (0.05) $^{b}$ | < LOQ | 0.56 (0.03) | 0.34 (0.04) | 250 (70) |
| 20CB | 6.7 | 11.15 (0.7) | 4.03 (0.0) | 6.30 (0.0) | 0.27 (0.01) $^{B}$ | 1.13 (0.05) | 0.84 (0.03) | 40.0 (2.4) | 10.3 (0.5) | 21.0 (1.6) | 6.27 (0.33) $^{ab}$ | < LOQ | 0.56 (0.04) | 0.31 (0.05) | 250 (16) |
| 20F | 6.7 | 10.81 (0.7) | 4.23 (0.0) | 6.40 (0.1) | 0.27 (0.00) $^{B}$ | 1.27 (0.05) | 0.87 (0.04) | 41.0 (0.8) | 9.9 (0.1) | 23.0 (0.8) | 6.40 (0.45) $^{a}$ | < LOQ | 0.54 (0.04) | 0.36 (0.05) | 283 (56) |
| 38C | 6.7 | 10.85 (0.1) | 4.10 (0.2) | 6.37 (0.2) | 0.29 (0.01) $^{AB}$ | 1.23 (0.05) | 0.83 (0.02) | 43.0 (0.8) | 11.0 (0.0) | 20.7 (0.9) | 5.70 (0.16) $^{b}$ | < LOQ | 0.62 (0.03) | 0.27 (0.02) | 187 (17) |
| 38CB | 6.7 | 10.28 (0.3) | 4.00 (0.1) | 6.10 (0.2) | 0.30 (0.00) $^{AB}$ | 1.20 (0.00) | 0.82 (0.01) | 39.7 (0.5) | 10.7 (0.5) | 20.7 (0.5) | 5.77 (0.12) $^{ab}$ | < LOQ | 0.58 (0.03) | 0.26 (0.02) | 163 (31) |
| 38F | 6.7 | 10.84 (0.3) | 4.07 (0.0) | 6.37 (0.2) | 0.27 (0.02) $^{AB}$ | 1.13 (0.05) | 0.85 (0.01) | 39.7 (1.2) | 9.9 (0.1) | 21.7 (0.5) | 6.23 (0.25) $^{a}$ | < LOQ | 0.57 (0.02) | 0.35 (0.04) | 277 (45) |
| 7C+N | 6.8 | 10.98 (0.4) $^{A}$ | 4.03 (0.1) $^{A}$ | 6.40 (0.1) $^{A}$ | 0.29 (0.00) $^{B}$ | 1.20 (0.00) | 0.79 (0.02) | 59.3 (24.6) | 11.3 (0.5) | 19.0 (0.0) | 5.27 (0.34) $^{A}$ | < LOQ | 0.69 (0.05) | 0.56 (0.38) | 217 (24) $^{B}$ |
| 7CB+N | 6.8 | 10.97 (0.5) | 3.73 (0.0) $^{A}$ | 6.27 (0.1) $^{A}$ | 0.28 (0.00) $^{B}$ | 1.13 (0.05) | 0.79 (0.01) | 39.7 (3.4) | 11.3 (0.5) | 19.7 (0.9) | 5.40 (0.28) $^{A}$ | < LOQ | 0.66 (0.07) | 0.27 (0.07) | 203 (17) $^{B}$ |
| 7F+N | 6.8 | 11.08 (0.4) | 3.83 (0.1) $^{A}$ | 6.27 (0.4) $^{A}$ | 0.29 (0.01) $^{B}$ | 1.17 (0.05) | 0.76 (0.03) | 39.7 (0.9) | 11.0 (0.8) | 19.3 (0.5) | 5.13 (0.53) $^{A}$ | < LOQ | 0.66 (0.10) | 0.24 (0.01) | 163 (33) $^{B}$ |
| 20C+N | 6.7 | 11.08 (0.2) | 3.77 (0.0) $^{A}$ | 6.03 (0.1) $^{AB}$ | 0.27 (0.00) $^{AB}$ | 1.17 (0.05) | 0.74 (0.02) | 40.0 (2.2) | 10.7 (0.5) | 18.0 (0.8) | 4.73 (0.24) $^{AB}$ | < LOQ | 0.59 (0.04) | 0.25 (0.01) | 200 (16) $^{AB}$ |
| 20CB+N | 6.8 | 10.78 (0.4) | 3.80 (0.2) $^{A}$ | 6.23 (0.2) $^{AB}$ | 0.28 (0.01) $^{AB}$ | 1.17 (0.05) | 0.75 (0.02) | 40.0 (2.9) | 10.6 (0.6) | 18.3 (0.5) | 4.87 (0.05) $^{AB}$ | < LOQ | 0.62 (0.02) | 0.26 (0.03) | 220 (22) $^{AB}$ |
| 20F+N | 6.7 | 10.93 (0.6) | 3.87 (0.1) $^{A}$ | 6.23 (0.3) $^{AB}$ | 0.27 (0.01) $^{AB}$ | 1.23 (0.05) | 0.77 (0.02) | 43.0 (2.4) | 10.7 (0.5) | 19.3 (1.2) | 5.03 (0.12) $^{AB}$ | < LOQ | 0.60 (0.02) | 0.30 (0.03) | 200 (22) $^{AB}$ |
| 38C+N | 6.8 | 10.39 (0.2) | 3.47 (0.2) $^{B}$ | 5.83 (0.1) $^{B}$ | 0.29 (0.02) $^{A}$ | 1.13 (0.05) | 0.73 (0.03) | 42.0 (7.1) | 10.7 (0.5) | 17.3 (0.5) | 4.10 (0.08) $^{B}$ | < LOQ | 0.65 (0.02) | 0.28 (0.12) | 280 (50) $^{A}$ |
| 38CB+N | 6.7 | 11.56 (0.1) | 3.70 (0.1) $^{B}$ | 5.87 (0.2) $^{B}$ | 0.29 (0.00) $^{A}$ | 1.13 (0.05) | 0.77 (0.01) | 38.0 (2.9) | 11.3 (0.5) | 18.3 (0.5) | 4.67 (0.09) $^{B}$ | < LOQ | 0.64 (0.01) | 0.25 (0.04) | 227 (45) $^{A}$ |
| 38F+N | 6.7 | 11.25 (0.1) | 3.67 (0.2) $^{B}$ | 5.93 (0.2) $^{B}$ | 0.29 (0.00) $^{A}$ | 1.13 (0.05) | 0.78 (0.02) | 38.7 (2.1) | 11.0 (0.8) | 19.0 (1.4) | 5.23 (0.57) $^{B}$ | < LOQ | 0.62 (0.04) | 0.27 (0.07) | 240 (45) $^{A}$ |

* Limit of quantification (LOQ) for boron is 0.5 mg kg$^{-1}$.
Uppercase letters indicate means that are significantly different from the rest due to the effect of temperature and lowercase due to mixtures.

concentration in the grain was highest in the mineral fertilizer control with the highest N application (150 kg N $ha^{-1}$). However, in all other treatments, the concentration of available N in the fertilizer/amendment did not affect N content in the grain consistently. In general, an increase in mineral fertilizer N resulted in lower concentrations of other nutrients in the grain, with the exception of Mn and Mo. Among the controls, the highest nutrient concentrations were thus found in the unfertilized treatments (S and SP). The uptake of nutrients per area (Tables S4 and S5) showed significant variation between the treatments, which reflected the differences in the yield.

A comparative evaluation with ANOVA and Tukey's test of variations between the amendments indicated significant differences only for Ca and Cu (Table 4). In the treatments with amendments and supplementary mineral N, differences were detected for Ca, Cu, P, K, and Na between the amendments that were composted/fermented at 7°C and 38°C (Table 4). However, compared to the concentrations in the controls, the variations are small and could be due to differences in the yield.

No obvious signs of nutrient deficiencies were detected during the growth experiment, besides the effect of the limited N availability on the growth. The concentrations in the grain for all fertilized and amended treatments showed little variation and are therefore discussed together. For the macronutrients, the content of P, K, Ca, and Mg in the grain is within normal ranges for barley (Chappell et al., 2017; Rogers et al., 2017). For the micronutrients Zn, Mn, and B, concentrations in the grains of the amended treatments are below the normal ranges. Concentrations below 20 mg $kg^{-1}$ for Zn and 15 mg $kg^{-1}$ for Mn in general plant tissue are indicative of deficiency (Havlin, 2014). The low Zn concentrations in the amended treatments show that in our study, the high Zn content in the organic amendments (265 – 335 mg $kg^{-1}$) did not result in higher uptake and accumulation in the plant, which could be due to limitations of the uptake rate in the plant at a higher yield, lower Zn mineralization, or due to lower availability of the Zn. High levels of available P can also limit the Zn uptake (Thorne, 1957). Similarly to Zn, Mn present in the amendments did not contribute substantially to the uptake of Mn, as shown by higher concentrations in grain from the control and mineral fertilizer treatments compared to those with organic amendments. One of the main factors controlling its availability in the soil is pH, and the slightly lower pH in the controls with peat (Table 4) can be the explanation for the higher uptake in the control treatments. Except for few treatments, B was below the quantification limit of 0.43 mg $kg^{-1}$ and accordingly is below what is typically measured in barley grain (Kabata-Pendias and Pendias, 2001), which also suggests deficiency. B is little mobile within the plants, and seeds and grains have the lowest concentrations which might explain the generally lower content in all treatments.

The trend of lower nutrient concentrations with the increase in yield is explained by a higher rate of plant growth and yield than the rate of uptake and is referred to as a dilution effect (Jarrell and Beverly, 1981). The uptake of nutrients and their concentration in plant tissues is regulated by complex interactions involved in the availability of different elements in the soil and the functionality of the plant's nutrient uptake mechanism. Increased growth under more favourable conditions alters the availability in the soil but can also be restricted by the rate of uptake and its genetic regulation in the plant. In our study, the method of fertilization had only a minor effect on nutrient contents in the grain but there was a pattern of higher nutrient concentrations in the treatments with lower yields. Therefore, the grain concentrations probably reflect to a greater extent the uptake capacity of the barley variety and to a lesser extent the availability from the applied amendments.

### 3.4. General discussion

In this study, we tested different treatment products derived from excreta to evaluate their fertilizer potential. The results showed that all amendments had a fertilization effect, though not always a statistically significant one. The variability in yield between the amendments was well correlated with available mineral N. The materials that were initially subjected to active composting supported by high ambient temperatures of 38°C had the greatest amounts of mineral N. Accordingly, they increased yields by 80 to 90% compared to no fertilization, whereas the other amendments resulted in an increase of only 10 to 46%. In the 38C and 38CB, higher loss of biomass (labile carbon) during composting also resulted in greater accumulation and thus concentration of other macro- and micronutrients, except for Na, which followed the opposite trend.

The fertilization potential of the amendments was therefore determined mostly by the initial treatment and less affected by the secondary treatment of vermicomposting. A probable explanation is the transformation and retention of N during the active composting phase. During microbial transformations of organic matter, N is the nutrient with the highest turnover and losses. Higher retention of N in composting is linked to higher nitrification which is dependent on the concentration of $NH_4$ (Tiquia, 2002). A higher rate of degradation in the 38°C composting can be expected to result in greater ammonification and to increase the porosity of the composting matrix, both of which are factors facilitating nitrification. Vermicomposting transformed and stabilized the treatment products that were not actively composted under 7°C and 20°C or thoroughly fermented during the LAF. It reduced the concentrations of $NH_4$ but does not seem to have enhanced nitrification to the extent that would match the $NO_3$ content after active composting at 38°C. Another explanation may be that the vermicomposting of those treatments resulted in greater losses.

At 20 t $ha^{-1}$, even the 38C and 38CB amendments only produced yields comparable to a relatively low level of mineral N fertilization, but increasing the application rate of the organic amendments would lead to excessive amounts of other nutrients. Based on the relatively high content of Zn, the amendments already fall within the category of Class 1 of the current Norwegian national guidelines for organic amendments/fertilizers (Norwegian Ministry of Agriculture and Food, 2003), which limits the application rate in agriculture to 40 t DM $ha^{-1}$ over a 10-year period. The amount of P applied with higher doses of organic amendments may cause overfertilization and losses to water bodies due to leaching or erosion. Therefore, planning the fertilization according to the amounts of P and K in the organic amendments with additional mineral N might be a better strategy to achieve more balanced fertilization and minimize potential negative effects on the environment. However, more detailed investigations will be necessary to evaluate the full environmental impact of different fertilization strategies based on excreta, taking into account energy use, greenhouse gas emissions, and other losses during all phases of the composting process and the use in agriculture. Any fertilization strategy has environmental impacts, e.g., due to greenhouse gas emissions in the production of the fertilizer/-amendment, but those will vary depending on multiple factors (Walling and Vaneeckhaute, 2020). The application rate and strategy will also affect gaseous emissions and leaching losses. However, those effects have to be considered within the specific context of the soil characteristics, climate, crop, and type of application (Nicholson et al., 2017; Walling and Vaneeckhaute, 2020).

The uptake of nutrients in our study did not indicate that disparities in the concentration of nutrients in the grain are due to the method of fertilization except for N. The relatively low concentrations of Mn, Zn, and B in the grains imply a low response to their content in the amendments, which is indicative of a low mineralization rate of these nutrients. The clear trend to higher nutrient concentrations in the grain in the unfertilized controls suggests that there is a dilution effect in treatments with higher yields that might overshadow a potential variation in nutrient uptake between the amendments.

## 4. Conclusions

This study demonstrated the agronomic value of different products

derived from human excreta processed under different conditions. The quantification of macro- and micronutrients showed that all amendments will contribute to soil enrichment with all required elements for plant growth. Upon application to fertile soil, the amendments showed slow mineralization of nitrogen but contributed to higher growth than treatments without fertilizer. Due to the low availability of nitrogen, the yields were lower in comparison to mineral fertilizer. The main determinant of fertilizer potential was nitrogen, which was higher in the amendments obtained by active composting at high ambient temperatures (38°C). There was no clear indication that the uptake of nutrients was affected by the type of fertilizer. Our results demonstrate the agronomic value of the products from different on-site sanitation systems and determined the effect of the treatment method on the availability of nitrogen. We identified that supplementing the amendments with mineral nitrogen is a suitable approach for balanced fertilization, which limits the potential for excessive fertilization and its associated environmental impacts.

## Availability of data and materials

The datasets used and/or analysed during the current study are stored in the institutional repository at Norwegian University of Life Sciences (NMBU), Faculty of Environmental Sciences and Natural Resource Management, and so are not publicly available. Data are however available from the corresponding author on reasonable request.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.resconrec.2021.105748.

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**Human excreta as a resource in agriculture – Evaluating the fertilizer potential of different composting and fermentation-derived products**

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**Electronic Supplementary Material**

**Table S1**. Macro- and micronutrient concentrations in initial mixtures (n=2, (SD)) before treatment and in the resulting amendments (n=3, (SD)) estimated per ash content in g $kg^{-1}$ ash. ANOVA-1 is comparison between initial mixtures and vermicomposting products. ANOVA-2 is comparison between the different treatments after vermicomposting.

| | N | P | K | Ca | Mg | S | Fe | Mn | Zn | Cu | B | Mo | Ni | Na | Ash % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 167.1 (4.6) | 96.1 (7.4) | 83.6 (2.0) | 160.1 (9.2) | 43.4 (0.8) | 27.4 (0.8) | 7.10 (0.62) | 4.85 (0.23) | 2.11 (0.15) | 0.19 (0.01) | 0.20 (0.01) | 0.01 (0.00) | 0.03 (0.00) | 61.3 (2.7) | 11.3 (0.3) |
| CB | 178.0 (10.6) | 84.6 (1.5) | 85.0 (0.7) | 151.7 (1.5) | 39.6 (1.6) | 25.7 (0.1) | 10.21 (2.16) | 4.70 (0.28) | 2.16 (0.04) | 0.19 (0.00) | 0.21 (0.00) | 0.01 (0.00) | 0.03 (0.00) | 54.6 (2.0) | 11.3 (0.4) |
| F | 165.5 (8.9) | 79.9 (10.2) | 78.5 (4.4) | 142.1 (20.0) | 37.3 (5.0) | 23.5 (3.5) | 9.67 (1.66) | 4.16 (0.02) | 2.26 (0.01) | 0.18 (0.02) | 0.20 (0.03) | 0.01 (0.00) | 0.03 (0.00) | 59.5 (9.8) | 11.5 (0.2) |
| 7C | 142.3 (36.3) | 73.3 (8.5) | 64.3 (5.3) | 141.5 (13.7) | 34.1 (3.3) | 24.4 (2.6) | 9.63 (1.28) | 4.28 (0.43) | 2.10 (0.19) | 0.19 (0.01) | 0.17 (0.01) | 0.01 (0.00) | 0.02 (0.00) | 40.7 (3.4) | 13.4 (1.2) |
| 7CB | 121.1 (7.4) | 67.5 (6.9) | 62.8 (2.6) | 147.5 (15.4) | 32.9 (2.2) | 22.3 (1.4) | 11.49 (0.45) | 3.96 (0.39) | 1.95 (0.14) | 0.19 (0.02) | 0.17 (0.01) | 0.01 (0.00) | 0.03 (0.00) | 34.8 (1.8) | 15.0 (0.9) |
| 7F | 134.2 (4.6) | 71.7 (6.6) | 67.9 (2.3) | 155.6 (7.3) | 36.0 (2.9) | 23.6 (2.1) | 17.31 (6.79) | 4.15 (0.02) | 2.37 (0.16) | 0.20 (0.01) | 0.19 (0.01) | 0.01 (0.00) | 0.03 (0.00) | 43.2 (3.2) | 13.7 (0.3) |
| 20C | 121.5 (4.6) | 73.2 (5.2) | 66.9 (3.8) | 145.5 (8.8) | 34.4 (2.4) | 25.2 (1.7) | 9.56 (0.24) | 4.30 (0.21) | 2.13 (0.14) | 0.19 (0.01) | 0.18 (0.01) | 0.01 (0.00) | 0.02 (0.00) | 42.0 (2.9) | 13.3 (0.6) |
| 20CB | 125.1 (15.5) | 72.5 (2.6) | 66.6 (2.9) | 153.4 (10.4) | 35.4 (1.7) | 24.4 (1.6) | 11.00 (0.36) | 3.98 (0.35) | 2.11 (0.16) | 0.20 (0.01) | 0.19 (0.01) | 0.01 (0.00) | 0.03 (0.00) | 37.8 (1.9) | 13.1 (1.1) |
| 20F | 136.0 (2.8) | 73.3 (4.8) | 66.6 (2.7) | 153.7 (6.4) | 35.5 (1.6) | 24.4 (1.7) | 12.04 (1.23) | 3.83 (0.11) | 2.22 (0.13) | 0.20 (0.01) | 0.19 (0.01) | 0.01 (0.00) | 0.03 (0.00) | 43.8 (1.9) | 14.1 (0.5) |
| 38C | 161.3 (14.0) | 75.5 (7.1) | 61.0 (1.4) | 153.2 (12.3) | 33.3 (1.0) | 26.6 (2.0) | 11.09 (1.64) | 4.38 (0.15) | 2.11 (0.08) | 0.21 (0.02) | 0.18 (0.01) | 0.01 (0.00) | 0.03 (0.00) | 38.2 (0.6) | 12.7 (0.7) |
| 38CB | 165.8 (18.0) | 75.0 (12.6) | 63.0 (8.7) | 162.1 (24.2) | 35.4 (5.0) | 25.0 (3.6) | 13.57 (1.67) | 4.33 (0.47) | 2.26 (0.21) | 0.22 (0.03) | 0.19 (0.03) | 0.01 (0.00) | 0.03 (0.00) | 35.3 (5.8) | 16.8 (2.0) |
| 38F | 129.5 (10.5) | 68.9 (9.0) | 65.5 (5.8) | 146.5 (12.8) | 34.6 (4.3) | 23.1 (3.0) | 11.53 (2.08) | 3.89 (0.34) | 2.21 (0.25) | 0.21 (0.04) | 0.18 (0.02) | 0.01 (0.00) | 0.03 (0.00) | 42.1 (4.9) | 15.1 (1.0) |
| ANOVA-1 | log ** | *** | *** | ns | ** | ns | K-W test | ** | ns | ns | * | * | * | *** | *** |
| ANOVA-2 | K-W test | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | * | ns |

Significance codes: '***' 0.001 , '**' 0.01, '*' 0.05 , '.' 0.1 ns = not significant

**Table S2**. Amounts of nutrients that would be applied per ha with application rate of 20 t $ha^{-1}$ amendment.

| | N kg ha$^{-1}$ | P kg ha$^{-1}$ | K kg ha$^{-1}$ | Ca kg ha$^{-1}$ | Mg kg ha$^{-1}$ | S kg ha$^{-1}$ | Fe kg ha$^{-1}$ | Mn kg ha$^{-1}$ | Zn g ha$^{-1}$ | Cu g ha$^{-1}$ | B g ha$^{-1}$ | Mo g ha$^{-1}$ | Ni g ha$^{-1}$ | Na kg ha$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7C | 396 | 205 | 181 | 397 | 96 | 68 | 27 | 12 | 5900 | 520 | 487 | 26 | 70 | 114 |
| 7CB | 340 | 189 | 177 | 413 | 92 | 63 | 32 | 11 | 5467 | 523 | 487 | 25 | 74 | 98 |
| 7F | 357 | 190 | 180 | 413 | 96 | 63 | 46 | 11 | 6300 | 530 | 503 | 27 | 75 | 115 |
| 20C | 334 | 201 | 184 | 400 | 95 | 69 | 26 | 12 | 5867 | 530 | 493 | 26 | 62 | 115 |
| 20CB | 338 | 197 | 181 | 417 | 96 | 66 | 30 | 11 | 5733 | 547 | 507 | 26 | 68 | 103 |
| 20F | 372 | 200 | 182 | 420 | 97 | 67 | 33 | 10 | 6067 | 550 | 510 | 28 | 70 | 120 |
| 38C | 438 | 206 | 166 | 417 | 91 | 72 | 30 | 12 | 5733 | 570 | 497 | 26 | 68 | 104 |
| 38CB | 453 | 203 | 171 | 440 | 96 | 68 | 37 | 12 | 6200 | 603 | 520 | 26 | 79 | 96 |
| 38F | 351 | 186 | 177 | 397 | 93 | 62 | 31 | 11 | 5967 | 570 | 487 | 25 | 68 | 114 |

**Table S3.** Changes in $NH_4$-N during 90-day incubation (n=3, (SD)) expressed in mg $NH_4$-N kg$^{-1}$ dry soil

| Day: | 0 | 1 | 3 | 7 | 14 | 29 | 42 | 60 | 90 |
|---|---|---|---|---|---|---|---|---|---|
| 7C | < LOQ | 0.25 (0.4) | < LOQ | 0.83 (0.2) | 0.11 (0.0) | < LOQ | < LOQ | 0.18 (0.1) | < LOQ |
| 7CB | < LOQ | 0.25 (0.4) | < LOQ | 0.19 (0.0) | 0.12 (0.1) | 0.08 (0.1) | 0.11 (0.2) | 0.20 (0.1) | < LOQ |
| 7F | < LOQ | < LOQ | < LOQ | 0.63 (0.3) | 0.11 (0.0) | < LOQ | 0.09 (0.1) | 0.14 (0.1) | < LOQ |
| 20C | < LOQ | < LOQ | < LOQ | 0.61 (0.5) | 0.18 (0.0) | < LOQ | 0.08 (0.1) | 0.21 (0.2) | < LOQ |
| 20CB | < LOQ | < LOQ | < LOQ | 0.39 (0.1) | 0.14 (0.1) | < LOQ | 0.21 (0.2) | 0.06 (0.1) | < LOQ |
| 20F | < LOQ | < LOQ | < LOQ | 0.24 (0.0) | 0.15 (0.1) | < LOQ | 0.09 (0.1) | < LOQ | < LOQ |
| 38C | 0.33 (0.2) | < LOQ | < LOQ | 0.11 (0.0) | 0.23 (0.0) | < LOQ | < LOQ | < LOQ | < LOQ |
| 38CB | < LOQ | < LOQ | < LOQ | 0.26 (0.1) | 0.09 (0.1) | < LOQ | 0.07 (0.1) | < LOQ | 0.04 (0.0) |
| 38F | < LOQ | < LOQ | < LOQ | 0.11 (0.0) | 0.11 (0.1) | 0.08 (0.1) | 0.09 (0.1) | < LOQ | 0.15 (0.1) |
| Control | < LOQ | < LOQ | < LOQ | 0.10 (0.1) | 0.11 (0.0) | 0.25 (0.2) | < LOQ | < LOQ | < LOQ |

* Limit of quantification (LOQ) for $NH_4$-N is 0.063 mg kg$^{-1}$ dry soil

**Table S4**. The uptake of macro- and micronutrients nutrients in the barley grain estimated per area (n=3, (SD)). ANOVA with one factor for comparison with the control treatments. S and SP are soil and soil with peat controls. 30N, 60N, 100N and 150N are treatments with mineral fertilizer with the corresponding levels of applied kg N $ha^{-1}$.

| | **N** kg $ha^{-1}$ | **P** kg $ha^{-1}$ | **K** kg $ha^{-1}$ | **Ca** kg $ha^{-1}$ | **Mg** kg $ha^{-1}$ | **S** kg $ha^{-1}$ | **Fe** g $ha^{-1}$ | **Mn** g $ha^{-1}$ | **Zn** g $ha^{-1}$ | **Cu** g $ha^{-1}$ | **B*** g $ha^{-1}$ | **Mo** g $ha^{-1}$ | **Ni** g $ha^{-1}$ | **Na** g $ha^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | 35.9 (1.2)g | 14.1 (0.8)e | 22.5 (1.8)def | 1.1 (0.2)ef | 4.3 (0.3)de | 2.9 (0.1)f | 241 (50.1)bcd | 36 (3.5)g | 117 (68.5) | 21.5 (0.53)f | 2.6 (0.30)ab | 1.7 (0.06)e | 3.2 (1.90) | 1383 (183)ab |
| SP | 34.3 (0.7)g | 13.2 (0.8)e | 20.0 (1.4)f | 1.0 (0.1)f | 3.9 (0.3)e | 2.9 (0.1)f | 182 (24.2)de | 36 (1.1)g | 76 (3.1) | 22.1 (0.75)f | 2.3 (0.31)ab | 1.6 (0.11)e | 1.8 (0.38) | 794 (155)b |
| 30 N | 50.9 (1.1)def | 18.8 (0.7)bcde | 30.6 (0.5)cdef | 1.4 (0.1)cdef | 6.0 (0.1)bcd | 4.1 (0.1)cde | 224 (8.1)bcde | 53 (3.7)cdef | 100 (2.5) | 30.5 (0.94)cde | 2.7 (0.30)ab | 2.4 (0.06)cde | 2.4 (0.12) | 721 (11)b |
| 60 N | 67.7 (5.8)bc | 22.3 (2.2)abcd | 38.7 (4.1)bc | 1.8 (0.3)abc | 7.3 (1.0)abc | 5.3 (0.6)bc | 291 (34.8)abc | 73 (10.2)bc | 128 (14.7) | 37.6 (5.26)bc | 2.2 (1.57)ab | 3.6 (0.76)bc | 2.5 (0.27) | 1082 (34)ab |
| 100 N | 86.5 (12.0)b | 24.0 (3.7)ab | 47.1 (8.7)ab | 2.3 (0.4)ab | 8.8 (1.8)ab | 6.7 (1.2)ab | 317 (49.4)ab | 101 (22.0)ab | 157 (22.9) | 46.9 (6.09)ab | 4.0 (0.57)a | 4.3 (0.45)ab | 2.6 (0.08) | 1638 (716)ab |
| 150 N | 112.7 (3.1)a | 27.4 (2.2)a | 56.5 (6.4)a | 2.4 (0.0)a | 9.9 (0.7)a | 8.4 (0.4)a | 352 (17.4)a | 111 (3.5)a | 180 (6.0) | 56.0 (3.57)a | < LOQ | 5.6 (0.46)a | 2.6 (0.25) | 1850 (346)a |
| | | | | | | | | | | | | | | |
| 7C | 41.6 (1.4)fg | 16.8 (0.4)cde | 26.4 (1.7)cdef | 1.1 (0.1)ef | 5.1 (0.3)cde | 3.4 (0.1)ef | 199 (36.7)cde | 40 (1.7)efg | 85 (3.2) | 23.0 (0.91)ef | 1.7 (0.14)ab | 2.8 (0.72)cde | 5.7 (6.30) | 1272 (436)ab |
| 7CB | 34.6 (1.0)g | 13.9 (0.9)e | 21.4 (1.4)ef | 1.0 (0.1)ef | 4.1 (0.3)de | 2.9 (0.0)f | 155 (9.4)de | 34 (1.3)g | 76 (1.0) | 20.1 (0.68)f | 1.0 (0.73)ab | 1.9 (0.09)e | 1.4 (0.05) | 859 (125)ab |
| 7F | 43.8 (2.3)fg | 16.8 (0.8)cde | 26.0 (1.6)cdef | 1.2 (0.1)def | 5.0 (0.4)cde | 3.5 (0.1)def | 159 (9.4)de | 42 (2.1)defg | 89 (2.3) | 25.4 (1.20)def | 0.6 (0.83)b | 2.4 (0.16)cde | 1.6 (0.33) | 943 (25)ab |
| 20C | 45.1 (3.6)efg | 17.5 (2.5)bcde | 28.4 (3.5)cdef | 1.2 (0.1)def | 5.2 (0.8)cde | 3.6 (0.2)def | 189 (17.2)de | 47 (4.1)defg | 93 (6.1) | 26.0 (1.36)def | 1.8 (1.30)ab | 2.5 (0.25)cde | 1.5 (0.20) | 1125 (379)ab |
| 20CB | 39.6 (4.7)fg | 14.4 (2.3)e | 22.6 (3.8)def | 1.0 (0.2)f | 4.0 (0.5)e | 3.0 (0.4)f | 142 (18.2)e | 37 (7.2)fg | 74 (8.5) | 22.3 (3.35)f | 1.3 (0.96)ab | 2.0 (0.44)de | 1.1 (0.03) | 904 (198)ab |
| 20F | 37.7 (3.2)g | 14.8 (1.5)e | 22.4 (2.4)def | 1.0 (0.1)f | 4.4 (0.6)de | 3.0 (0.2)f | 143 (15.1)e | 34 (3.4)g | 80 (5.9) | 22.3 (1.99)f | 1.2 (0.83)ab | 1.9 (0.26)e | 1.2 (0.14) | 992 (211)ab |
| 38C | 59.3 (6.9)cde | 22.3 (2.2)abcd | 34.7 (3.2)bcde | 1.6 (0.2)cde | 6.7 (0.8)bc | 4.6 (0.6)cde | 235 (25.5)bcde | 60 (7.5)cde | 113 (12.4) | 31.1 (3.05)cde | 0.7 (1.02)ab | 3.4 (0.45)bcd | 1.5 (0.21) | 1017 (123)ab |
| 38CB | 59.5 (2.7)cd | 23.2 (2.1)abc | 35.4 (3.2)bcd | 1.7 (0.1)bcd | 6.9 (0.4)abc | 4.8 (0.2)cd | 230 (13.2)bcde | 62 (5.7)cd | 119 (4.0) | 33.3 (1.33)cd | 2.0 (1.45)ab | 3.3 (0.37)bcd | 1.5 (0.20) | 953 (221)ab |
| 38F | 42.8 (2.7)fg | 16.1 (1.2)de | 25.2 (2.5)cdef | 1.1 (0.1)ef | 4.5 (0.3)de | 3.4 (0.2)ef | 157 (6.5)de | 39 (3.0)fg | 86 (4.1) | 24.7 (2.15)def | 1.8 (1.27)ab | 2.2 (0.23)cde | 1.4 (0.11) | 1107 (264)ab |
| | log | | | | log | log | | log | K-W test | log | | | K-W test | log |
| | *** | *** | *** | *** | *** | *** | *** | ** | *** | *** | * | *** | ** | * |

Significance codes: '***' 0.001 , '**' 0.01, '*' 0.05 , '.' 0.1 ns = not significant

* Limit of quantification (LOQ) for boron is grain concentration of 0.5 mg $kg^{-1}$

**Table S5**. The uptake of macro- and micronutrients nutrients in the barley grain estimated per area (n=3, (SD)). +N indicate the amendments with supplementary 60 kg N $ha^{-1}$. ANOVA with one factor for comparison with the control treatments. S and SP are soil and soil with peat controls. 30N, 60N, 100N and 150N are treatments with mineral fertilizer with the corresponding levels of applied kg N $ha^{-1}$.

| | **N** kg $ha^{-1}$ | **P** kg $ha^{-1}$ | **K** kg $ha^{-1}$ | **Ca** kg $ha^{-1}$ | **Mg** kg $ha^{-1}$ | **S** kg $ha^{-1}$ | **Fe** g $ha^{-1}$ | **Mn** g $ha^{-1}$ | **Zn** g $ha^{-1}$ | **Cu** g $ha^{-1}$ | **B*** g $ha^{-1}$ | **Mo** g $ha^{-1}$ | **Ni** g $ha^{-1}$ | **Na** g $ha^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | 35.9 (1.2)e | 14.1 (0.8)d | 22.5 (1.8)de | 1.1 (0.2)c | 4.3 (0.3)cd | 2.9 (0.1)e | 241 (50.1)bc | 36 (3.5)e | 117 (68.5) | 21.5 (0.53)e | 2.6 (0.30) | 1.7 (0.06) | 3.2 (1.90) | 1383 (183)abcd |
| SP | 34.3 (0.7)e | 13.2 (0.8)d | 20.0 (1.4)e | 1.0 (0.1)c | 3.9 (0.3)d | 2.9 (0.1)e | 182 (24.2)c | 36 (1.1)e | 76 (3.1) | 22.1 (0.75)de | 2.3 (0.31) | 1.6 (0.11) | 1.8 (0.38) | 794 (155)cd |
| 30 N | 50.9 (1.1)de | 18.8 (0.7)cd | 30.6 (0.5)cde | 1.4 (0.1)bc | 6.0 (0.1)bcd | 4.1 (0.1)de | 224 (8.1)bc | 53 (3.7)de | 100 (2.5) | 30.5 (0.94)cde | 2.7 (0.30) | 2.4 (0.06) | 2.4 (0.12) | 721 (11)d |
| 60 N | 67.7 (5.8)cd | 22.3 (2.2)bc | 38.7 (4.1)bcd | 1.8 (0.3)ab | 7.3 (1.0)ab | 5.3 (0.6)bcd | 291 (34.8)abc | 73 (10.2)bcd | 128 (14.7) | 37.6 (5.26)bc | 2.2 (1.57) | 3.6 (0.76) | 2.5 (0.27) | 1082 (34)bcd |
| 100 N | 86.5 (12.0)bc | 24.0 (3.7)abc | 47.1 (8.7)abc | 2.3 (0.4)a | 8.8 (1.8)ab | 6.7 (1.2)ab | 317 (49.4)ab | 101 (22.0)ab | 157 (22.9) | 46.9 (6.09)ab | 4.0 (0.57) | 4.3 (0.45) | 2.6 (0.08) | 1638 (716)abc |
| 150 N | 112.7 (3.1)a | 27.4 (2.2)ab | 56.5 (6.4)a | 2.4 (0.0)a | 9.9 (0.7)a | 8.4 (0.4)a | 352 (17.4)ab | 111 (3.5)a | 180 (6.0) | 56.0 (3.57)a | < LOQ | 5.6 (0.46) | 2.6 (0.25) | 1850 (346)ab |
| 7C + N | 83.7 (4.1)bc | 30.7 (1.3)a | 48.8 (1.4)ab | 2.2 (0.0)a | 9.1 (0.2)a | 6.0 (0.3)bc | 449 (176.8)a | 86 (2.0)abc | 145 (2.6) | 40.2 (3.24)bc | 1.1 (1.61) | 5.3 (0.39) | 4.2 (2.81) | 1647 (148)ab |
| 7CB + N | 76.2 (1.9)bc | 26.0 (1.1)abc | 43.7 (2.8)abc | 1.9 (0.1)ab | 7.9 (0.7)ab | 5.5 (0.3)bcd | 277 (36.8)abc | 79 (7.3)abcd | 137 (13.6) | 37.6 (3.03)bc | < LOQ | 4.6 (0.70) | 1.9 (0.51) | 1415 (135)abcd |
| 7F + N | 78.7 (6.9)bc | 27.2 (2.2)ab | 44.6 (4.9)abc | 2.0 (0.2)ab | 8.3 (0.7)ab | 5.4 (0.5)bcd | 281 (12.4)abc | 78 (9.3)abcd | 137 (10.1) | 36.6 (5.44)bc | 1.0 (1.46) | 4.7 (0.94) | 1.7 (0.05) | 1170 (298)abcd |
| 20C + N | 80.3 (3.4)bc | 27.3 (1.8)ab | 43.8 (3.5)abc | 2.0 (0.1)ab | 8.5 (0.7)ab | 5.4 (0.4)bcd | 291 (33.2)abc | 78 (7.1)bcd | 131 (13.4) | 34.4 (3.30)bcde | 1.1 (1.59) | 4.3 (0.59) | 1.8 (0.06) | 1450 (135)abc |
| 20CB + N | 77.5 (12.7)bc | 27.2 (4.0)ab | 44.8 (7.2)abc | 2.0 (0.2)ab | 8.3 (1.1)ab | 5.4 (0.7)bcd | 285 (31.0)abc | 76 (9.0)bcd | 132 (19.8) | 34.9 (5.03)bcd | < LOQ | 4.4 (0.48) | 1.8 (0.12) | 1568 (223)ab |
| 20F + N | 69.3 (3.9)bcd | 24.6 (1.8)abc | 39.6 (3.5)abc | 1.7 (0.2)ab | 7.8 (0.4)ab | 4.9 (0.3)cd | 273 (19.2)abc | 68 (2.7)cde | 122 (2.1) | 31.9 (1.60)cde | 1.1 (1.55) | 3.8 (0.34) | 1.9 (0.30) | 1279 (226)abcd |
| 38C + N | 82.7 (9.0)bc | 27.5 (2.2)ab | 46.4 (4.9)abc | 2.3 (0.1)a | 9.0 (0.8)a | 5.8 (0.6)bcd | 328 (11.7)ab | 85 (9.7)abcd | 139 (19.2) | 32.6 (3.52)cde | < LOQ | 5.1 (0.47) | 2.1 (0.62) | 2186 (109)a |
| 38CB + N | 93.0 (9.0)ab | 29.8 (3.0)ab | 47.3 (5.9)abc | 2.3 (0.2)a | 9.1 (1.2)a | 6.2 (0.5)bc | 306 (39.1)abc | 91 (11.6)abc | 147 (10.4) | 37.5 (2.82)bc | 1.3 (1.91) | 5.2 (0.54) | 2.0 (0.43) | 1845 (505)ab |
| 38F + N | 69.8 (5.1)bcd | 22.8 (1.9)abc | 36.8 (3.0)bcde | 1.8 (0.1)ab | 7.1 (0.8)abc | 4.8 (0.3)cd | 240 (24.6)bc | 68 (5.9)cde | 117 (2.7) | 32.3 (2.92)cde | < LOQ | 3.9 (0.31) | 1.7 (0.45) | 1501 (342)abc |
| | | | | | | | log | | K-W test | | K-W test | K-W test | K-W test | log |
| | *** | *** | *** | *** | *** | *** | ** | *** | * | *** | * | ** | ns | *** |

Significance codes: '***' 0.001 , '**' 0.01, '*' 0.05 , '.' 0.1 ns = not significant

* Limit of quantification (LOQ) for boron is grain concentration of 0.5 mg $kg^{-1}$

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