Human urine and frass as fertilizers

Nitrogen availability over time

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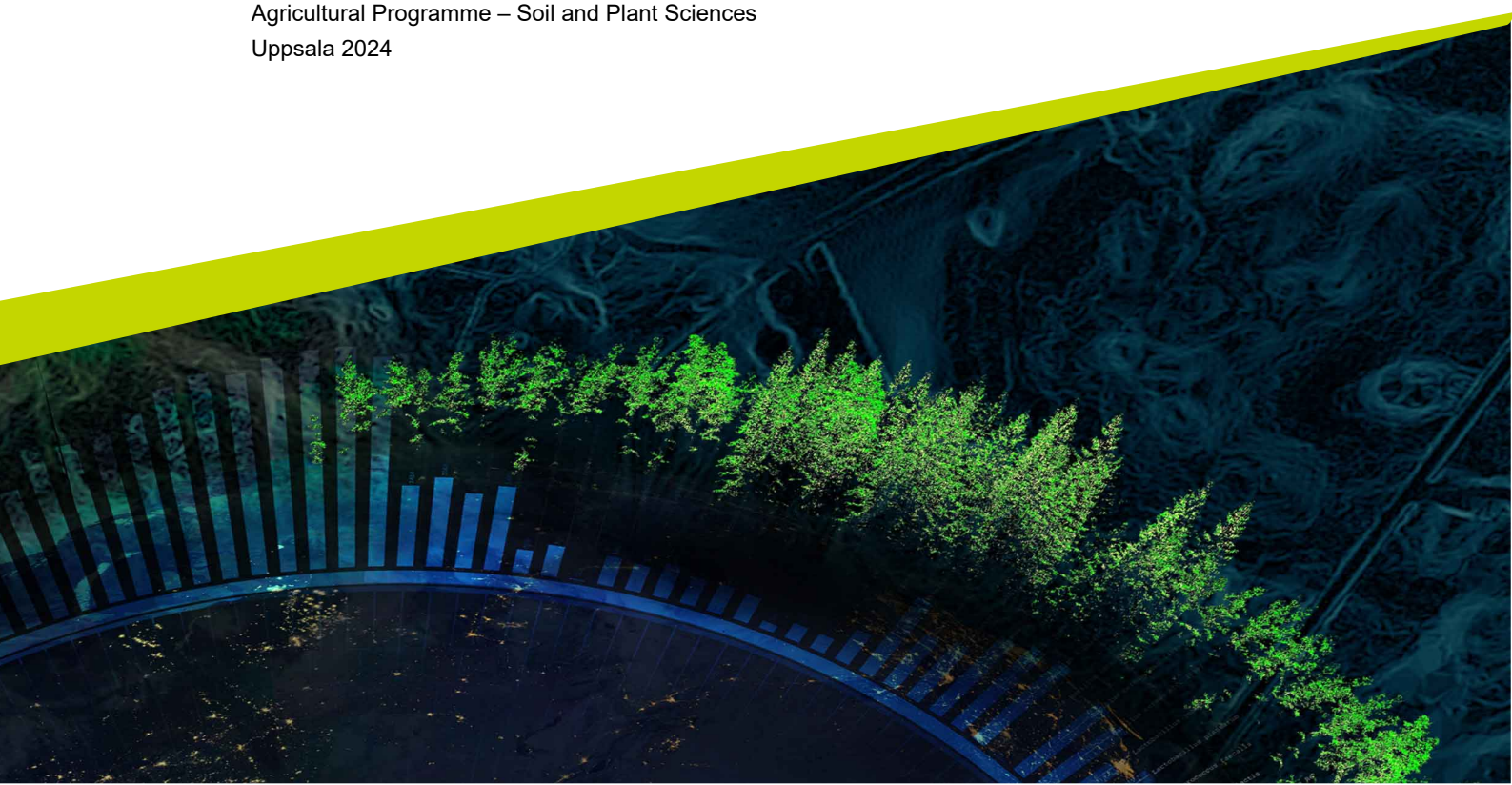

Human urine and frass as fertilizers. Nitrogen availability over time

Erica Aronsson

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Abstract

There is a pressing need for implementing more sustainable fertilizers in agriculture. Through a laboratory incubation trial and subsequent data analysis of a previously perfomed field trial, this study aimed to evaluate the nitrogen (N) release dynamics of soil treated with novel fertilizers derived from human urine and black soldier fly larvae (BSFL) frass, and to assess their potential compared to commercial NPK fertilizer. Soil samples underwent various treatments, including urine pellets, BSFL frass, commercial NPK fertilizer, and no fertilizer. Incubated at 15 °C, samples were periodically sampled over a 63-day period for nitrate-N and ammonium-N extraction analysis. The findings suggested that urine pellets demonstrated promise as a viable alternative to commercial NPK fertilizer, exhibiting comparable mineral N release dynamics. This observation was reinforced by field trial results, which revealed significantly higher barley yields in urine fertilized soil compared to unfertilized soil, with yields similar to those of NPK fertilized barley. Additionally, BSFL frass showed potential as a slow-release N fertilizer in both the laboratory and field trials. Moving forward, continued research is essential to optimize the utilization of urine pellets and BSFL frass among various crops and soil types.

Keywords: nitrogen mineralization, urea hydrolysis, black soldier fly larvae, incubation trial, nitrogen recovery, nutrient recycling, nitrate, ammonium, barley

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Abbreviations

ANOVA	Analysis of variance
BSFL	Black soldier fly larvae
°C	Degree Celsius (0 °C = 273.15 Kelvin)
cm	Centimeter (1×10^{-2} m)
CO_2	Carbon dioxide
$CO(NH_2)_2$	Urea
DM	Dry matter
dw	Dry weight
EC	Electrical conductivity
Eq.	Equation
g	Gram
GHG	Greenhouse gas
GS	Zadoks Growth Stage
h	Hour (3600 s)
ha	Hectare (10 000 m^2)
H_2	Hydrogen gas
H^+	Hydrogen ion
K	Potassium
KCl	Potassium chloride
kg	Kilogram (1000 g)
KOH	Potassium hydroxide
LCA	Life cycle assessment
m	Meter
M	Molar
mg	Milligram (1×10^{-3} g)
Mg	Magnesium
mL	Millilitre (1×10^{-6} m^3)
mm	Millimeter (1×10^{-3} m)

m^2	Square meter
m^3	Cubic meter
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammmonium
nm	Nanometer (1×10^{-9} m)
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NPK	Nitrogen-Phosphorus-Potassium
N_r	Reactive form of nitrogen
NUE	Nitrogen use efficiency
N_2O	Nitrous oxide
N_2	Dinitrogen
O_2	Oxygen gas
P	Phosphorus
rpm	Rounds per minute
S	Sulfur
SD	Standard deviation
SOM	Soil organic matter
SLU	Swedish University of Agricultural Sciences
Tg	Teragram (1×10^{12} g)
WEEE	Waste from electrical and electronic equipment
WHC	Water holding capacity
µm	Micrometre (1×10^{-6} m)
µS	Microsiemens

Introduction

The planetary boundaries include nine critical environmental processes for humanity to stay within to uphold resilient and safe Earth systems (Rockström et al. 2009). In 2023, six of these had already been transgressed, with the biogeochemical flows of nitrogen (N) and phosphorus (P) being among the affected boundaries (Richardson et al. 2023). The N biogeochemical cycle is strongly affected by agricultural systems (Sutton et al. 2013), where the industrial fixation of reactive N (N_r), *i.e.*, to produce synthetic fertilizers, is the dominating driver of the cycle (Fowler et al. 2013) (Figure 1). The N_r includes the mineral forms ammonium (NH_4^+) and nitrate (NO_3^-), both of which are important plant nutrients and enter the agricultural sector through three primary sources: i) biological fixation, mainly driven by legume cultivation, ii) atmospheric deposition, or iii) Haber-Bosch synthesized fertilizers. Additionally, N_r can originate from the mineralization of soil organic matter (SOM) or the application of organic N fertilizers (Bodirsky et al. 2014). However, N_r is often lost from agricultural systems as NO_3^- through leaching or transferred to the atmosphere as ammonia (NH_3), nitric oxide (NO), dinitrogen gas (N_2), or nitrous oxide (N_2O). Once lost or transferred, the N atom can trigger a cascade of environmental effects. For instance, excessive N_r, in combination with other nutrients, can disrupt aquatic and terrestrial ecosystems through eutrophication (Galloway et al. 2003). In the atmosphere, it can lead to stratospheric ozone depletion (Ravishankara et al. 2009; Billen et al. 2021) and contribute to global warming (Galloway et al. 2003).

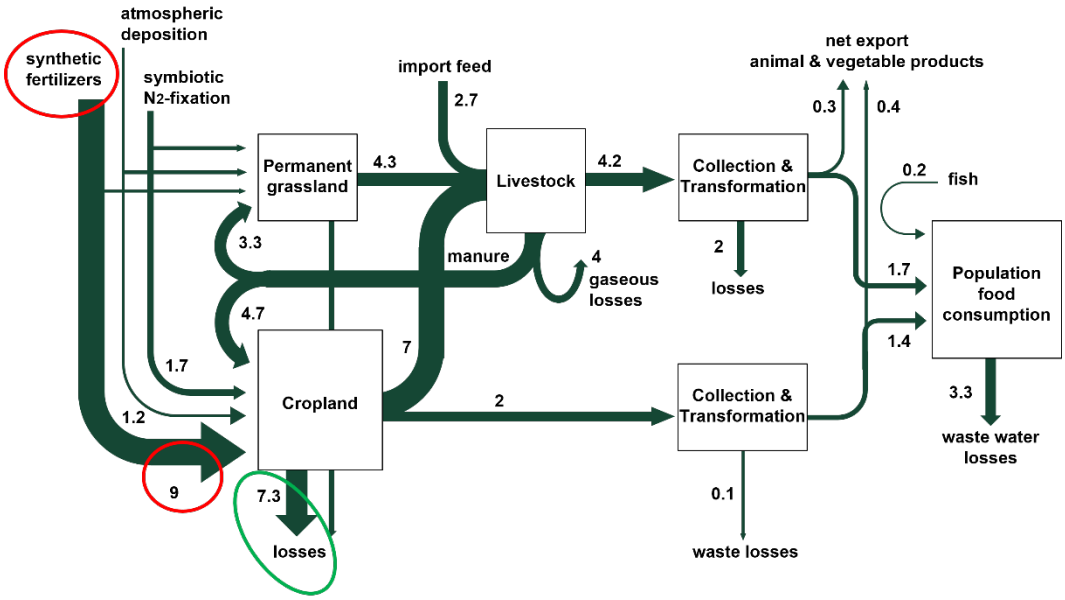

Figure 1. Illustration of the key N pathways and dynamics (Tg N) within the agro-food system of Europe during the period 2009−2013. The figure highlights major inputs from synthetic fertilizers (red circles) and losses from cropland (green circle). Figure adapted from Billen et al. (2021).

While the production of synthetic fertilizers is on the rise, so is the generation of waste, both within the food supply chain and in other fronts. According to the definition in the Swedish Environmental Code (SFS 1998:808), waste encompasses any object or matter that the bearer disposes of, is obliged to dispose of, or intends to dispose of. In Sweden, each person generated an average of 449 kg of household waste in 2022, comprising 36% bulky waste, 34% residual waste, 15% packaging waste, 10% food waste, and 5% hazardous waste and waste from electrical and electronic equipment (WEEE) (Avfall Sverige 2022). Food waste in particular represents an opportunity to utilize valuable resources like N and return them to agricultural land, promoting circularity (Dobermann et al. 2022). Similarly, human excreta (urine and feces) is another waste product that contains valuable resources. Urine for instance is rich in N, yet its agricultural potential remains significantly undervalued (Heinonen-Tanski & van Wijk-Sijbesma 2005).

Various technologies exist for recycling human excreta and organic waste materials, such as food waste, to improve circularity. In Sweden, it is most common to treat sorted-out food waste by anaerobic digestion to produce biogas and biofertilizer (Avfall Sverige 2022). Additionally, sewage sludge from wastewater treatment can be repurposed as fertilizer, although it typically contains only a small proportion of the N present in the wastewater, with most being lost during treatment (Ostermeyer 2022). The development of more alternative fertilizers that close the N cycle and minimize N_r loss is essential. This approach is vital for enhancing the sustainability of agricultural systems and society at large.

1. Background

1.1 Nitrogen in plants

Nitrogen is an essential macronutrient for plants, constituting a component in chlorophyll, proteins, and nucleic acids. The nutrient is required by plants in significant amounts for several metabolic processes, implying that N deficiency is one of the most limiting factors to growth for most crops (Campbell et al. 2018). The atmosphere contains 78% N, but it is in an inert form as N_2 (Sutton et al. 2013). Only a minority of plants can use this form of N, through symbiotic relations with specific bacteria, and therefore, mainly the mineral forms NH_4^+ and NO_3^- are absorbed by the roots of most plants (Campbell et al. 2018; Lasa et al. 2001), with NO_3^- being the preferred source for most species (Li et al. 2012). Hence, plants rely on the availability of N in these forms, either from the mineralization of organic N existing in soils or from the application of fertilizers containing N to optimize their growth and development (Dari et al. 2019).

Approximately 40% of the arable land in Sweden is dedicated to cereals, primarily barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) (Jordbruksverket n.d.). A shortage of N in cereals can result in decreased crop protein content and yield losses (IFA 2007), particularly during phases of vigorous growth (Shafi et al. 2011). During the early spring growth stages of barley up to Zadoks Growth Stage (GS) 31 (Figure 2), N uptake is relatively low. The highest demand for N occurs during stem elongation (GS 31–39). As the canopy reaches its maximum size and ears start to emerge, N uptake slows down again, typically from GS 39 to GS 59. Following ear emergence, N uptake becomes very limited as N stored in leaves and stems is redistributed to support the development of grain protein. The N accumulation in <spring barley at harvest is typically 25–30% lower compared to that of winter barley due to spring barley's shorter growing season (Yara 2024).

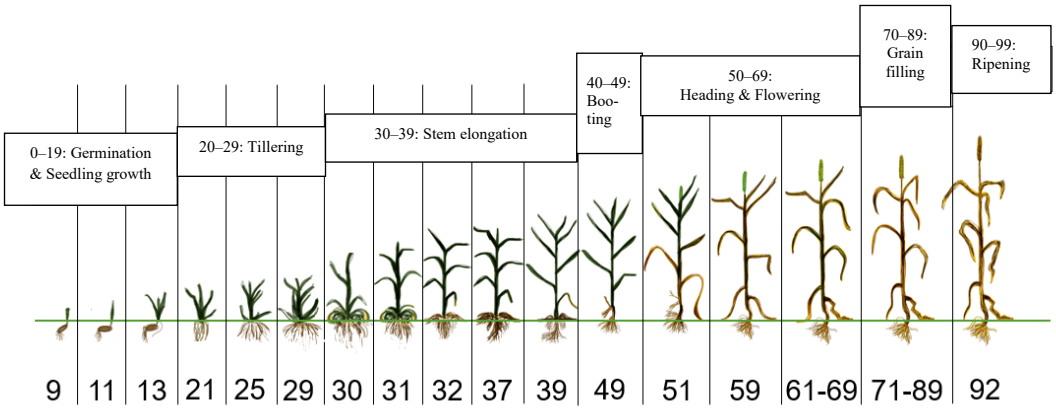

Figure 2. Zadoks cereal development scale. Adapted from Alqudah (2015).

Consequently, adequate N availability early in the growing season generally results in higher yields, while higher N levels later in the growing period tend to increase protein content. For instance, Giordano et al. (2022) observed a linear increase in wheat protein content with higher proportions of late N and later applications. Additionally, Sardana and Zhang (2005) found that N application at the initial stages is crucial for achieving desired growth in barley, but N application at the boot stage did not contribute to yield significantly. Conversely, excessive N application can lead to lodging (Shafi et al. 2011), pests, diseases (Gómez-Trejo et al. 2021), and environmental losses. The release of organically bound N late in the season after annual crops have completed their N uptake also increases the risk of losses. Therefore, the timing of N release from fertilizers is crucial and should guide fertilizer selection and application timing. To achieve optimal crop growth and yield, it is essential for N mineralization patterns to align with fluctuations in crop nutrient requirements and uptake, thereby ensuring synchrony between N supply and N demand (Johnson et al. 2012).

1.2 Nitrogen in soils

Nitrogen compounds undergo several transformations, including mineralization, immobilization, nitrification, and denitrification (Figure 3). The compounds are exchanged between the soil and the atmosphere through processes such as volatilization, denitrification, biological N fixation and atmospheric deposition. Exchanges also occur between the soil and the hydrosphere through leaching, erosion/runoff, and irrigation (IFA 2007). The stable SOM typically contains about 5−6% N, constituting the primary N stock in most soils. Approximately 1−3% of this N can be gradually made available to plants each year through microbial processes. Organically bound N within the soil is also found in various organic forms, such as soil-living organisms, fungal hyphae, plant roots, and rhizomes (Eriksson et al. 2011).

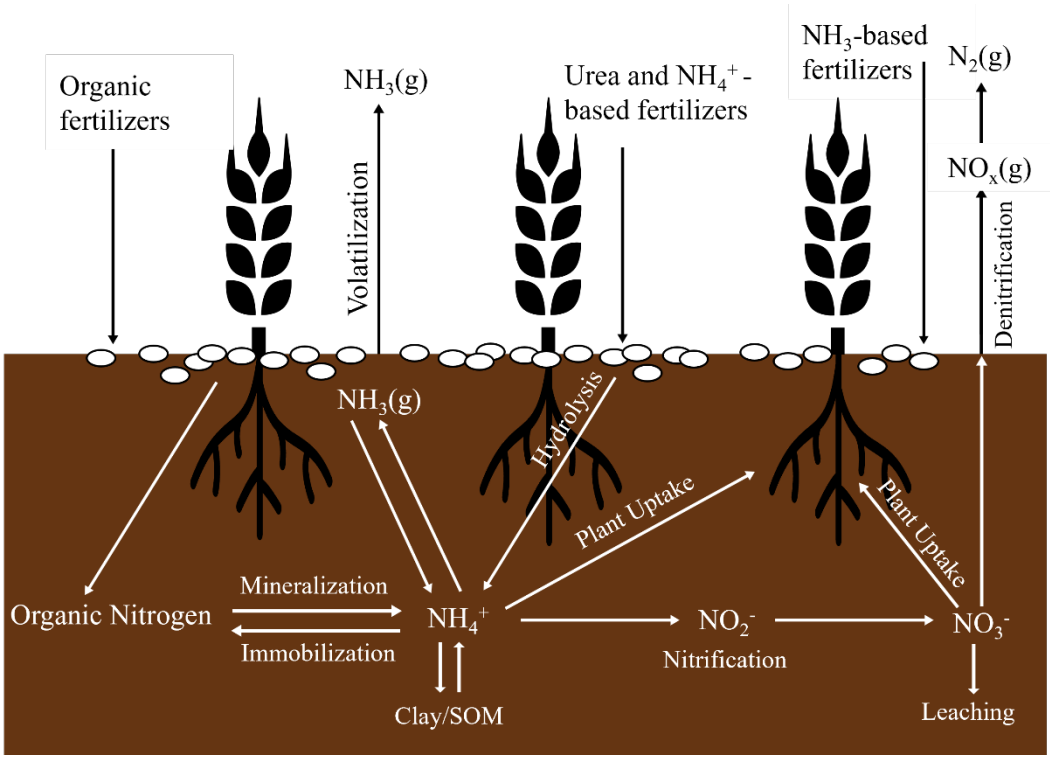

Figure 3. Schematic of N cycling. Adapted from Dari et al. (2019).

The N mineralization process is characterized by the conversion of N from organic to inorganic (mineral) forms during the turnover of organic material by soil microorganisms, and the reverse process is known as immobilization (see Equation 1). Specific enzymes produced by fungi and bacteria catalyze mineralization by facilitating the transformation and breakdown of organic molecules. Mineralization and immobilization occur simultaneously. The available N supply for growing crops is determined by the net mineralization or net immobilization. Whether the turnover of organic material results in net mineralization or net immobilization depends on several factors, including the microorganisms' access to energy-rich and easily exploited material for synthesizing building blocks of the cells for microbial growth, as well as the N content of this material (Eriksson et al. 2011). The ratio of the mass of C to the mass of N in the material, *i.e.* the C:N ratio, can also have a significant effect on decomposition. Soil microbes typically have a C:N ratio near 8:1 and must acquire enough C and N from their environment to maintain that ratio in their bodies. That corresponds to a diet with a C:N ratio near 24:1, which will be consumed relatively quickly by the microorganisms with essentially no excess C or N left over (USDA 2011). Microbes use C as a source of energy, and an adequate supply of N promotes the growth of microbial biomass. When the available N exceeds microbial demand and the C:N ratio is lower than 24:1, it leads to net N mineralization, resulting in the release of mineral N into the soil.

Conversely, if the available N is insufficient for microbial needs and the C:N ratio is higher than 24:1, additional mineral N must be immobilized from the soil to allow microbial growth and facilitate the completion of the decomposition process (Cabrera et el. 2004; USDA 2011).

$$R{-}NH_2 + 2\,H_2O \underset{\text{immobilization}}{\overset{\text{mineralization}}{\rightleftharpoons}} OH^- + R{-}OH + NH_4^+ \qquad \textit{(Eq. 1)}$$

The mineralization process is also influenced by other aspects of the chemical composition of organic matter, such as contents of cellulose, hemicellulose, lignin, and polyphenols (Mohanty et al. 2011). These compounds are differently metabolized by the microbes present in the soil, which is reflected in the rate of carbon dioxide (CO_2) production, *i.e.*, soil respiration. Other than providing insight into the organic matter and its decomposition, the respiration reflects the soil's capacity to support soil life, including microorganisms, invertebrates, plants and other living organisms interacting with the soil. Additionally, soil respiration can serve as a tool for estimating soil microbial biomass and for inferring the processes involved in nutrient cycling in the soil (USDA 2009).

As shown in Equation 1, NH_4^+ is formed during the mineralization, which in turn can be oxidized to NO_3^- under aerobic conditions, a process known as nitrification. Ammonium is in equilibrium with NH_3 as follows:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad \textit{(Eq. 2)}$$

The equilibrium is pH-dependent (with a pKa of 9.9 at 10 °C), shifting towards the production of NH_3 as the pH value increases. Ammonia is oxidized as follows in two main steps:

$$2NH_3 + 3O_2 \rightarrow 2NO_2^- \text{ (nitrite)} + 2H^+ + 2H_2O + \text{energy} \qquad \textit{(Eq. 3a)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy} \qquad \textit{(Eq. 3b)}$$

The NO_2^- concentration is typically very low in soils because NO_2^- does not normally accumulate and is oxidized to NO_3^- at a rate similar to its formation (Eriksson et al. 2011).

1.3 Nitrogen fertilizers

An increased fertilization level of N generally enhances plant growth more significantly than other nutrients (Maheswari et al. 2016), although plant growth can also be constrained by deficiencies in other essential nutrients. Consequently, global demand, production, usage, and losses are most significant for N (FAO 2019). Fertilizers can contain organic (carbon-containing) or inorganic nutrients and other compounds, but the minerals a plant absorbs are in the same form, NH_4^+ and NO_3^- when it comes to N. Thus, N in organic materials needs to be mineralized to become plant available (Campbell et al. 2018).

1.3.1 Synthetic fertilizers

There are three primary forms of N in synthetic fertilizers: ammoniacal, nitric and ureic ($CO(NH_2)_2$), being used either individually or in combinations (Dari et al. 2019). The invention of the Haber-Bosch process in the early 20th century revolutionized the production of synthetic fertilizers on an industrial scale, enabling an intensification of agricultural production and contributing to global growth of the human population, as illustrated in Figure 4. Erisman et al. (2008) estimate "that the number of humans supported per hectare of arable land has increased from 1.9 to 4.3 persons between 1908 and 2008", primarily due to the use of Haber-Bosch derived N. This method converts inert atmospheric N_2 into NH_3 by reacting N_2 with hydrogen gas (H_2) at high temperatures and pressures (Wan et al. 2023). Ammonia can further be combined with CO_2 to create urea (Ding et al. 2023).

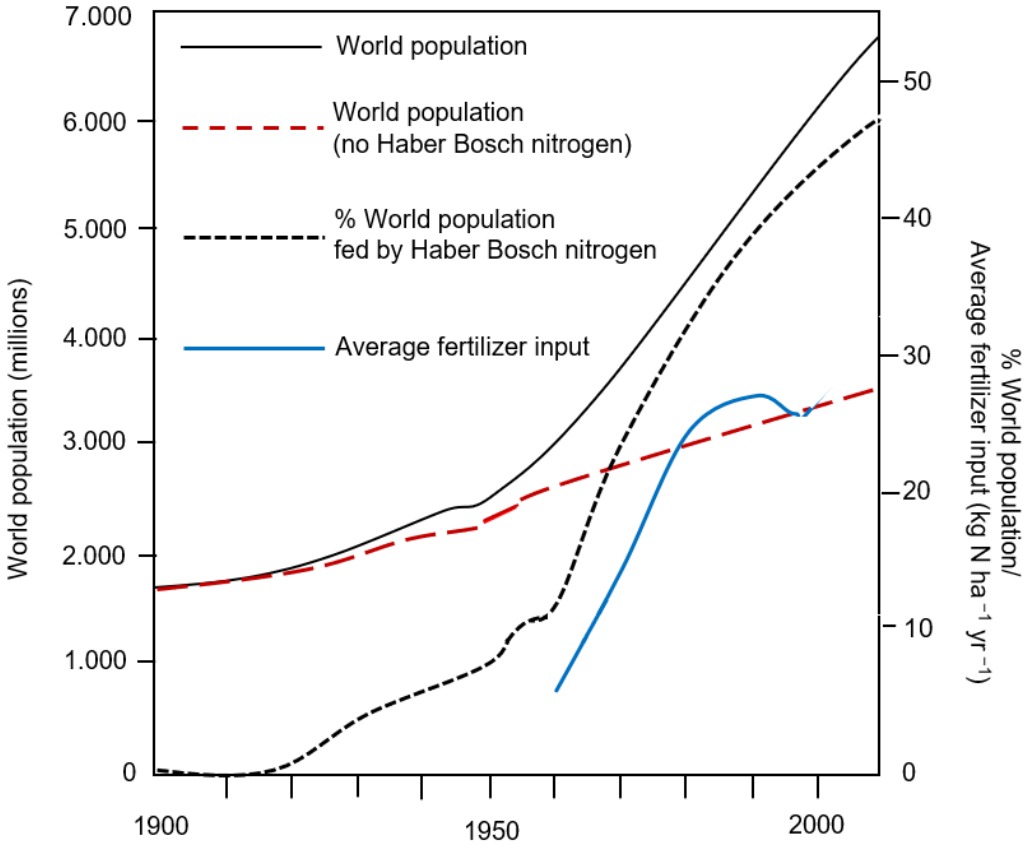

Figure 4. Patterns in world population and N utilization across the 20th century. The solid line represents the registered world population, while the long-dashed line depicts the estimated world population that could be supported without N_r from the Haber-Bosch process. Adapted from Erisman et al. (2008).

Synthetic fertilizers allow precise application in terms of nutrient supply, dosage, and timing. Its known N content and uniform composition enables accurate application, and the applications can be divided to meet the changing needs of a crop as it develops. Using the right fertilizer product at the right time, right place and right rate is a fundamental principle for improving the N use efficiency (NUE) (IFA 2007; Govindasamy et al. 2023), *i.e.,* the ratio of N output (in the harvested plant) to N input. Consequently, synthetic fertilizers can theoretically ensure a high NUE in crop production. However, in practice the use of N fertilizers in agriculture results in substantial losses to the environment through leaching, volatilization, and runoff, leading to a lower NUE (Govindasamy et al. 2023). For instance, the cereal NUE in 2015 was 35% for the world (Omara et al. 2015). Considering food losses during harvesting, transport, and consumption, only 4−14% of N_r used globally in agricultural systems ends up as consumable plant-based and animal protein (Pikaar et al. 2017).

Additionally, due to the significantly higher energy content in NH_3 compared to N_2 (Heinonen-Tanski & van Wijk-Sijbesma 2005), the Haber-Bosch process demands excessive energy inputs, accounting for approximately 2% of the global energy expenditure (Sutton et al. 2013). Moreover, the process currently relies on non-renewable resources such as natural gas as the source of H_2 (Martin et al. 2022).

1.3.2 Organic fertilizers

Organic fertilizers were defined by Huntley et al. (1997) as naturally occurring compounds containing plant nutrients produced from waste materials or by-products, where only the processing steps or physical extraction are anthropogenically assisted. Commonly used organic fertilizers include farmyard manure, green manure, compost, sewage sludge, bone meal, and food processing wastes (Huntley et al. 1997). The use of dried pelletized human urine and black soldier fly larvae (BSFL) frass as fertilizers is relatively new.

The application of organic materials contributes to the improvement of various soil properties. For instance, Bulluck et al. (2002) compared the influence of organic and synthetic fertilizers on soil microbial, physical and chemical properties. The results showed that the utilization of organic fertilizers improved soil quality by enhancing beneficial soil microorganisms, reducing pathogen populations, increasing SOM and total C, and lowering bulk density.

The N content in organic materials can vary widely depending on factors such as the origin and type of wastes, the fertility of the soil where a crop residue or a green manure crop is grown, the diet of the animals producing manure, and the methods used for storage and application (IFA 2007). Moreover, predicting the pattern and quantity of N released from organic N sources during the growing season is challenging because the release of nutrients is highly dependent on the degree and rate of mineralization. The release of N varies considerably, affected by soil moisture, temperature, and texture among many other factors, as demonstrated by Agehara and Warncke (2005), who performed a comparable study on N release from four organic fertilizers. In general, the mineralization rate was positively correlated with soil moisture (up to 90% water holding capacity (WHC)) and temperature (up to 25 °C), due to an increase in microbial activity. Furthermore, variations in N release patterns among the fertilizers could also be explained by their chemical composition. Fertilizers with high N content in a readily mineralizable form, low C:N ratio, and low lignin:N ratio exhibited a more rapid N release. The net N release followed this order: urea > blood meal > alfalfa pellets > partially composted chicken manure. For urea, the mineralization rate was suggested to depend on the activity of and contact with the enzyme urease in

soil, facilitating the hydrolysis of urea to NH_4^+ when applied to soil (Agehara & Warncke 2005), as shown in Figure 3.

1.3.2.1 Human urine pellets

Every year, a healthy adult on average produces around 500 L of urine (Heinonen-Tanski & van Wijk-Sijbesma 2005), containing 5000−7000 mg L^{-1} of total N (Karak & Bhattacharyya 2011), which varies based on factors such as climate, water intake, body weight, and dietary protein content (Heinonen-Tanski & van Wijk-Sijbesma 2005). Human urine also contains 2000−3000 mg L^{-1} of potassium (K) and 300−500 mg L^{-1} of phosphorus (P) (Karak & Bhattacharyya 2011). Approximately 85% of N in fresh urine is present as urea, 10% as other organic N, and 5% as ammoniacal N (Udert et al. 2006). Urea is a by-product of protein metabolism in the body (Korrapati & Mehendale 2014). An artificially produced version of urea is often used in synthetic fertilizers (see 1.3.1 Synthetic fertilizers). Whether excreted or synthetically produced, the chemical composition is the same, yet the concentration can differ, and the concentration of the synthetic fertilizer will be higher and more consistent (Kishor et al. 2020).

Given that urine contains some of the essential macronutrients required for plant growth (N, P and K), it serves as a viable fertilizer (Heinonen-Tanski & van Wijk-Sijbesma 2005; Winker et al. 2009). Despite its potential, the use of urine as fertilizer is currently constrained, leading to poor nutrient recycling and return to cropland (Martin et al. 2022). The recovery of all human excreta, of which urine is the primary source of N (Vinnerås et al. 2006) that is not recycled today could potentially reduce the global inputs of synthetic N fertilizers in agriculture by 16−21% (Trimmer et al. 2019).

Several studies have shown that human urine can be used as a liquid fertilizer (Kirchmann & Pettersson 1994; Lindén 1997; Ranasinghe et al. 2015; Sene et al. 2018). However, this direct pathway comes with several challenges, such as the need to collect and store urine at the household level, transport it over long distances and apply large volumes to cropland (Larsen & Gujer 2013). Moreover, urea tends to hydrolyze (Mazzei et al. 2019) and volatilize from urine as NH_3 during storage (Kirchmann & Pettersson 1995), potentially leading to N losses (Dari et al. 2019). To overcome these obstacles, various treatment technologies have been developed over the last 20 years to reduce the volume of the collected urine, stabilize N and avoid NH_3 volatilization (Maurer et al. 2006). One such technology is alkaline dehydration, in which urine is alkalized (pH > 10) and dehydrated by warm air circulation to concentrate and reduce its volume into a dry product (Simha et al. 2020). These conditions hinder urea degradation, facilitate water evaporation, and minimize N losses (Senecal 2020). A further possibility for facilitating the use of dry urine is by pelletizing it, transforming

urine into an easy-to-handle fertilizer, which simplifies storage and transport and allows the farmers to use their existing conventional farming equipment.

The environmental impacts of producing wheat with three different urine-based fertilizers (stored urine, alkaline dehydrated urine, and nitrified concentrated urine) were evaluated in a comparative analysis by Martin et al. (2022). This evaluation was performed using a life cycle assessment (LCA), with synthetic fertilizer used for wheat production serving as the reference scenario. The study identified that, in terms of greenhouse gas (GHG) emissions, eutrophication, water consumption, and fossil resources, the environmental impacts of the scenarios utilizing urine-based fertilizers were lower than those of the reference scenario. However, the study also revealed that both stored and alkaline dehydrated urine could lead to higher NH_3 volatilization compared to synthetic fertilizers. Additionally, the nitrified concentrated urine and alkaline dehydrated urine fertilizers exhibited higher electricity consumption values associated with their production than the reference system. The need to optimize the energy consumption of these technologies has been addressed in other studies as well (*e.g.,* Gunnarsson et al. 2022).

Considerable attention has been dedicated to the development of techniques for producing urine-based fertilizers, as compiled by Harder et al. (2019), Martin et al. (2020), and Chipako and Randall (2020). Moving forward, N fertilizing value and mineralization patterns need to be evaluated, to adapt fertilization strategies with these novel fertilizers, mitigate pollution and enhance the NUE by plants.

1.3.2.2 Black soldier fly larvae frass

Various methods exist for treating organic waste materials such as food waste, including thermophilic composting and anaerobic digestion. Mertenat et al. (2019) stated that “one of the most innovative biowaste treatment options” is to feed waste to insect larvae, such as the black soldier fly larvae (*Hermetica illucens* L.) (BSFL). Through this method, waste undergoes a composting-like process, resulting in its conversion into two primary products: firstly, a larval biomass that is rich in fats and proteins, suitable for animal feed (Surendra et al. 2020), and secondly, a residual by-product known as frass that contains several plant nutrients and microorganisms (both bacteria and fungi) (Lopes et al. 2022). BSFL frass constitutes a mixture of substrate residues (*i.e.*, uneaten feed materials), and BSFL feces and shed exoskeletons (Schmitt & de Vries 2020; Basri et al. 2022). In comparison with conventional composting processes, BSFL composting is faster and more efficient in terms of nutrient recycling (Lalander et al. 2015). According to recent studies, frass can be considered a multifaceted fertilizer with various benefits (Beesigamukama et al. 2020b; Quilliam et al. 2020;

Klammsteiner et al. 2020). It can improve soil health by suppressing plant pathogens (Choi & Hassanzadeh 2019) and fungal diseases, reducing soil acidity, increasing microbial abundance and diversity (Beesigamukama et al. 2021), resulting in higher crop growth and yield (Beesigamukama et al. 2020b). Frass may also contribute to an increased nutrient uptake efficiency as it has been reported to contain microbial strains that produce auxins (Poveda et al. 2019), which are regarded as the primary group of hormones crucial for the process of adventitious root formation (Rout 2006). On the contrary, other studies have reported that frass has no significant effect on crop growth (Gebremikael et al. 2022) and that frass can have negative effects on crop growth and soil quality, such as phytotoxicity if not post-treated and stabilized (Alattar et al. 2016). Frass is often biologically unstable and immature due to the rapid composting process with BSFL, which is related to a decreased availability of nutrients and increased content of potentially phytotoxic compounds (Song et al. 2021). In this sense, frass could be post-treated by thermophilic composting or anaerobic digestion, aiming to increase its maturity degree and safety for use.

The quality of frass appears to vary depending on the specific substrate fed to the larvae. According to a review conducted by Lopes et al. (2022), micronutrient levels vary depending on the feed substrate, while pH (around 7.5) and C:N ratio (around 15) show less variation. However, the frass always exhibits a lower C:N ratio compared to the feed substrate provided (Sarpong et al. 2019). Palma et al. (2020) demonstrated that high levels of protein and starch/sugar, along with lower levels of fiber in food waste, have a positive effect on BSFL frass composition and promote nutrient release. Klammsteiner et al. (2020) showed that BSFL fed with chicken feed obtained higher N concentrations than larvae fed with grass waste and fruit/vegetable waste. When larvae are fed bread waste, the N concentration is lower than the ones reported by Klammsteiner et al. (2020), in comparison to findings of Lopes et al. (2020). Basri et al. (2022) suggest that a mixture of two or different types of substrates may be preferable to produce high-nutrient BSFL frass.

The quality of frass utilized as fertilizer has been reported to influence the mineralization patterns of N when it is applied to the soil. For instance, most of the inorganic N in frass is in the form of NH_4^+ (Lalander et al. 2015; Beesigamukama et al. 2020a; Kawasaki et al. 2020). The conversion of NH_4^+ into NO_3^- through nitrification processes requires time. Concurrently, NH_4^+ is highly absorbed by soil microorganisms, increasing the likelihood of immobilization. This has been reported by several studies, showing an initial phase of immobilization that lasted 30−70 days after the application of frass (Beesigamukama et al. 2021; Gebremikael et al. 2022). Consequently, this may

limit N availability to plants during early growth stages, depending on the timing of frass application.

1.4 Field trial

Human urine pellets and BSFL frass have previously been utilized in a field trial conducted by an external actor on the Swedish island of Gotland during the 2023 growing season to assess their impact on yields. Barley (*Hordeum vulgare* L. Planet) was sown on May 26 and harvested on August 27. The crop received fertilization at sowing, with the following fertilizers and doses applied: 567 kg ha^{-1} of pelletized dried urine, 1000 kg ha^{-1} of frass, and 370 kg ha^{-1} of NPK. Thus, all treatments received 85 kg N ha^{-1}. The NPK treatment was employed as the positive control to provide a reference for expected outcomes, while an untreated plot served as the negative control.

The field trial rendered interesting results in terms of crop productivity, with the following yields: 7.3 tons ha^{-1} from urine pellets, 7.0 tons ha^{-1} from frass, 7.4 tons ha^{-1} from NPK, and 5.2 tons ha^{-1} from the unfertilized treatment (Table 1), demonstrating a significant potential of the fertilizers. However, the dynamics of N from the fertilizers in that agricultural soil were not yet assessed, leading to the formulation of the hypotheses and aim of this study.

Table 1. Yield results from the field trial.

	Dried urine + P20	BSFL frass + NS 27−4	NPK 24−4−5	Un-fertilized
Yield (kg ha^{-1})	7 359a	7 048a	7 429a	5 248b

P20 refers to a phosphorus (P) fertilizer containing 20% P. NS 27−4 denotes a fertilizer with 27% nitrogen and 4% sulfur. Mean values that share a letter are not significantly different at $p \geq 0.05$. Statistical differences were assessed using ANOVA followed by Tukey's test.

1.5 Aim, objectives, and hypotheses

This study aimed to provide data on the N release patterns, under laboratory conditions, from human urine-based fertilizer and BSFL frass over time. The specific objectives were to answer the following questions:

i) What nitrogen release dynamics are observed in soil treated with fertilizers derived from human urine and BSFL frass?

ii) Can the nitrogen release patterns observed during incubation explain any variations in yield of the barley in the field trial?

Urine pellets were hypothesized to function as short-term N fertilizers similar to synthetic ones, as evidenced by initial high mineral N concentrations observed early in the incubation period. Conversely, frass was hypothesized to act as a long-term fertilizer with a gradual release of N, as indicated by slower N release observed during the incubation.

1.6 Delimitations

This study focused on analyzing soil samples treated with distinct fertilizers regarding their mineral N release over time. The fertilizers tested included urine pellets and BSFL frass, with a positive control (commercial NPK fertilizer) and a negative control (unfertilized soil) also included. The study did not address hygiene-related aspects, the presence of undesirable substances (*e.g.*, heavy metals, pharmaceuticals, hormones, and pathogens), or practical feasibility and limitations (*e.g.*, regarding logistics, legal aspects, and socio-cultural acceptance). Neither was the different techniques regarding urine diversion, urine treatment, or BSFL treatment examined.

2. Material and Methods

2.1 Soil

The soil used in this experiment was collected in November 2023 from a field (57°39'13.7"N, 18°28'30.0"E) near Roma on the Swedish island of Gotland (Figure 5), where barley (*Hordeum vulgare* L. Planet) was grown last season (2023). The location is situated in an area dominated by Calcaric Cambisols (Soil Atlas of Europe 2005).

Figure 5. The geographical location of the field trial. (Image source: Pixabay (n.d.).)

The 0−10 cm topsoil was sampled to ensure no subsoil with higher pH was included in the soil sample. The soil was partially air-dried at room temperature to allow sieving and then passed through a 2 mm sieve to remove coarse particles, plant residues, and stones. Earthworms and all signs of lime (white vague spots) were also removed. The dry matter (DM) of the soil was determined by weighing the soil before and after oven-drying at 105 °C until constant weight. To

determine the WHC, dried soil was weighed, saturated with water, allowed to drain, and weighed again. The DM content was found to be 88%, while the WHC was determined to be 51%. Additionally, three samples (250 g) were taken and sent to an accredited laboratory (Eurofins, Kristianstad) for further analysis of the soil characteristics, showing an electrical conductivity (EC) of 270 µS cm^{-1} and pH value of 6.7. The chemical properties of the soil are shown in Table 2.

Table 2. Chemical properties of the soil, urine pellets, BSFL frass, and commercial NPK pellets used in the current study.

	NO_3^--N	NH_4^+-N	Urea-N	Mineral N	Tot-N	P	K	S	Ca	Mg	Na	Zn	Mn	Cu
	mg kg^{-1}										µg kg^{-1}			
Soil	69.9	~ 0*	−	69.9		0.78	28.8	19.5	~ 0*	74.8	16.9	~ 0*	~ 0*	~ 0*
Fertilizers	%													
Urine pellets	−	2.45	11.1	2.45	13.55	1.25	8	−	2.4	0.3	−	−	−	−
NPK pellets	10.3	13.3	−	23.6	23.6	3.6	4.6	3.0	−	0.5	−	−	−	−
BSFL frass	~ 0*	0.68	−	0.68	3.8	1.10	1.31	0.71	6.69	0.48	0.86	0.057	0.051	0.0096

* Below detection limit

2.2 Fertilizers

The experiment involved three types of fertilizers: urine pellets, BSFL frass, and NPK pellets. The frass used in this experiment was obtained from a BSF colony that has been maintained since 2015 at the Swedish University of Agricultural Sciences (SLU) in Uppsala. The colony primarily serves to provide young larvae for scientific experiments, and its management procedures are based on the second edition of the publication "Black Soldier Fly Biowaste Processing: A Step-by-Step Guide" by the Swiss Federal Institute of Aquatic Science and Technology − Eawag (Dortmans et al. 2021), with adaptations. To supply young larvae for experiments, adult flies are kept in reproduction cages for egg collection. Most of the produced larvae are used for experiments, while a small portion is retained in the colony to complete their development until they reach adulthood, thus perpetuating the colony. After egg collection, the young larvae hatch within 3 days. These larvae are then fed for five days (when they reach a size that is possible to count them manually and utilize them in known proportions for waste treatment) with a commercial chicken feed diet (Granngården Hönsfoder Start) mixed with water, aiming to achieve an approximate moisture content of 70%. Subsequently, when they reach a desired size, the larvae are fed with the same

diet for another 12 days until they reach the last larval stage (6th instar). During this period, all feed substrate is consumed by the larvae and transformed into what is known as frass. At this stage, larvae and frass are separated by sieving (using a 5−10 mm mesh) and stored for further use (−20 °C). The chemical properties of the frass are shown in Table 2, together with the ones of the urine pellets and NPK pellets.

Since the production of urine fertilizer is under development, the companies involved were unable to share the specific details regarding the technology and pelletization process. However, the overview is that for the collection of urine, a stabilizer was added to the urinals' urine collection tank. This step limits the hydrolysis of the urea to NH_3. After this, the stabilized urine was condensed 25 times through drying with warm air. The condensed liquid was then mixed with a binding agent (organic waste product) and further dried until <5% moisture remained. The dried product was pelletized and sieved to achieve particles with a diameter ranging from 2 to 5 mm.

The NPK 24−4−5 pellets were sourced from Yara (YaraMila 24−4−5) and served as a positive control to provide a reference for expected outcomes. This fertilizer, in addition to N, P, and K, also contained sulfur (S) and magnesium (Mg). The N content consisted of approximately equal parts of NH_4^+ and NO_3^-. As a negative control, an unfertilized treatment was utilized.

2.3 Experimental design

The quantities of fertilizers (urine pellets, BSFL frass, and NPK pellets) applied in the incubation trial were intended to reflect the fate of N in the fertilizer at the doses applied in the field (excepting the presence of plants). Thus, an effort was made to estimate the amount of soil interacting with the fertilizers in the field and using the same relation between fertilizer and in the incubation. The doses used in the incubation trial were thus determined by considering a known plant row distance of 12.5 cm and an estimated width of the fertilizer band in the field (Figure 6), which was approximately 2 cm as estimated from photographs of the equipment. To calculate the proportion of field surface area interacting with the applied fertilizer, the width of the fertilizer band was divided by the row distance, resulting in a total of 16%. Translated to area per hectare (10 000 m^2), 1600 m^2 per hectare were thus assumed to be supplied with fertilizers in the field trial. Moreover, it was assumed that the fertilizers were uniformly mixed into the topsoil with a depth of 20 cm and a bulk density of 1.2 tons m^{-3}. Consequently, 384 tons of soil per hectare were assumed to interact with the fertilizers in the field. The doses utilized in the field trial (85 kg N ha^{-1} per treatment: 567 kg ha^{-1}

of urine pellets, 1000 kg ha^{-1} of frass, and 370 kg ha^{-1} of NPK) was recalculated to usage rates of 1.47 kg urine, 2.60 kg frass, and 0.96 kg NPK per ton soil, considering the soil interacting with the fertilizers. Finally, using the same proportions between soil and fertilizer to the incubation trial with soil portions of 40 g gave the following application rates: 0.059 g of urine pellets, 0.104 g of frass, and 0.039 g of NPK pellets. The doses in terms of total N, NO_3^-, and NH_4^+ in each sample are shown in Table 3.

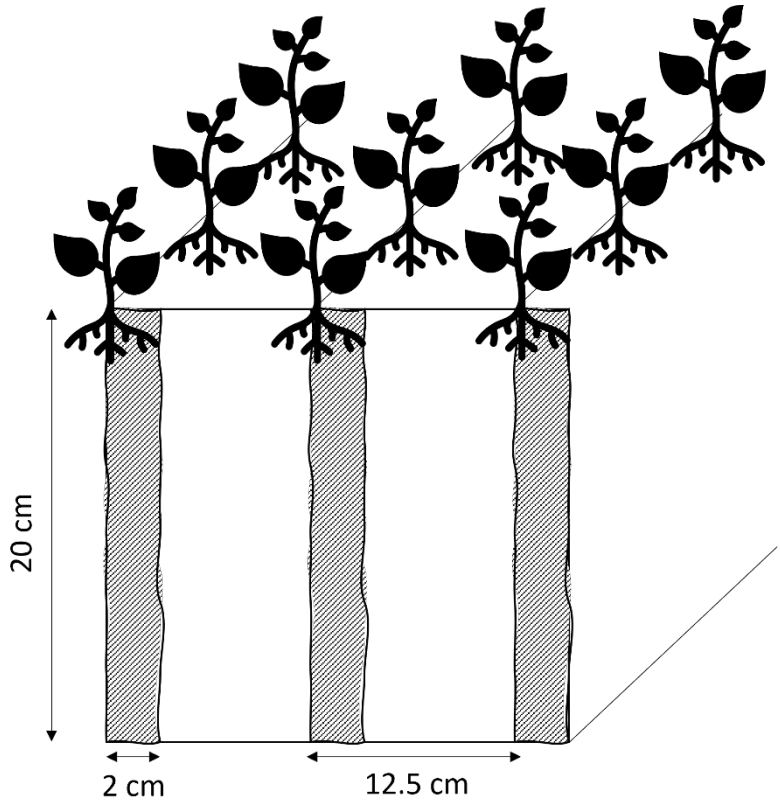

Figure 6. Schematic illustration of the field in cross-section, with a row distance of 12.5 cm, fertilizer band of 2 cm, and topsoil depth of 20 cm. The figure is not made to scale and the plant depicted is not barley.

Table 3. Concentrations of N pools in samples of 40 g soil from urine pellets, BSFL frass, and NPK pellets.

Treatment	Tot-N (mg g^{-1})	Urea (mg g^{-1})	NO_3^- (mg g^{-1})	NH_4^+ (mg g^{-1})
Urine pellets	8.0	6.5	–	1.4
BSFL frass	4.3	–	0*	0.7
NPK pellets	9.2	–	4.0	5.2

* Below detection limit <50 mg kg^{-1}

2.3.1 Soil respiration pilot test

A preliminary test was conducted to evaluate whether respiration could be measured. Two samples of urine pellets, BSFL frass, and NPK pellets, respectively, were mixed separately with 40 g (fresh weight) of soil in 250 mL plastic jars and placed at room temperature. Two samples of 40 g of unfertilized soil were also included. In each jar, 12 mL of deionized water was added as well to further wet the soil. One CO_2 trap consisting of 10 mL of 0.5M KOH solution pipetted into scintillation vials was placed in each jar to absorb the evolved CO_2. The conductivity and temperature in the traps were measured on days one, two, five, and six using a conductivity electrode (ProfiLine Cond 3310 Portable Conductivity Meter, WTW, Weilheim, Germany). The traps were replaced with identical, but unspent, traps when the previous set of traps were removed for measurements of the conductivity and temperature. The CO_2 content was calculated using the following formula:

$$N(CO_2) = A \times \frac{C(t_0) - C(t_1)}{C(t_0)} \qquad \textit{Eq. (4)}$$

where $C(t_0)$ is the conductance at the beginning of the incubation (time t_0) before any absorption of CO_2 by KOH, $C(t_1)$ is the conductance at time t_1, and A is an empirically determined constant expressing the theoretical maximum amount of CO_2 absorbed. A value of A=174.5 mg CO_2 was used. The results showed that respiration could not be determined due to carbonate content in the soil which inflated the CO_2 release from the soil and resulted in overestimated values of respired C. After this, the incubation therefore focused solely on N.

2.3.2 Incubation procedure

Portions of 40 g dw soil were weighed into 250 mL plastic jars and adjusted to 60% of the WHC and the lids closely sealed. The soil samples then underwent a pre-incubation period of two weeks at 22.3 ± 2.4 °C and two days in an incubation chamber at 14.7 ± 0.2 °C. The jars were opened for approximately 45 minutes on days three and six to allow gas exchange. Weekly weight checks were conducted to monitor potential weight loss, revealing no losses.

After the pre-incubation, urine pellets, BSFL frass, and NPK pellets were added to the pre-incubated soil samples and mixed gently using a spatula. A fourth treatment was also prepared, where no fertilizer was added but otherwise treated the same way as the other treatments. All treatments had four replicates. In total, 96 samples were prepared to allow destructive sampling of four replicates at each of the seven sampling times. The soil samples were then incubated at an air

temperature of 14.7 ± 0.2 °C. A temperature logger was placed by the jars to keep track of the temperature.

2.3.3 Mineral nitrogen analysis

After 1, 2, 3, 5, 7, and 9 weeks of incubation, four replicates of each treatment were removed from the incubation chamber for determination of the NO_3-N and NH_4^+-N concentrations. For the initial sampling (week zero), four additional samples of unfertilized soil were used. After removal from the incubation chamber, the samples (in their individual jars) were placed in a freezer for a minimum of 24 h. For the extraction, they were removed from the freezer and the extraction solution (100 mL 2M KCl) immediately added to each jar, as standard procedure in the laboratory (Lindén 2013). The jars were subsequently shaken on a horizontal shaker for 18 h. Afterward, 50 mL of the homogenized mixture from each jar was separately poured into falcon tubes and centrifuged at 2800 rpm and 20 °C for ten minutes. Finally, the mixture was filtered using a syringe filter (0.20 μm), after which 10 mL of the extract was stored in falcon tubes at –18 °C until analysis.

Ammonium-N and NO_3^--N were measured in the Thermo Fisher Scientific Gallery Discrete Analyzer, according to the manufacturer instructions. Thus, NH_4^+-N was measured by means of a colorimetric test based on Berthelot reaction, where phenol is substituted with salicylate (performed at 37 °C using a 660 nm filter). The principles of the procedure are that all NH_4^+-N is converted to NH_3, which subsequently reacts with hypochlorite ions produced through the alkaline hydrolysis of sodium dichloroisocyanurate. This reaction results in the formation of monochloramine, which then reacts with salicylate ions in the presence of sodium nitroprusside at approximately pH 12.6, leading to the formation of a blue compound. The absorbance of this compound is measured spectrophotometrically and is related to the NH_3 concentration by means of a calibration curve. Similarly, NO_3^--N was also measured by a colorimetric (hydrazine) method. The principle of the procedure for this ion is that NO_3^--N is reduced to NO_2^- by hydrazine under alkaline conditions. The total NO_2^- ions are then reacted with sulphanilamide and N−1−naphthylethylenediamine dihydrochloride under acidic conditions to form a pink azo-dye. The absorbance was measured at 540 nm and is related to the total oxidized N concentration by means of a calibration curve.

2.3.4 Calculations of nitrogen release

The recovery of N throughout the incubation period describes the percentage of N from each fertilizer that was retained as mineral N in the soil after the period. It

was calculated by subtracting the mineral N obtained in the control treatment from the mineral N (NH_4^+ + NO_3^-) accumulated in the fertilized soils, and then expressing that quantity as a proportion of the total N existing in the fertilizers, according to their characterization.

The net mineralization/nitrification per subperiod (days) aimed to describe the transformation of N between different mineral forms from one measurement to the next. It was calculated by subtracting the amount of N (NH_4^+, NO_3^-, and total mineral N, respectively) accumulated in one period from the amount accumulated in the subsequent period.

2.4 Statistical analysis

Prior to the statistical analysis, data (concentrations and accumulated NH_4^+, NO_3^- and total mineral N) were submitted to either the Ryan-Joiner or Kruskal Wallis tests to assess normality of errors or to the Levene's test for assessing the homoscedasticity of variances. The data that fitted normal distribution and homogeneity were evaluated by a one-way analysis of variance (ANOVA). In case significant differences among groups were observed, a multiple comparison of means test of Tukey was applied subsequently at a 5% significance level. In case the data did not present a normal distribution, the non-parametric test of Kruskal Wallis was performed followed by the Dunn's test. The statistical analyses of ANOVA and Tukey's test were conducted using Minitab Statistical Software, version 21.4.2 (Minitab LLC 2023). Kruskal Wallis test and Dunn's test were performed in R (R Core Team 2020).

3. Results

3.1 Concentrations of ammonium, nitrate, and total mineral nitrogen

The concentrations of NH_4^+-N were significantly higher in the soils fertilized with NPK pellets and urine compared to the BSFL frass treated soil and the unfertilized soil until day 21. After that, the concentrations of all treatments remained relatively consistent around 4 mg NH_4^+-N kg^{-1} (Figure 7). The NPK treatment initially exhibited a high level since much of the N was applied as NH_4^+. In contrast, the urine treatment started at a lower level compared to the NPK treatment and then increased from the start to 7 days.

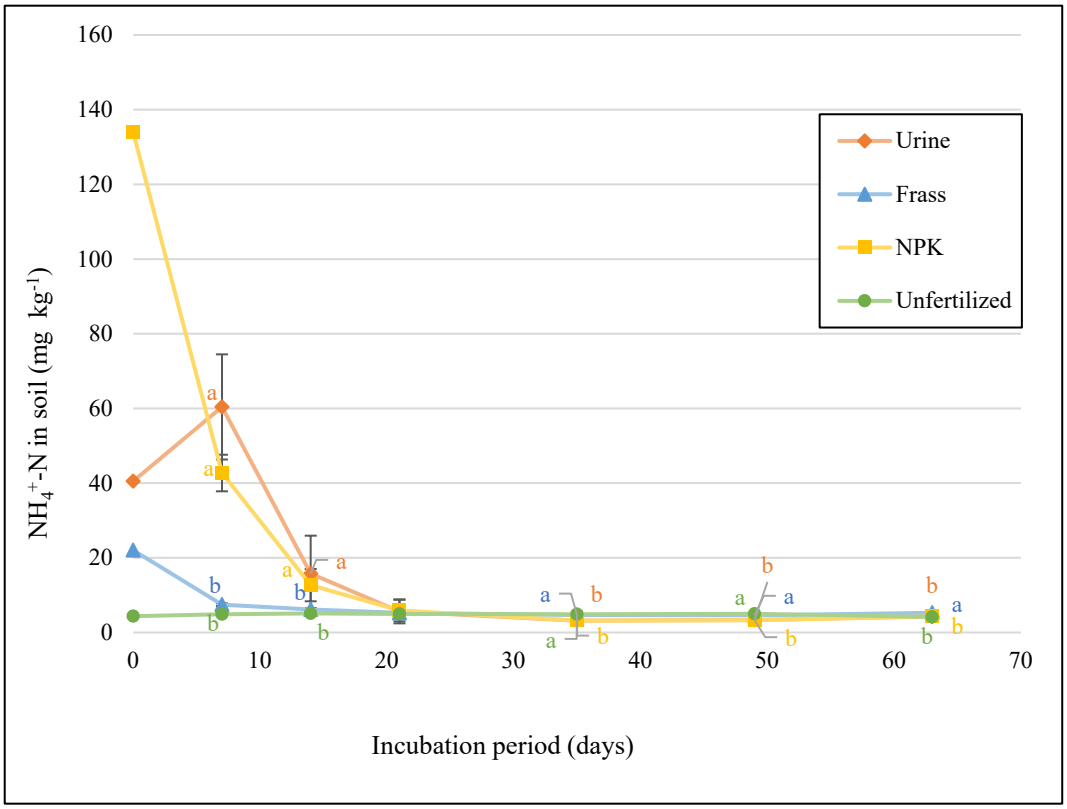

Figure 7. Concentrations of NH_4^+-N in soil fertilized with urine pellets and BSFL frass during 63 days of incubation. The data at t=0 are theoretical calculations based on the basic soil at t=0 and the application rates. Soil fertilized with NPK and unfertilized soil are controls. Values are the means of quadruplicates and error bars indicate standard deviations (SDs) of the means. Within the same day, mean values that share a letter are not significantly different at $p \geq 0.05$.

Application of urine and NPK pellets resulted in significantly higher concentrations of NO_3^--N during the entire incubation period compared to soil fertilized with BSFL frass and unfertilized soil (Figure 8). The concentrations increased for all treatments throughout the period, with the most significant change observed in the urine fertilized soil.

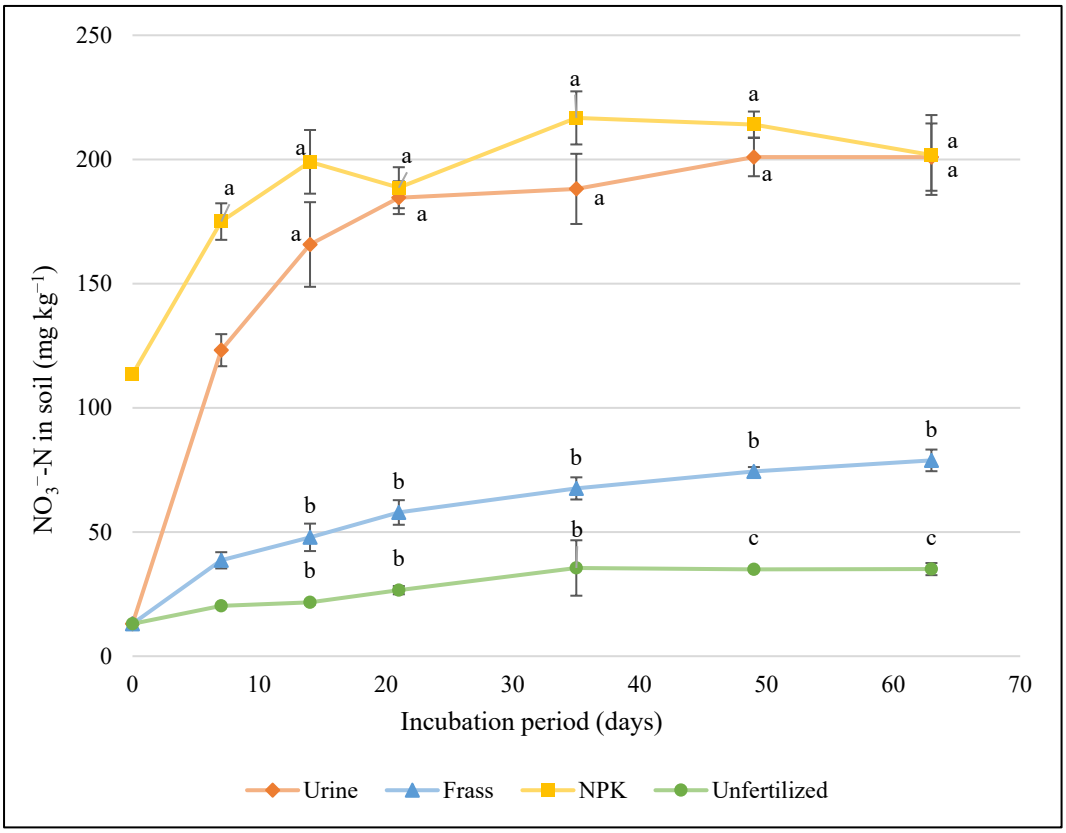

Figure 8. Concentrations of NO_3^--N in soil fertilized with urine pellets and BSFL frass during 63 days of incubation. The data at t=0 are theoretical calculations based on the basic soil at t=0 and the application rates. Soil fertilized with NPK and unfertilized soil are controls. Values are the means of quadruplicates and error bars indicate SDs of the means. Within the same day, mean values that share a letter are not significantly different at $p \geq 0.05$.

The concentrations of total mineral N were significantly higher throughout the incubation period in the urine and NPK fertilized soils compared to soil fertilized with BSFL frass and unfertilized soil (Figure 9). At the end of the experiment, the soil fertilized with NPK exhibited the highest concentration of accumulated total mineral N (206 mg N kg^{-1}) with 98% present in nitric form and 2% in ammoniacal form. Following closely behind NPK, the soil fertilized with urine pellets showed a statistically similar concentration of accumulated N (205 mg N kg^{-1}), with the same proportions between NO_3^- and NH_4^+ as the NPK fertilized soil. The soil fertilized with BSFL frass exhibited significantly lower accumulation of total mineral N at the end (84 mg N kg^{-1}) in comparison to NPK and urine, with 94% present as NO_3^- and the remaining as NH_4^+. Conversely, the unfertilized soil showed the significantly lowest concentration of accumulated N (39 mg N kg^{-1}), with 89% in nitric form and 11% in ammoniacal form.

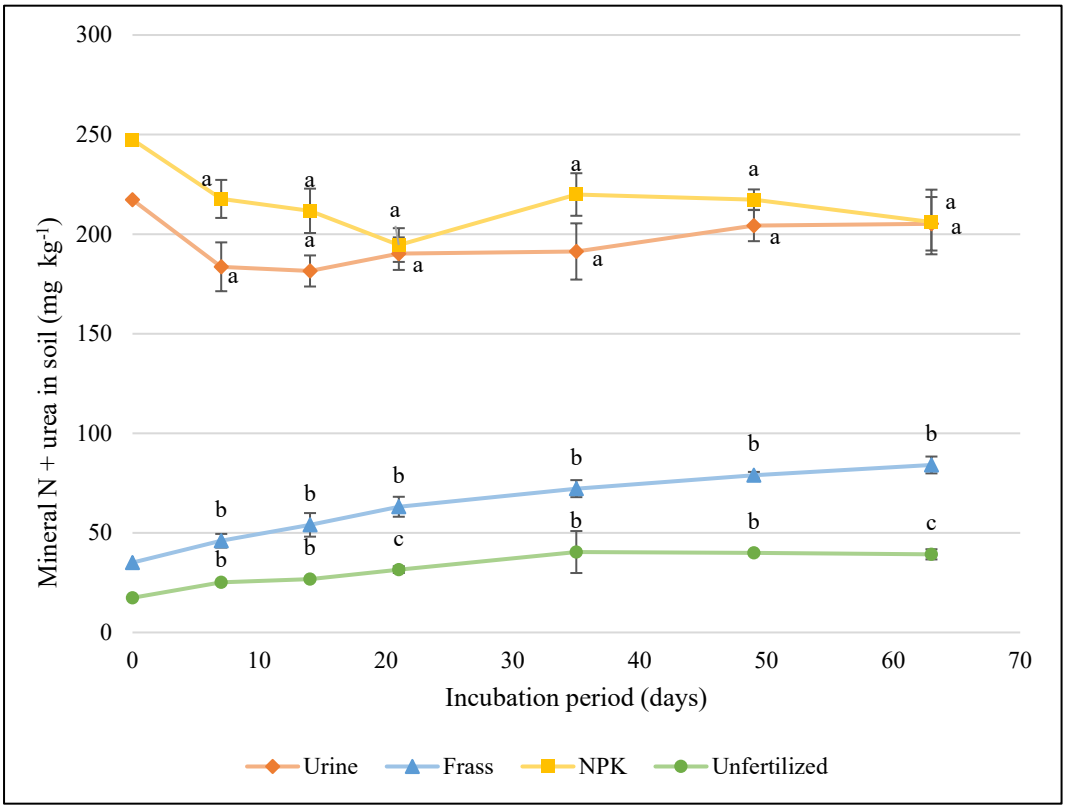

Figure 9. Concentrations of total mineral N and urea in soil fertilized with urine pellets and BSFL frass during 63 days of incubation. The data at t=0 are theoretical calculations based on the basic soil at t=0 and the application rates. Soil fertilized with NPK and unfertilized soil are controls. Values are the means of quadruplicates and error bars indicate SDs of the means. Within the same day, mean values that share a letter are not significantly different at $p \geq 0.05$.

The recovery of the applied N in the mineral N pool was calculated to be 83% for urine pellets, 73% for NPK pellets, and 45% for frass.

3.2 Net mineralization and nitrification per subperiod

The net changes in NH_4^+-N concentrations of all treatments were overall negative, with the most significant decrease occurring within the first 14 days of incubation for all treatments (Table 4). From day 14 onwards, the urine treated soil exhibited net changes statistically similar to those of the NPK fertilized soil.

Table 4. Net changes per subperiod (days) of ammonium, nitrate, and total mineral + urea nitrogen concentrations observed in unfertilized and soil fertilized with urine pellets, BSFL frass and a commercial NPK fertilizer. Values are presented as means (n = 4).

Parameter	Time (days)	Urine	Frass	NPK	Unfertilized	*p*-value
	0	40.51	22.05	134.05	4.37	
	7	19.89a	−14.60b	−91.34a	0.51b	**
NH_4^+-N	14	−44.65c	−1.28ab	−30.03bc	0.22a	***
(mg kg^{-1})	21	−10.15b	−0.92ab	−6.76b	−0.15a	*
	35	−2.44	−0.58	−2.73	−0.08	ns
	49	0.18	−0.07	0.10	0.16	ns
	63	0.92a	0.67a	1.04a	−0.86b	*
	0	13.04	13.04	113.47	13.04	
	7	110.17a	25.57b	61.53a	7.22b	**
NO_3^--N	14	42.55a	9.25bc	24.05ab	1.44c	**
(mg kg^{-1})	21	18.85a	10.01a	−10.42b	4.88a	*
	35	3.54	9.69	28.11	8.93	ns
	49	12.80	6.83	−2.67	−0.55	ns
	63	0.00	4.46	−12.26	0.10	ns
	0	217.26	35.08	247.50	17.40	
Total	7	−33.65a	10.97b	−29.80a	7.74b	**
mineral N	14	−2.09	7.98	−5.98	1.67	ns
+ urea	21	8.70a	9.09a	−17.18b	4.73a	**
(mg kg^{-1})	35	1.10	9.10	25.38	8.85	ns
	49	12.98	6.75	−2.57	−0.39	ns
	63	0.92	5.13	−11.22	−0.76	ns

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant at $p \geq 0.05$. Per parameter and within the same row, mean values that share a letter are not significantly different at $p \geq 0.05$. Statistical differences were assessed using ANOVA followed by Tukey's test for parametric data, or the Kruskal-Wallis test followed by Dunn's test for non-parametric data.

The application of fertilizers had a significant impact on the net nitrification during the first 21 days of incubation, as presented in Table 4. The most significant net nitrification among the treatments was observed during the first 7 days of incubation in the soil fertilized with urine, which did not receive NO_3^- as part of the N application. Similarly, in the BSFL frass treatment, where NO_3^- was not included in the N application as well, the largest net nitrification also occurred during the first 7 days of incubation.

The net mineralization, as revealed by the total mineral N, remained relatively steady in the frass treated soil throughout the incubation period (Table 4). In contrast, the net changes varied more in the urine and NPK fertilized soils. Specifically, application of frass resulted in net changes 1−5 times higher compared to urine fertilized soil during days 14−35 and day 63.

4. Discussion

This study aimed to provide data on N mineralization dynamics in soil treated with fertilizers derived from human urine and BSFL frass, under laboratory conditions, and to give insight to the results observed in a field trial. However, translating results from laboratory experiments to field conditions presents challenges. Laboratory incubation conditions optimize the mineralization process but overlook environmental fluctuations that significantly influence mineralization, such as precipitation, soil moisture content, temperature, and microbial abundance (Beesigamukama et al. 2021). For example, the WHC was adjusted to 60%, a level previously reported to maximize microbial activity (Linn & Doran 1984), and that varies significantly under field conditions, not being as stable as it was in this study. Neither was the complex interactions between crops and soil considered. Despite these limitations, integrating laboratory findings with field trial data can provide valuable insights into the efficacy of urine pellets and frass as N fertilizers.

4.1 Human urine pellets

The results showed that the urine fertilized soil exhibited significantly higher concentrations of mineral N ($NH_4^+ + NO_3^-$) compared to both the soil treated with BSFL frass and unfertilized soil. Furthermore, the mineral N from the urine pellets was released most rapidly in the first week of incubation, probably due to the high proportion of initial total N (14%), with the majority present in ureic form (11%). In comparison to the NPK fertilized soil, the urine pellets displayed similar concentrations of mineral N and a higher recovery of the applied N (83% and 73%, respectively), indicating efficient hydrolysis of urea and subsequent nitrification of NH_4^+. Urea has been previously reported to hydrolyze fast in soil (Tomar & Soper 1981; MacLean & McRae 1987). Baldi and Toselli (2014) observed that applications of urea resulted in a peak of NH_4^+ concentration within the first 7 days, consistent with the findings in the current study. Additionally, the results align with the observations in the field trial where the yield of urine fertilized barley was statistically similar to that of NPK fertilized barley (7.3 and 7.4 tons ha^{-1}, respectively).

However, the patterns of urea hydrolysis are soil dependent, for instance regarding soil moisture (Vlek & Carter 1983) and temperature (Sadeghi et al. 1988). Studies have also shown that urea hydrolysis increases with increasing soil organic-C, because the soil urease activity depends on the microbial biomass, which is proportional to SOM (McGarity & Meyers 1967; García et al. 1993). This indicates that urine pellets need to be evaluated in soils with different characteristics, to optimize the fertilizing strategies.

The results showed that the most significant net nitrification occured during the first 7 days of incubation in the soil fertilized with urine. Nitrate serves as the preferred source for most species (Li et al. 2012), yet it also constitutes the form in which a majority of N is lost (Stenberg et al. 1998), posing a risk of environmental losses if the application is not timed to align with plant uptake (Masunga et al. 2016). This is particularly relevant in this case given that N uptake is relatively low at this initial growth stage, with higher demands observed later in the season, particularly during stem elongation (Yara 2024). The Swedish Board of Agriculture (2023) recommends splitting N fertilizer applications to adjust fertilization throughout the growing season, ensuring the crop's N demands are met while minimizing losses and enhancing both quantity and quality of the crop. For example, the optimal protein level for malting barley falls within the range of 9.0−11.5% (Paynter 1996), while wheat destined for bread making typically requires a protein content exceeding 10% (preferably >12%) (Rosenqvist & Thylén 2002). Consequently, further research on the effects of split applications of urine pellets is warranted.

4.2 Black soldier fly larvae frass

The results revealed that the application of BSFL frass resulted in significantly lower concentrations of mineral N compared to the soils fertilized with urine pellets and NPK, with net mineralization remaining relatively steady throughout the incubation period. Despite this, the accumulated concentration at the end of the experiment was also significantly lower than in the urine and NPK treatments, though proportions of NH_4^+ and NO_3^- were similar to those in urine and NPK fertilized soils, suggesting aerobic conditions within the jars throughout the experiment. Moreover, the recovery of N for frass was 45%, contrasting with the 73% recovery observed for NPK. These findings suggest that the N availability of BSFL frass may not be optimally aligned with the N demands for the growth of annual crops in a cool temperate climate, depending on application rates. Nonetheless, it may serve as a slow-release N fertilizer when immediate plant N demands are not critical. For short-term N supply, very large application rates would likely be required to satisfy the needs of the crop (and those of the

microbial community). This was also verified in a lettuce production trial conducted under greenhouse conditions, where the authors applied frass to the soil according to the N demand of the plant and observed that the nutrient requirements of this short-cycle crop were not met over a 42-day period due to low mineralization (Esteves et al. 2022).

On the contrary, the results contrast with prior research, where strong net immobilization was observed following the application of BSFL frass, likely due to a higher soluble C content and C:N ratio of the frass used in those studies (Beesigamukama et al. 2021; Gebremikael et al. 2022). Moreover, Lopes et al. (2022) argued that it is plausible that the behavior of frass in the soil is highly variable due to the variability of frass compositions and their associated structural matrices. For instance, Masunga et al. (2016) reported that low rates of N mineralization are associated with composts containing a high proportion of stable compounds, with a high C:N ratio and N complexed in organic forms. If the soil respiration test had been conducted, the results could have provided insights into the stability of the frass. This is because moderately biodegradable compounds tend to generate lower rates of CO_2 production compared to when abundant amounts of easily degradable compounds are present, which results in an intense microbial activity (Hue & Liu 1995).

The results from the field trial showed a slightly lower yield when frass was used (7.0 tons ha^{-1}) compared to the yields of barley fertilized with urine pellets and NPK (7.3 and 7.4 tons ha^{-1}, respectively), yet significantly higher than the unfertilized barley (5.2 tons ha^{-1}). However, it is important to note that barley was cultivated for several months compared to the 63 days of incubation in the laboratory study, indicating that under field conditions, mineralization processes were likely close to releasing almost all of the N in frass, or at least more than the 45% recovered in this study. Furthermore, no definitive conclusions can be drawn solely from the field trial results regarding an exclusive use of BSFL frass as fertilizer, as frass was also supplemented with synthetic fertilizer (NS 27−4) in the field. Studies have reported that a combination of synthetic N fertilizer and organic fertilizer can enhance the net mineralization and nitrification rates of the organic-N (Kaleem Abbassi & Khaliq 2016), regardless of soil characteristics (Han et al. 2004). Moreover, as previously reported, frass has been associated with higher yields through improvements in soil health (Beesigamukama et al. 2021) and enhanced nutrient uptake efficiency (Poveda et al. 2019). However, these effects were not measured or evaluated in the current study.

Ultimately, it is essential to recall that applications of organic materials such as frass have several benefits other than only providing nutrients. For instance, they can improve soil quality and fertility by enhancing beneficial soil

microorganisms, reducing pathogen populations, increasing SOM and total C, and lowering bulk density, benefits that synthetic fertilizers do not offer (Bulluck et al. 2002).

5. Conclusion

In summary, this 63-day incubation trial demonstrated the potential of urine pellets as a viable alternative to commercial NPK fertilizer, exhibiting comparable mineral N release dynamics. Field trial results further supported this, showing significantly higher yields in urine fertilized barley compared to unfertilized soil, with yields similar to those of NPK fertilized barley. Additionally, BSFL frass displayed promising results as a slow-release N fertilizer, although further research is needed to fully understand its efficacy when used exclusively, under both laboratory and field conditions. Moreover, the outcomes of this study provide a foundation for developing tailored fertilization strategies across diverse crops and soil types, or at least the beginning steps in optimizing N fertilization for these novel fertilizers.

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Popular science summary

Fertilizers are essential in agriculture to achieve high-quality yields. While synthetic fertilizers, which are industrially produced, deliver nutrients predictably, they do not enhance soil quality like organic fertilizers, which originates from animals or vegetables. However, predicting nutrient release from organic fertilizers is more complex. This study focused on understanding the nitrogen release from two novel organic fertilizers derived from human urine and black soldier fly larvae (BSFL) frass. Nitrogen is vital for plant growth and required in substantial quantitites. The primary objective of implementing these fertilizers is to produce more sustainable options for agriculture.

Human urine has potential as a fertilizer due to its high nitrogen content. Recovering all human urine that is not recycled today could potentially reduce global inputs of synthetic nitrogen fertilizers in agriculture by 16−21%. However, using liquid urine poses challenges, such as collection, storage, and transportation. Moreover, urea (the primary nitrogen component in urine) tends to convert into the volatile gas ammonia, requiring techniques to stabilize nitrogen. Pelletizing dry urine offers a solution by making the product easier to handle and transport.

BSFL frass, a byproduct of insect larvae fed with organic waste such as food waste, results from a composting-like process yielding two primary outputs: larval biomass suitable for animal feed, and frass. Frass constitutes a mixture of uneaten feed materials, BSFL feces, and exoskeletons, containing several plant nutrients. Frass can improve soil health and crop growth. However, its quality depends on the larvae's diet and the composting duration.

This research aimed to study nitrogen release dynamics from urine pellets and BSFL frass in soil under laboratory conditions, and to give insight into the results observed in a previously performed field trial where the yields of barley fertilized with urine pellets and frass were investigated. This study aimed to reflect the field trial. Thus, soil samples were fertilized with either urine pellets or BSFL frass. Additionally, samples fertilized with commercial synthetic fertilizer and non-fertilized samples were included as references. The samples were incubated at 15 °C and periodically sampled for nitrogen extraction over 63 days.

The study revealed urine pellets to be a promising alternative to synthetic fertilizer, showing similar nitrogen release dynamics. Field trials confirmed higher barley yields with urine fertilizer than unfertilized soil, and similar yields as synthetic fertilized barley. Additionally, BSFL frass showed potential as a slow-release nitrogen fertilizer in both laboratory and field conditions. Further research is needed to optimize the utilization of urine pellets and BSFL frass among various crops and soils.

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