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Long-term soil health effects of human urine and other bio-based fertilizers: A comprehensive field study

Veronika Hansen [a,*], Franziska Häfner [b], Bianca Messmer [a], Luca Bragazza [c], Carsten Tilbæk Petersen [a], Jakob Magid [a]

[a] Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, Frederiksberg 1871, Denmark
[b] Agroscope, Soil Quality and Soil Use Group, Reckenholzstrasse 191, Zürich 8046, Switzerland
[c] Agroscope, Field-Crop Systems and Plant Nutrition Group, Route de Duillier 60, Nyon 1260, Switzerland

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ABSTRACT

Bio-based fertilizers (BBFs) are gaining attention as sustainable alternatives to mineral fertilizers due to their potential not only to enhance soil health and crop productivity, but also to close nutrient cycling. Derived from urban, agricultural or industrial origin, BBFs vary widely in composition, and their long-term impact on soil health and crop productivity remains insufficiently understood. To fill this gap, we evaluated the long-term effects (> 20 years) of various BBF applications, including compost, sewage sludge, cattle slurry and human urine on soil physical, chemical and biological properties, comparing them with conventional mineral NPK fertilizer in the CRUCIAL field experiment in Denmark. Our results showed that while mineral NPK fertilizers support crop productivity and improve certain soil properties, they do not deliver the broader soil health benefits associated with organic amendments. Indeed, compost and sewage sludge notably increased soil organic carbon (SOC), cation exchange capacity, soil porosity, water content at field capacity and microbial activity, while reducing bulk density and clay dispersibility. Human urine exhibited a comparable nitrogen fertilizer effect in terms of crop yields to the NPK treatment, highlighting its potential for urban nutrient recycling. Although values for human urine treatment did not exceed any critical thresholds, a higher sodium adsorption ratio than mineral NPK treatment and a negative trend for bulk density were observed, indicating a need for complementary organic matter input. Multifunctional soil health assessments confirmed superior performance of organic amendments (compost and sewage sludge) across chemical, physical, and biological indicators, primarily driven by sustained organic matter inputs. These findings underscore the positive effect of long-term application of BBFs - especially compost and sewage sludge – not only on SOC increment but also on soil physical, chemical and biological properties that are essential for sustainable crop production.

1. Introduction

Soil health is a critical component of global food security and other essential ecosystem services such as water supply, biodiversity conservation, erosion control and climate regulation, but soil is also threatened by degradation processes such as organic matter loss, nutrient imbalance, erosion and compaction (Bünemann et al., 2018; Davis et al., 2023). Soil health refers to the capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans (Lehmann et al., 2020). Due to its multifunctionality, soil health assessment of agro-ecosystems is complex and integrates physical, chemical, and biological properties, each of which plays a role in supporting key soil functions such as soil structure maintenance, water and nutrient cycling, carbon (C) sequestration, and biological productivity. Intensive agriculture focusing on high-yielding crops under high inputs of non-renewable or energy-demanding fertilizers is often associated with soil degradation and biodiversity loss (Wittwer et al., 2021). Indeed, the production of mineral nitrogen (N) fertilizers relies on the energy-intensive Haber-Bosch process, which accounts for 1–2 % of the global energy demand (Hargreaves, 2014), emphasizing the importance of circular resource use in fertilization. Identifying sustainable soil management practices without compromising yields is then decisive to both combat soil degradation and strengthen a circular economy.

In recent decades, increasing attention has been given to the use of

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* Corresponding author.
E-mail address: veha@plen.ku.dk (V. Hansen).

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bio-based fertilizers as a sustainable alternative to mineral fertilizers. Bio-based fertilizers (BBFs) have the potential to enhance soil organic carbon (SOC), stimulate microbial activity, and improve soil structure and nutrient availability, ultimately contributing to the improvement of soil health (Obriot et al., 2016). Bio-based fertilizers derive from various waste streams of urban, agricultural or industrial origin, and include compost, animal manures, sewage sludge, and more recently also human urine. Therefore, the effect of different bio-based fertilizers on soil health can vary widely depending on the type, quality, and quantity of applied BBF. The effect on the soil can be direct, according to the intrinsic properties of the products, or indirect by modifying soil physical, biological and chemical properties (Larney and Angers, 2012; Steffens and Bünemann, 2025). In a recent meta-analysis, Steffens and Bünemann (2025) found that solid and C-rich BBFs with wide C:N ratio were more effective than liquid BBFs in increasing SOC concentration. Similarly, in a long-term field study, Peltre et al. (2015) observed that repeated applications of organic amendments over 11 years increased SOC and soil porosity, and decreased bulk density compared to the mineral fertilization. These changes also resulted in decreased draught force potentially leading to reduction of fuel consumption during soil tillage. The strongest effects were observed with compost treatments, whereas human urine tended to increase specific draught force. In addition, other field trials also suggested that organic amendments improve soil biodiversity, biological activity, and physical properties compared to mineral fertilizers (Obriot et al., 2016). The most biodegradable organic amendments appeared to be most efficient in enhancing soil biological properties, while the mineral fertilizers resulted in highest crop productivity (Obriot et al., 2016).

Despite growing interest in circular nutrient management, the use of human urine is still rather underexplored even if it could potentially be a valuable nutrient source due to its high N content and availability. However, little is known about its effects on key soil health indicators. The recycling of human urine as fertilizer and its use as an untapped urban nutrient resource has re-gained attention since the 90 s (Larsen and Gujer, 1996; Drangert, 1998). The N fertilizer value of human urine has already been demonstrated (Heinonen-Tanski and Van Wijk-Sijbesma, 2005), but it may vary depending on the treatment or processing technique (Martin et al., 2021). Human urine also contains sodium chloride (NaCl), which has been identified as a potential risk for plant growth if salts accumulate in the soil after repeated applications (Krause et al., 2021). Therefore, long-term studies are necessary to evaluate the extent of Na accumulation and its effect on the cation balance and structural stability of soil.

Both sewage sludges and organic household waste compost can contain a profile of contaminants that is different from animal fertilizers, e.g. with respect to microplastics (Johansen et al., 2024), medicinal residues, xenobiotics and heavy metals (Magid et al., 2020; Vuaille et al., 2022). However, there is a notable knowledge gap regarding the long-term effect of field applications of sewage sludge, making it unclear if and how such contaminant profiles accumulate or influence soil functionality over time. It would be expected that biological measures of soil health are particularly sensitive to negative impacts of contaminant profiles (Kurniawati et al., 2023).

To understand how repeated applications of bio-based fertilizers affect soil health and crop productivity over time, long-term field trials with appropriate baseline or reference value are essential (Bünemann et al., 2018). For this goal, the long-term CRUCIAL (C̲losing the R̲ural-U̲rban Nutrient C̲ycle - Investigations through A̲gronomic L̲ong-term experiments) experiment was conceptualized and designed to assess the long-term risks of recycling urban residues and it was therefore chosen for this study.

The overall aim of the present study was to assess the effect of long-term (> 20 years) application of several bio-based fertilizers on soil physical, chemical and biological parameters. To our current knowledge, no long-term field data are available for human urine fertilization and its impact on soil health and crop production. The objective was to compare the application of organic fertilizers (compost, sewage sludge and cattle slurry) and human urine to mineral fertilizer (NPK). We hypothesized that: 1) bio-based fertilizers enhance SOC in proportion to their C inputs, and thereby improving soil physical, chemical and biological parameters relative to mineral fertilizer; 2) all the studied BBFs result in lower crop yield per unit N relative to mineral fertilizer; and 3) application of human urine, due to its high NaCl concentration and lack of C inputs, negatively affects soil structural properties compared with organic and mineral fertilizers. In addition, we integrated previous experimental findings on soil indicators from the CRUCIAL trial to provide a broader perspective on the effects of BBFs on soil quality and resilience, particularly in the context of biological functioning and contaminant exposure.

2. Materials and methods

2.1. Field site and experimental design

This study used data and soil samples from the CRUCIAL field trial that is located 20 km west of Copenhagen (Denmark) on the experimental farm of the University of Copenhagen. According to the FAO classification, the soil can be described as a Luvisol with a sandy loam texture (Table 1). The CRUCIAL experiment was established in 2003 and designed in randomized blocks with 11 fertilizer treatments arranged in triplicates across 33 plots. Fertilizer rates were based on mineral fertilizer equivalents (MFE) and were adjusted to comply with Danish regulations for maximum fertilizer rates (Peltre et al., 2015). The effective N application rates were intended to be approximately 110 kg N ha^{-1} year^{-1}. Total N utilization efficiencies based on legal requirements were: 70 % for cattle slurry and human urine, 45 % for sewage sludge, and 40 % for compost. The fertilizers were applied every year and incorporated by ploughing. For this study, 6 treatments with 3 replicates were chosen: unfertilized (U), human urine (HU), sewage sludge (S), composted household waste (CH), inorganic fertilizer (NPK) and cattle slurry (CS) (Lekfeldt et al., 2017). Human urine was sourced from a settlement in Albertslund (Hyldespjældet) and from a community in Trekroner (Munksøgård). The compost was produced by a private company (BioVækst, Solum) using the AIKAN technology 1 and receiving separated organic wastes from households (Joernsgaard et al., 2013). The household waste was combined with garden and park wastes that were packed into closed containers for 20 days, where soluble carbon was extracted for biogas production. Subsequently, the material was aerated and composted in a closed space for one month. Finally, the material was composted in the open air over approximately 3 months until maturity.

The sewage sludge was obtained from a public treatment plant (BIOFOS, Avedøre) receiving mixed wastewaters from industries (not hazardous) and private households from Copenhagen. The mean residence time in the BIOFOS sewage treatment plant was ca. 20 days, and subsequently, the sludge was flocculated with ferric salts (currently $FeIIICl_3$) and then degassed for methane production for approximately 24 days.

2.2. Soil sampling

Undisturbed soil samples were collected for physical analysis (bulk density, porosity as well as water content at both field capacity and wilting point) in November 2022. In each plot, 12 undisturbed soil cores (100 cm^3) were extracted randomly at 10 cm soil depth and kept at 4°C prior to the analysis.

Minimally disturbed soil samples were also gently collected for aggregate size distribution. In each plot, 3 soil samples were collected into stiff plastic containers after removing the top 10 cm of soil layer.

For chemical analysis and soil texture, 3 samples were collected randomly and bulked to one composite sample per plot in November 2022.

Table 1
Soil texture analysis in the different long-term field treatments. Values (%) of organic matter, clay, silt and sand are presented as means with standard error in parentheses ($n = 3$). Different letters indicate significant differences among the treatments ($p < 0.05$).

	Organic matter %		Clay (< 0.002 mm)		Silt (0.002–0.02 mm)		Sand (0.02–2 mm)	
Unfertilized	1.76 (0.09)	a	16.1 (0.67)	a	14.8 (0.66)	a	67.3 (1.39)	a
NPK	2.30 (0.05)	ab	15.8 (0.30)	a	14.6 (0.59)	a	67.3 (0.87)	a
Cattle slurry	2.46 (0.10)	b	17.4 (0.95)	a	15.5 (0.38)	a	64.7 (1.01)	a
Sewage sludge	2.77 (0.11)	b	17.0 (1.39)	a	15.4 (0.60)	a	64.9 (2.03)	a
Human urine	2.26 (0.14)	ab	16.5 (0.99)	a	15.4 (0.20)	a	65.9 (1.12)	a
Compost	4.41 (0.24)	c	17.0 (1.30)	a	14.5 (1.10)	a	64.2 (2.32)	a
Treat effect	<0.0001		0.4063		0.5985		0.2067	
Block effect	0.9404		0.0006		0.1821		0.0171	

2.3. Soil physical properties

The volumetric water content at field capacity (FC) of undisturbed soil cores was measured using a sand table. The soil cores were placed on the sand table, saturated with water from below and equilibrated at −100 cm water pressure potential (pF 2.0). The samples were subsequently dried at 105°C to determine water content and bulk density. The volumetric water content at wilting point was measured on a saturated kaolinite plate in a pressure container. A pressure of 15 000 hPa was applied for 3 weeks to obtain equilibrium at pF 4.2. Available water capacity (AWC) was calculated as the difference between water contents at PF 2.0 and PF 4.2. The remaining soil samples were washed on a 2 mm sieve to isolate stones for correction of the water content and bulk density. The gravimetric water content was calculated by dividing the mass of water in each sample by the mass of the dry soil.

Soil porosity was estimated from bulk density and a particle density of 2.65 g cm^{-3}.

Aggregate size distribution was determined using a nest of sieves (0.5, 1, 2, 4, 8, and 16 mm). The results are presented as geometric mean diameter (GMD in mm). Soil texture was analyzed by Agrolab using standardized methods DIN EN 15936: 2012–11 (Dumas) and DIN ISO 11277: 2002–08.

2.4. Soil chemical properties

Soil samples were air-dried and sieved to 2 mm for laboratory analyses. All nutrient analyses were performed according to Swiss reference methods of the Federal Agricultural Research Stations for Analysis of Soils (Agroscope, 1996). Soil pH was measured by mixing 15 g of dry soil with 50 mL of 0.01 M $CaCl_2$ solution. Total carbon (C) and nitrogen (N) were determined using the Dumas method with an elemental analyzer (Thermo Flash 2000, NF ISO 13878). Soil organic carbon (SOC) was calculated by subtracting carbonate-C, which was measured via the gas-volumetric Scheibler method, (ISO 10693:1995) from total C. Total phosphorus (P) was measured with ICP-OES following an aqua regia digestion.

Nutrient availability for P, potassium (K), calcium (Ca), and magnesium (Mg) was assessed using various extraction methods. The directly plant-available fractions of these nutrients were extracted in H_2O (1:10) for P, K, and Mg, with additional extraction for K and P (K-CO_2 and P-CO_2) using CO_2-saturated water (0.08 M CO_2, 1:2.5). Magnesium availability (Mg-$CaCl_2$) was also assessed in a 0.00125 M $CaCl_2$ (1:10) solution. Potassium and Mg concentrations were measured using atomic absorption spectroscopy (F-AAS AA 240 FS, Varian), and P was determined photometrically (Photometer Evolution 220, Thermo Scientific). The reserve nutrient fractions for P, K, Ca, and Mg, along with trace elements copper (Cu), zinc (Zn), and manganese (Mn), were determined by 1:10 extraction with ammonium acetate (0.5 M) and EDTA (0.02 M) and measured via ICP-OES. Exchangeable P was measured using the Olsen method and extraction with 0.5 M sodium bicarbonate (Olsen, 1954).

The potential cation exchange capacity (CEC) and base saturation was measured by extraction with 0.1 M $BaCl_2$ following Swiss standard methods (Agroscope, 2020) where the base cations are determined via atomic absorption spectrophotometer. Sodium adsorption ratio (SAR) was calculated based on exchangeable cation measurements using the formula:

$$SAR = \frac{[Na]}{\sqrt{\frac{[Ca]+[Mg]}{2}}}$$

2.5. Soil biological properties

Multiple substrate-induced Respiration (MSIR) was analyzed using the MicroResp™ method (Campbell et al., 2003). Soil samples were sieved (<2 mm) and filled into 96-deep-well plates (Abgene Storage, Thermo Fisher Scientific, MA, USA) with approximately 420 mg soil per well. The plates were then sealed and incubated for 7 days at room temperature in the dark. Agar plates with cresol red indicator (31.5 mg L^{-1}) were prepared for colorimetric quantification of CO_2 released from the deep well plates. A standard curve was obtained by placing indicator well strips in bottles with known CO_2 concentrations from 0.039 % to 5 % and measuring absorbance at 590 nm using a plate reader (EON, BioTek, Winooski, USA) after 4 h of incubation at 25 °C. The optimal soil water content was determined according to Wakelin et al. (2013). Based on that, 57 µL of Milli-Q water were added to the soil in each well to establish the soil moisture where the highest respiration rate was found on average. A total of 15C-sources were included in the analysis and added to provide 1.54 mg C $well^{-1}$ (corresponding to 12 mg of glucose per g water in well). The substrate-induced respiration rate (µg CO_2-C g^{-1} h^{-1}) was calculated from the absorbance of the indicator plates before and after 4 h of incubation at 25 °C also considering basal respiration from samples with only H_2O. A heatmap was generated to visualize the patterns of multiple substrate-induced respiration. The heatmap was created in Microsoft Excel using conditional formatting with color scale applied to numerical data. In the color scheme, red represented lower values and green represented higher values.

The activities of four soil enzymes related to C-cycling, i.e. β-glucosidase (BG) and cellobiohydrolase (CH), to C and N-cycling, i.e. β -N-acetylglucosaminidase (NAG), and to P-cycling, i.e. acid phosphatase (AP), were measured using synthetic fluorogenic substrates according to a modified procedure by (Marx et al., 2001; German et al., 2011). Specifically, the fluorogenic 4-methylumbelliferone (MUF)-based substrate was used to determine the activities of the four enzymes (Sigma-Aldrich, St. Louis, MO). The fluorescence was measured by a microplate reader (BioTek, Instruments, US) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm after incubation at room temperature. For calibration and quenching effects, a set of standards were prepared with 200 µL of soil slurry solution (for each individual sample) with a range of increasing concentrations of MUF standards. Enzyme activities were calculated from the regression slopes of the standard measurements along with the fluorescence average values of the triplicates for each sample and they were reported as µmol substrate (MUF) g^{-1} dry soil h^{-1}.

2.6. Calculation of SOC stock, and the relative SOC production from input materials

The SOC stocks were estimated based on an equivalent soil mass of 360 kg m^{-2} in the plow layer. This reference value was based on the NPK treatment that had an unchanging C concentration and bulk density based on measurements from this study and a previous one (Peltre et al., 2015). The plow layer depth of the NPK treatment was determined to 24 cm, whereas in some other treatments it increased due to swelling resulting from a decrease in bulk density. It is assumed that the soil mass of the plow layer is comparable across all treatments.

The Equivalent Soil Mass (ESM) of the NPK plow layer was calculated as follows:

$$ESM = BD\left(1.5\quad t\quad m^{-3}\right) \times PLD(0.24\quad m) = 0.36\quad t\quad m^{-2} \tag{1}$$

where BD is the bulk density of the NPK treatment and PLD the depth of the plow layer in that treatment.

The SOC stock was calculated as follows:

$$SOC\ stock\left(t\quad ha^{-1}\right) = ESM \times 10.000\quad \times SOC/100 \tag{2}$$

Where ESM (t m^{-2}) is the equivalent soil mass of the NPK plow layer (see [1]) and SOC (%) is the soil organic carbon concentration in the respective treatments.

The relative SOC production from input materials was calculated as follows:

$$SOC_{productivity\ factor} \quad = \quad \frac{SOC\ stock - SOC\ stock\ outset}{TreatmentCinput} \tag{3}$$

Where *SOC stock* is the SOC stock of the respective treatment, *SOC stock outset* is the SOC stock at the beginning of the experiment and *Treatment C input* is the total C input to the treatment over the experimental period.

2.7. Crop yield

The field has been managed in continuous crop rotation dominated by spring crops (barley, oilseed rape, wheat, oats), with only three years of winter crops (oilseed rape, wheat, barley) since 2003. Spring crops were established in April and winter crops in September. Grain yield was estimated by harvesting an area of 33 × 1.5 m in each plot using an experimental combine harvester. Samples were oven-dried to determine dry matter content. Spring barley was cultivated in the field in 2003, 2004, 2017, 2019 and 2022, and was therefore selected to evaluate the long-term effect of bio-based fertilizers on crop yield. The average yield for years 2003 and 2004 was used to represent the initial phase of cultivation, while the average of 2017, 2019 and 2022 reflected the effect after 13–20 years of continuous application.

2.8. Statistical analysis

Statistical analysis of the data was performed in R, version 3.1.1. (R Core Team, 2017). The data for soil and yield analysis were analyzed using a linear mixed effects model in the *lme4* package, with fertilizer treatments as fixed effect and field block as a random effect. To assess changes in yield at two time points (mean of 2003–2004 as "start" and mean of 2017–2022 as "end"), time and treatment × time interaction were included as additional fixed effects, and block was treated as a random effect to account for the repeated measurements within each block. By using this model structure, the overall temporal changes in yield, treatment effects across both time points, and within-treatment changes between start and end can be evaluated. The repeated-measures analyses for yield were conducted using linear mixed-effects models in the *nlme* package. Prior to model fitting, data were tested for homogeneity of variance using the Levene`s test and for normality of residuals using the Shapiro-Wilk test and transformed where necessary. The statistical differences among the treatments were analyzed using estimated marginal means (emmeans) with the R package *lsmeans* (Lenth et al., 2018). P values were adjusted according to the Tukey method. Differences at $p < 0.05$ were reported as significant.

For the multifunctional analyses, the indicators were scored and normalized based on division of each value by the highest value (Obriot et al., 2016) to make a simplified comparison across treatments and enable a direct quantitative comparison of percentage increases or decreases. For all indicators that were scored, the "more is better" assumption was applied, except for bulk density using the "less is better" approach. Indicators that could be used as proxy for a specific soil function were then averaged by the mean. For soil structure, the physical indicators GMD, clay dispersibility, bulk density, porosity and available water capacity were normalized and aggregated. Biological activity included MSIR and the four enzyme activities, while for the nutrient pool, SOC, Olsen-P, total N, available K (K-CO_2) and base saturation were aggregated.

A Pearson׳s correlation coefficients were calculated to assess linear relationship among the measured soil health parameters. Correlations were computed using pairwise complete observations, and statistical significance was tested for each pair of variables. Significance thresholds were set at $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$ (ns). Results were visualized as a heatmap, with color indicating correlation strength and direction and symbols denoting significance level.

3. Results

3.1. Soil physical properties

Addition of compost resulted in significantly lower bulk density and higher gravimetric water content and porosity than all other treatments, as well as lower clay dispersibility than the unfertilized treatment (Table 2). Sewage sludge and cattle slurry treatments significantly decreased bulk density, increased gravimetric water content and porosity compared to the unfertilized treatment, whereas sewage sludge also improved gravimetric water content compared to the NPK treatment (Table 2). Application of human urine led to results similar to the unfertilized and the NPK treatment apart from significant increase in gravimetric water content compared to the unfertilized treatment (Table 2). The unfertilized treatment significantly increased bulk density and decreased gravimetric water content and porosity compared with NPK treatment.

Compost and sewage sludge applications led to the highest increase in the water content at FC, whereas NPK, cattle slurry, human urine and the unfertilized treatment reached similar values (Table 3). Similarly, the available water capacity was highest in compost and sewage sludge treatments, however only the compost treatment was significantly higher than the unfertilized treatment. The addition of human urine led to results similar to the NPK treatment.

3.2. Soil chemical properties

Since the outset of the experiment (2003), SOC stocks had changed considerably across the treatments (Table 4). In the unfertilized treatment, the SOC stock decreased dramatically, based on equivalent soil mass, from 54 to approximately 42 t ha^{-1} in the plow layer. The human urine and NPK treatments decreased slightly, and this was also the case for the cattle slurry, despite having received a C input of an estimated 25.5 t ha^{-1} over the course of the long-term experiment. This entails that a negative relative SOC productivity must be assigned to the cattle slurry treatment. In the sewage sludge and the compost treatment, SOC stocks increased (Table 4). Relative to the C input over the course of the experiment, the organic treatments resulted in a relative SOC productivity where compost > sewage sludge > cattle slurry.

Compost addition increased most chemical soil parameters compared with the other bio-based fertilizers, particularly the contents

Table 2
Bulk density (n = 36), gravimetric water content (n = 36), porosity (n = 36), geometric mean diameter (GMD) (n = 6) and clay dispersibility (n = 6). Values are presented as means with standard error in parentheses. Different letters indicate significant differences among the treatments ($p < 0.05$).

	Bulk density		Water content		Porosity		GMD		Clay dispersibility	
Treatment	g cm^{-3}		%		%		mm		g g^{-1}	
Unfertilized	1.60 (0.02)	d	18.1 (0.18)	a	39.7 (0.63)	a	9.52 (1.56)	a	0.50 (0.08)	b
NPK	1.51 (0.01)	bc	19.5 (0.12)	b	42.9 (0.54)	bc	6.52 (1.08)	a	0.35 (0.02)	ab
Cattle slurry	1.51 (0.02)	bc	19.8 (0.17)	b	42.9 (0.61)	bc	7.34 (1.47)	a	0.39 (0.10)	ab
Sewage sludge	1.48 (0.01)	b	21.0 (0.18)	c	44.1 (0.55)	c	6.01 (2.27)	a	0.34 (0.09)	ab
Human urine	1.55 (0.02)	cd	19.3 (0.18)	b	41.6 (0.57)	ab	7.57 (1.46)	a	0.37 (0.03)	ab
Compost	1.35 (0.01)	a	23.3 (0.14)	d	49.2 (0.52)	d	4.12 (0.44)	a	0.25 (0.04)	a
Treat effect	< 0.0001		< 0.0001		< 0.0001		0.1263		0.0177	
Block effect	0.3233		0.0004		0.3233		0.3237		0.0112	

Table 3
Volumetric water content at field capacity (FC) and wilting point (WP) and available water capacity (AWC). Values are presented as means with standard error in parentheses (n = 36). Different letters indicate significant differences among the treatments ($p < 0.05$).

	Water content at FC		Water content at WP		AWC	
Treatment	%		%		%	
Unfertilized	28.9 (0.35)	a	15.1 (0.61)	a	13.7 (0.56)	a
NPK	29.3 (0.29)	a	14.8 (0.60)	a	14.5 (0.57)	ab
Cattle slurry	29.9 (0.40)	ab	15.3 (0.65)	a	14.6 (0.63)	ab
Sewage sludge	30.9 (0.51)	bc	14.9 (0.63)	a	16.0 (0.72)	ab
Human urine	29.8 (0.42)	ab	15.9 (0.65)	a	13.9 (0.61)	ab
Compost	31.5 (0.30)	c	15.0 (0.53)	a	16.4 (0.56)	b
Treatment effect	< 0.0001		0.8199		< 0.0001	
Block effect	< 0.0001		0.0012		0.5845	

Table 4
Soil organic carbon (SOC) stocks and estimated SOC productivity of input C based on total cumulative C inputs, and relative input C productivity (SOC productivity factor) during the experimental period from 2003 to 2022.

	SOC %	SOC stock[1] t ha^{-1}	C input (t)	SOC productivity factor
Outset value 2003	1.50	54.0		
Unfertilized	1.17	42.1	0	
NPK	1.45	52.2	0	
Cattle slurry	1.42	51.1	25.5	-0.1
Sewage sludge	1.67	60.1	21.9	0.3
Human urine	1.41	50.8	0	
Compost	2.62	94.3	83.0	0.5

[1] Equivalent soil-mass based calculations of SOC stocks

of SOC, total N and K-CO_2 (Table 5) as well as soil pH, CEC, basic cations and base saturation (Table 6). The sewage sludge treatment was characterized by significantly higher Olsen-P levels (Table 5). The effect of human urine addition on soil chemistry did not differ significantly from that of the NPK fertilization (Table 5). Phosphorus extractions showed significantly higher levels of directly plant-available P (P-CO_2 and P-H_2O) in sewage sludge compared to compost and the other treatments (Supplementary Table S3). In contrast, P-AAE, representing the medium to long term-available P, was comparable between sewage sludge and compost, and higher than in the remaining treatments. The different K extractions followed a similar trend, showing the highest values in compost, followed by cattle slurry, with significantly lower K availability in the other treatments. Directly plant-available Mg (Mg-$CaCl_2$) was also highest in compost, sewage sludge, and cattle slurry (Supplementary Table S3).

Urine application led to a significantly higher sodium absorption ratio (SAR) compared with NPK and the unfertilized treatment, and to a lower base saturation than compost (Table 6). However, the SAR for urine was not significantly different from that observed in the sewage sludge and cattle slurry treatments.

After scoring and aggregating the chemical indicators (SOC, base saturation, total N, Olsen-P, and available K), nutrient pool scores were highest for compost and decreased in the following order: compost > sewage sludge, cattle slurry ≥ NPK, human urine and unfertilized treatment (Supplementary Table S1).

Bulk density was negatively correlated with SOC ($r = -0.88$, $p < 0.001$) and positively correlated with clay dispersibility ($r = 0.69$, $p < 0.01$) and geometric mean diameter ($r = 0.72$, $p < 0.001$) (Fig. 1). Water content at FC and the available water capacity were positively correlated with SOC ($r = 0.49$, $p < 0.05$ and $r = 0.63$, $p < 0.01$, respectively). Soil pH and CEC also showed positive correlations with SOC ($r = 0.66$, $p < 0.01$ and $r = 0.85$, $p < 0.001$, respectively) (Fig. 1). CEC was positively correlated with water content at FC ($r = 0.61$, $p < 0.01$) and available water capacity ($r = 0.67$, $p < 0.01$).

3.3. Soil biological properties

Basal respiration ranged between 0.46 and 0.50 µg CO_2-C g soil^{-1} hour^{-1} and did not differ among the treatments (Table 7). The multiple substrate-induced respiration (MSIR) rates were between 7.75 and 14.93 µg CO_2-C g soil^{-1} hour^{-1} and were significantly affected by the soil treatments. Soil amended with compost and sewage sludge significantly increased the MSIR rates compared to the NPK treatment by 39 %, whereas the unfertilized treatments were lower than NPK treatment although not significantly different (Table 7). The MSIR rates were positively correlated with SOC and negatively to bulk density ($r = 0.66$, $p > 0.01$ and $r = -0.63$, $p > 0.01$, respectively) (Fig. 1).

Soil enzyme activity exhibited a slight increase in compost treatment, particularly for acid phosphatase, although the differences were not statistically significant (Supplementary Fig. S1). Enzyme stoichiometry analyses indicated an association of enzyme activity with SOC content, as the vector length (from lnC:P-enzymes plotted against lnC:N-enzymes) showed a negative correlation with SOC suggesting potential SOC limitations (Supplementary Fig. S2).

3.4. Nutrient input and crop yield

The average application rates of C, N, P and K by each of the studied BBFs clearly showed that human urine contributed to a similar degree of nutrient input as the NPK fertilizer, whereas compost was the amendment with the highest input of C and macronutrients (Table 8). Phosphorus input was particularly high in the compost and sewage sludge treatments, namely more than 120 kg ha^{-1} compared to the NPK treatment.

There was no significant difference in barley yields among the treatments at the beginning of the experiment (2003–2004). However, after 13–19 years (2017–2022), barley yields in the unfertilized control were significantly lower compared to the other treatments (Table 8). The highest yields at the end (2017–2022) were obtained with NPK and human urine, followed by compost, cattle slurry, and sewage sludge.

The treatment × time interaction was not significant (Table S4),

Table 5

Soil pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (N), total phosphorus (P), Olsen-P and potassium (K-CO_2). Values are presented as means with standard error in parentheses (n = 3). Different letters indicate significant differences among the treatments ($p < 0.05$).

Treatment	pH ($CaCl_2$)		EC (µS cm^{-1})		SOC (g kg^{-1})		Total N (g kg^{-1})		Total P (g kg^{-1})		Olsen-P (mg kg^{-1})		K-CO_2 (mg kg^{-1})	
Unfertilized	6.1 (0.18)	a	37.7 (1.67)	a	11.7 (0.17)	a	1.15 (0.02)	a	0.411 (0.02)	a	7.77 (0.56)	a	5.09 (0.29)	a
NPK	5.9 (0.17)	a	47.3 (3.28)	abc	14.5 (0.32)	b	1.41 (0.02)	ab	0.525 (0.06)	a	27.6 (12.8)	a	8.99 (1.06)	a
Cattle slurry	6.4 (0.04)	ab	50.3 (1.67)	bc	14.2 (0.54)	ab	1.41 (0.05)	ab	0.558 (0.02)	a	32.4 (1.66)	ab	30.0 (1.52)	b
Sewage sludge	6.1 (0.03)	a	55.3 (0.67)	c	16.7 (0.24)	b	1.68 (0.03)	b	1.06 (0.07)	b	148 (13.1)	c	6.03 (0.83)	a
Human urine	5.9 (0.13)	a	40.0 (2.08)	ab	14.1 (0.58)	ab	1.39 (0.09)	a	0.492 (0.01)	a	15.9 (0.4)	a	7.30 (0.38)	a
Compost	6.7 (0.01)	b	84.3 (3.18)	c	26.2 (0.96)	c	2.79 (0.1)	c	0.989 (0.03)	b	66.1 (4.46)	b	46.5 (6.46)	c
Treat effect	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Block effect	0.956		0.929		0.973		0.941		0.804		0.940		0.913	

indicating no overall differential development in yield among treatments between the initial and later period. Nevertheless, within-treatment comparisons suggested that yields in the unfertilized control were significantly lower ($p = 0.013$) at the end (2017–2022) than at the beginning of the experiment (2003–2004). This decline was visible in raw yield data, corresponding to a reduction in productivity of about 42 % (Table 8).

The addition of human urine did not result in significantly different barley yields compared to the NPK treatment, either at the beginning or at the end of the experiment, whereas barley yield was significantly higher than in the sewage sludge at the end of the study period (Table 8).

3.5. *Multifunctional analysis*

Multifunctional analysis is displayed in Fig. 2. Aggregation and scoring of physical soil indicators (GMD, clay dispersibility, bulk density, porosity and available water capacity), reflecting the state of soil structural quality, showed that compost reached the highest score (Supplementary Table S1). Soil structure scores for compost were 24–26 % higher than those of NPK, human urine or cattle slurry, and 35 % higher than the unfertilized control. For the biological activity, MSIR and enzyme activity were included as indicators, showing that all amendments tended to increase the biological activity compared to unfertilized treatment, where the organic amendments, particularly compost and sewage sludge, resulted in the highest increases (Supplementary Table S1). Only compost showed significantly higher biological activity than the unfertilized treatment. The aggregated indicators for the nutrient pool (SOC, Olsen-P, total N, K-CO_2, and base saturation) decreased in the following order: compost > sewage sludge ≥ cattle slurry ≥ NPK, human urine, unfertilized control. Human urine, NPK, and cattle slurry showed statistically comparable scores among all three soil function proxies. Compost, sewage sludge, and cattle slurry showed nutrient pool scores that were 49, 25 and 17 % higher, respectively, than those of the unfertilized control (Fig. 2, Supplementary Table S1).

4. Discussion

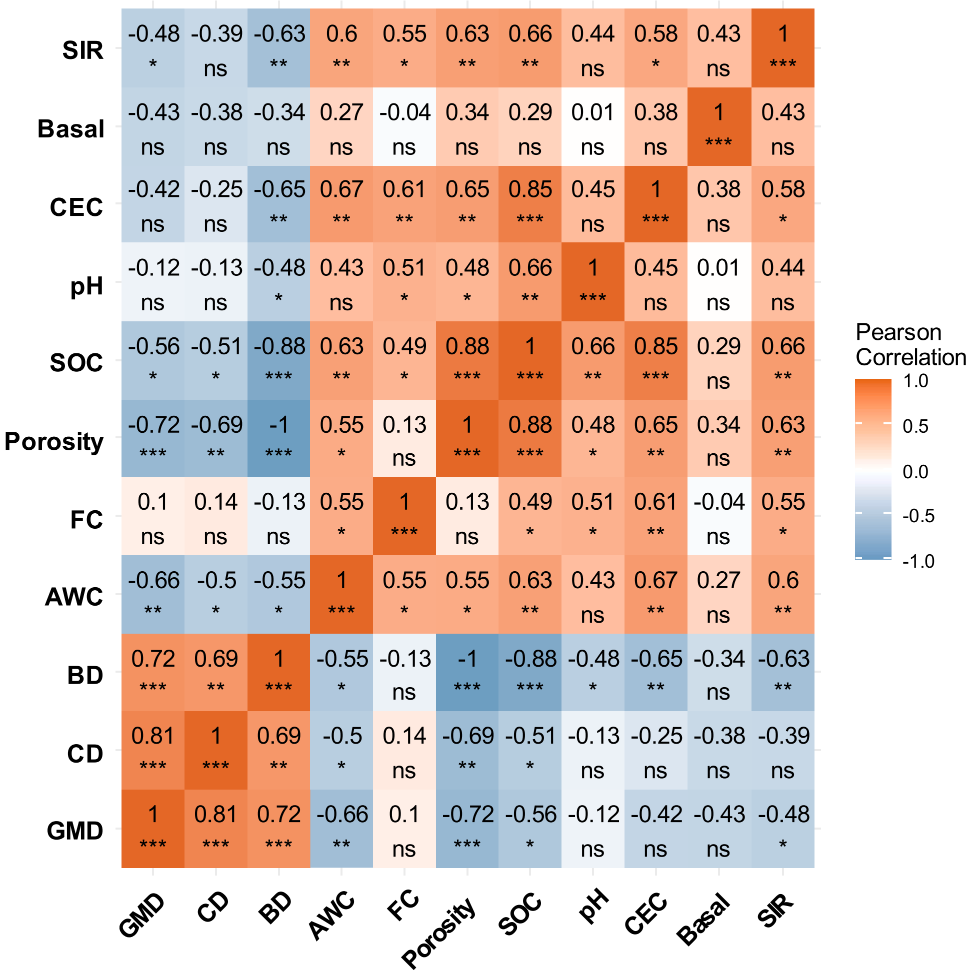
4.1. *The effect of bio-based fertilizers on soil health*

Our study evaluated the long-term application of various bio-based fertilizers on a set of soil health-related parameters. One of the most frequently measured soil health indicators is the SOC content, as it affects many soil functions and services (Bünemann et al., 2018). The observed changes in SOC stocks in the current study (Table 4) showed that the C input by organic fertilizers results in a SOC increase *that is not* proportional to their C content, thus refuting the first statement in hypothesis 1. On the contrary, as indicated by the subsequent estimate of the $SOC_{productivity\_factor}$ (cattle slurry < sewage sludge < compost), there is a substantial difference in the quality and recalcitrance of the added organic C. The feedstock for both cattle and sewage treatments is rather digestible (Owens et al., 2010; Poblete et al., 2022). However, the residence time in a cattle rumen is only a few days (Ellis et al., 1994), while the mean residence time in the BIOFOS sewage treatment plant is approximately 20 days. Subsequently, the sludge is degassed for methane production for approximately 24 days, as described in the materials and methods section. Thus, it is quite reasonable that the sewage sludge represents a highly degraded, and thus more recalcitrant C source than cattle slurry. This was also shown after 50 years of organic amendments in the Ultuna frame trial (Magid et al., 2010). By contrast, the feedstock for the compost was formed by largely lignified garden and park waste remaining after a biogas digestion of the waste mixture. As described in the materials and methods section, it was matured over a 4-month period prior to use, constituting the most recalcitrant organic matter input.

Thus, not all BBFs contributed equally to SOC stocks, as the quality

Table 6
Potential cation exchange capacity (CEC$_{pot}$), basic cations, base saturation and sodium adsorption ratio (SAR). Values are presented as means with standard error in parentheses (n = 3). Different letters indicate significant differences among the treatments ($p < 0.05$).

Treatment	CEC$_{pot}$ (cmol$^+$ kg^{-1})		Basic cations (cmol$^+$ kg^{-1})		Base saturation %		SAR	
Unfertilized	17.1 (0.89)	ab	13.4 (0.94)	a	78.3 (1.74)	ab	0.0144 (0.001)	a
NPK	17.0 (0.59)	ab	13.3 (0.59)	a	77.9 (2.44)	ab	0.0171 (0.001	a
Cattle slurry	16.1 (0.59)	a	13.4 (0.32)	a	83.3 (1.63)	ab	0.0266 (0.001)	ab
Sewage sludge	20.7 (1.07)	bc	15.9 (0.87)	a	76.9 (0.29)	ab	0.0287 (0.002)	ab
Human urine	18.6 (1.29)	ab	13.7 (1.64)	a	73.7 (6.79)	a	0.0413 (0.007)	b
Compost	24.0 (1.28)	c	21.3 (1.19)	b	88.73 (1.22)	b	0.0241 (0.001)	a
Treat effect	< 0.001		< 0.001		0.0659		< 0.001	
Block effect	0.386		0.407		0.3397		0.732	



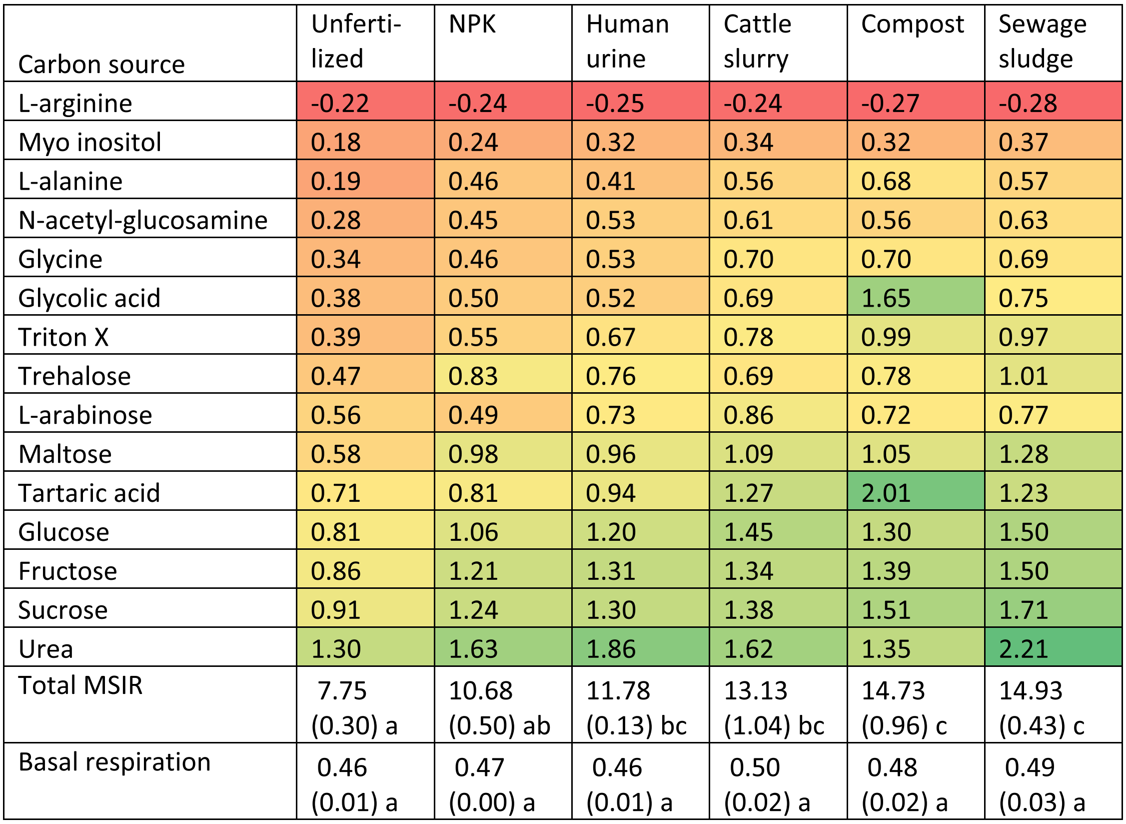
	GMD	CD	BD	AWC	FC	Porosity	SOC	pH	CEC	Basal	SIR
SIR	-0.48 *	-0.39 ns	-0.63 **	0.6 **	0.55 *	0.63 **	0.66 **	0.44 ns	0.58 *	0.43 ns	1 ***
Basal	-0.43 ns	-0.38 ns	-0.34 ns	0.27 ns	-0.04 ns	0.34 ns	0.29 ns	0.01 ns	0.38 ns	1 ***	0.43 ns
CEC	-0.42 ns	-0.25 ns	-0.65 **	0.67 **	0.61 **	0.65 **	0.85 ***	0.45 ns	1 ***	0.38 ns	0.58 *
pH	-0.12 ns	-0.13 ns	-0.48 *	0.43 ns	0.51 *	0.48 *	0.66 **	1 ***	0.45 ns	0.01 ns	0.44 ns
SOC	-0.56 *	-0.51 *	-0.88 ***	0.63 **	0.49 *	0.88 ***	1 ***	0.66 **	0.85 ***	0.29 ns	0.66 **
Porosity	-0.72 ***	-0.69 **	-1 ***	0.55 *	0.13 ns	1 ***	0.88 ***	0.48 *	0.65 **	0.34 ns	0.63 **
FC	0.1 ns	0.14 ns	-0.13 ns	0.55 *	1 ***	0.13 ns	0.49 *	0.51 *	0.61 **	-0.04 ns	0.55 *
AWC	-0.66 **	-0.5 *	-0.55 *	1 ***	0.55 *	0.55 *	0.63 **	0.43 ns	0.67 **	0.27 ns	0.6 **
BD	0.72 ***	0.69 **	1 ***	-0.55 *	-0.13 ns	-1 ***	-0.88 ***	-0.48 *	-0.65 **	-0.34 ns	-0.63 **
CD	0.81 ***	1 ***	0.69 **	-0.5 *	0.14 ns	-0.69 **	-0.51 *	-0.13 ns	-0.25 ns	-0.38 ns	-0.39 ns
GMD	1 ***	0.81 ***	0.72 ***	-0.66 **	0.1 ns	-0.72 ***	-0.56 *	-0.12 ns	-0.42 ns	-0.43 ns	-0.48 *

Fig. 1. Correlation matrix showing the relationships for the measured soil health parameters: substrate induced respiration (SIR), basal respiration (Basal), cation exchange capacity (CEC), soil organic carbon (SOC), water content at field capacity (FC), available water capacity (AWC), bulk density (BD), clay dispersibility (CD) and geometric mean diameter (GMD). The color legend represents the Pearson correlation coefficient values. Stars indicate the significance level of the correlation (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

and recalcitrance of organic input matters more than the quantity of C input. Similar results were recently observed in treatments from the CRUCIAL experiment (not analyzed in the current paper), which applied extremely high rates of amendment with farmyard manure, compost and sewage sludge (Magid et al., 2025). Our observations are further corroborated by findings from the long-term DOK trial in Switzerland, where after 42 years, the highest SOC contents were found under biodynamic management with composted manure compared to organic with rotted manure and conventional system with stacked manure (Krause et al., 2022). These results emphasize that not only the amount of organic matter, but also its quality, processing intensity (e.g. storage duration, aeration) and biological quality of the soil strongly influence SOC contents (Krause et al., 2022).

Human urine, the only bio-based fertilizer without additional C input, resulted in a minor decrease in the SOC stocks after 20 years (Table 4). Despite a substantial C input, the cattle slurry also resulted in a decrease in SOC stocks and did not improve any physical or chemical parameters compared to NPK and human urine treatments with no direct C input. While, as discussed above, the C in cattle slurry is the least recalcitrant, it is surprising that there is little or no benefit for physical or chemical parameters, considering that more than 25 tons C was applied during the experiment (Table 4). Interestingly, sewage sludge treatment

Table 7
Heat map of multiple substrate-induced respiration (MSIR) (μg CO^2-C g soil^{-1} hour^{-1}). Values for MSIR were corrected for water-induced respiration. Total MSIR and basal respiration, values are presented as means with standard error in parentheses (n = 3). Different letters indicate significant differences among the fertilization treatments ($p < 0.05$).



Carbon source	Unfertilized	NPK	Human urine	Cattle slurry	Compost	Sewage sludge
L-arginine	-0.22	-0.24	-0.25	-0.24	-0.27	-0.28
Myo inositol	0.18	0.24	0.32	0.34	0.32	0.37
L-alanine	0.19	0.46	0.41	0.56	0.68	0.57
N-acetyl-glucosamine	0.28	0.45	0.53	0.61	0.56	0.63
Glycine	0.34	0.46	0.53	0.70	0.70	0.69
Glycolic acid	0.38	0.50	0.52	0.69	1.65	0.75
Triton X	0.39	0.55	0.67	0.78	0.99	0.97
Trehalose	0.47	0.83	0.76	0.69	0.78	1.01
L-arabinose	0.56	0.49	0.73	0.86	0.72	0.77
Maltose	0.58	0.98	0.96	1.09	1.05	1.28
Tartaric acid	0.71	0.81	0.94	1.27	2.01	1.23
Glucose	0.81	1.06	1.20	1.45	1.30	1.50
Fructose	0.86	1.21	1.31	1.34	1.39	1.50
Sucrose	0.91	1.24	1.30	1.38	1.51	1.71
Urea	1.30	1.63	1.86	1.62	1.35	2.21
Total MSIR	7.75 (0.30) a	10.68 (0.50) ab	11.78 (0.13) bc	13.13 (1.04) bc	14.73 (0.96) c	14.93 (0.43) c
Basal respiration	0.46 (0.01) a	0.47 (0.00) a	0.46 (0.01) a	0.50 (0.02) a	0.48 (0.02) a	0.49 (0.03) a

Table 8
Inputs of fresh matter (FM), total C, N, P and K (Annual average input values for 2003–2022) and grain yield for spring barley (average for years (1) 2003 and 2004, and (2) 2017, 2019 and 2022). Different letters indicate significant differences among the fertilization treatments for years 2017–2022 ($p < 0.05$).

	Average application rates					Average grain yield							
	FM	Total C	Total N	Total P	Total K	2003–2004				2017–2022			
	t ha^{-1} y^{-1}	kg ha^{-1} y^{-1}				kg ha^{-1} y^{-1}				kg ha^{-1} y^{-1}			
Unfertilized	0	0	0	0	0	4287	±	544	a	2494	±	259	d
NPK		0	144	12	52	5918	±	837	a	6582	±	461	a
Cattle slurry	47	1344	86	30	100	4803	±	663	a	5432	±	567	bc
Sewage sludge	16	1154	166	156	15	4967	±	713	a	5184	±	604	c
Human urine	77	0	181	13	21	6186	±	347	a	6191	±	571	ab
Compost	26	4372	399	151	487	5435	±	413	a	5726	±	500	abc

with similar annual C inputs as cattle slurry improved many soil health parameters compared to NPK treatments, however significantly only for water content at FC and total MSIR. The NPK and human urine treatments resulted in similar SOC stocks as cattle slurry, probably due to indirect C input from crop residues, such as straw and root biomass, also indicated by higher yields of spring barley in these two treatments than in cattle slurry. The unfertilized treatment with no direct C inputs resulted in overall soil degradation, reflected by significantly lower SOC levels, water content, porosity and higher bulk density than in NPK treatments.

The compost treatment had a pronounced effect on soil structure by reducing bulk density and clay dispersibility and increasing porosity, water content at FC and available water capacity. These changes are likely caused by the high annual C input from the compost. These findings show that recalcitrant organic amendments, due to their high C content, increase SOC levels (Steffens and Bünemann, 2025), which in turn enhances soil structure (Obriot et al., 2016; Hartmann and Six, 2023).

Besides the direct effect of C input on soil structure, greater C availability may serve as energy source for soil microbes and increase microbial activity as reflected by high MSIR rates in compost and sewage sludge treatments in our study. This, in turn, contributes to further improvements in soil structure through the production of binding agents, such as glomalin and other extracellular polysaccharides, which help to stabilize soil aggregates and improve soil porosity (Hartmann and Six, 2023). The positive feedback loop between C input, biological activity and soil structure is evident when considering that microbial respiration correlates negatively with bulk density, indicating that increased microbial respiration helps to mitigate compaction by fostering soil aggregation. These biological processes not only improve soil structure but

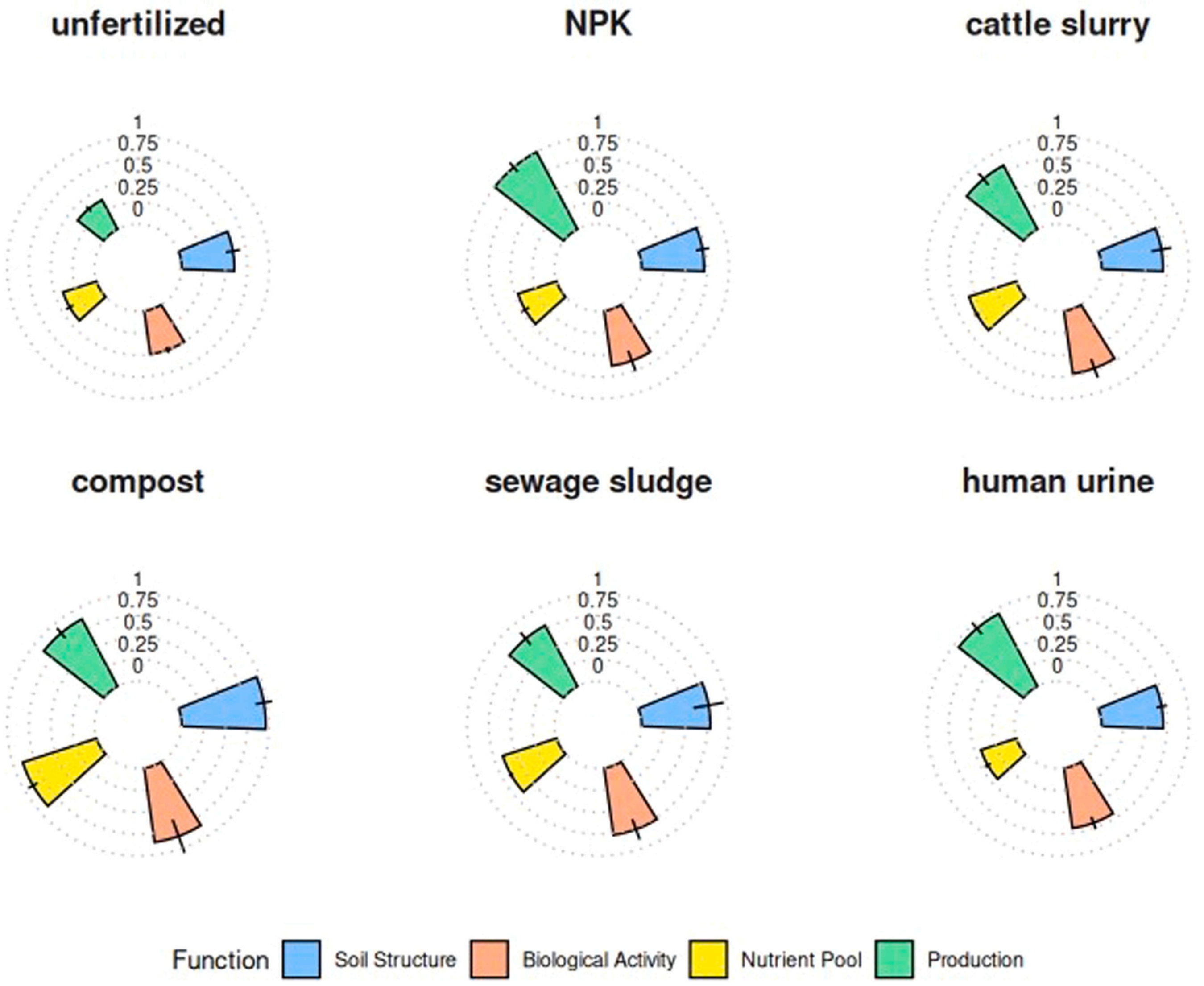


Fig. 2. Multifunctionality analyses of experimental treatments after 20 years. Scores are based on normalization and aggregation of soil indicators for biological activity, nutrient pool, and soil structure, whereas productivity is based on grain yield. Relative normalized scale based on the comparison to the highest or lowest indicator value, where appropriate.

also enhance the ability of the soil to retain water, as demonstrated by the significant increase in water content at field capacity and available water capacity in the compost treatments. These results indicate that biological activity plays a pivotal role in enhancing soil physical properties (Obriot et al., 2016). Thus, our results are partly consistent with the first hypothesis that C-rich bio-based amendments, such as compost and sewage sludge, but not cattle slurry, would lead to an increase in SOC content and overall soil health.

The multifunctional analyses revealed higher soil health scores in relation to chemical, physical, and biological properties for the organic amendments (compost and sewage sludge), primarily due to the organic matter input. On average, soil health scores were 22–43 % higher in compost compared to NPK, supporting our hypothesis. However, increases or decreases in soil indicator values do not necessarily reflect a negative or beneficial impact on soil functionality. Therefore, soil physical indicators were evaluated in relation to available soil quality thresholds (Häfner et al. in prep.), while for SIR and enzyme activity no applicable reference values were available. Regarding soil porosity, values below 40 % can indicate potential negative effects on root penetration (Huber et al., 2008). This appeared to be the case only for unfertilized treatment, which slightly dropped below this threshold (39.7 %). Regarding available water capacity, only sewage sludge and compost fell within the optimal range for plant growth, whereas the remaining treatments were slightly below the threshold of 15 %, which could potentially limit plant growth. The strong variation in bulk density also reflected functionally relevant influences of the amendments. None of the treatments exceeded the critical bulk density threshold for sandy loams (1.8 g cm^{-3}), above which root growth may be restricted (USDA NRCS, 2019). Only the unfertilized control approached the lower critical threshold of 1.63 g cm^{-3} which may affect root development. Compost was the only treatment within the ideal range for plant growth (<1.4 g cm^{-3}). The remaining treatments were suboptimal but remained below the lower threshold affecting root growth. The soil SOC to clay ratio, which indicates soil structural quality (Johannes et al., 2017), showed that only compost reached the optimum ratio of > 1:8. Sewage sludge had a ratio of 1:10, while the unfertilized treatment fell below the threshold of 1:13, indicating a degraded structure. The other treatments had ratios between 1:10 and 1:13. Considering the analysis of aggregate stability (GMD), no significant differences were found, which partially contradicts the other indicators on soil physical quality. However, for instance Johannes et al. (2017) suggested that the SOC: clay ratio should also be considered in combination with the visual evaluation of soil structure (VESS). Therefore, it may be beneficial to assess multiple indicators to better understand the implications of management practices on soil structural quality. Although the physical indicators showed trends suggesting soil structure degradation, particularly in the unfertilized treatment, the soil still maintained a certain level of functional resilience. Only compost and to some extent, sewage sludge, demonstrated a beneficial structural state when compared to reference values from literature.

4.2. Fertilizer efficiencies deviate from regulatory assumptions

Organic fertilizers with high C input are usually associated with a lower N availability per N unit, as N is partially organically bound (Larney and Angers, 2012; Gómez-Muñoz et al., 2017). In the CRUCIAL trial, the fertilization was based on the regulatory short-term N availability. This determined the mineral fertilizer equivalent (MFE), resulting in higher total N inputs to the soil. The yield in the organic treatments was still lower compared with both the human urine and the NPK treatments. In our trial we assumed that only 70 % of the total N in human urine was directly available, leading to an N input approximately 37 kg higher than for the NPK treatment. Crop yields were within a comparable range for both NPK and human urine, indicating an appropriate N supply from human urine. Although total N input in the urine treatment was higher than in NPK treatment, N losses via NH_3 volatilization may have reduced the N efficiency, explaining the slightly lower total soil N content in urine compared to NPK treatment. Indeed, Kirchmann and Pettersson (1994) found higher N losses via NH_3-N from stored human urine due to its high pH (8.9) and high ammonium N share ($>$ 90 %), as well as lower crop N uptake compared to ammonium nitrate fertilization. Acidification or nitrification of stored human urine could further increase its MFE and decrease NH_3 volatilization (Martin et al., 2021, 2023).

Interestingly, although MFE for both cattle slurry and human urine was assumed to be 70 %, human urine resulted in approximately 12 % higher average crop yields (2017–2022), indicating a higher N efficiency. Similarly, Martin et al. (2021) observed higher N use efficiencies for nine different urine-based fertilizers compared to cattle slurry, which aligned with the higher mineral N content in the human urine.

Productivity of compost was, respectively, 8 and 13 % lower than that of human urine and NPK, despite receiving more than twice the total N input, i.e. additional 250 kg N ha^{-1} (Table 8). This surplus could lead to substantial N losses (Reimer et al., 2025), highlighting the environmental trade-off between reduced N efficiency and productivity and the positive effect of compost on soil functionality. Ultimately, none of the bio-based fertilizers showed a negative effect on productivity after 13–20 years of repeated application. However, our second hypothesis, that BBFs lead to lower crop production per unit N relative to mineral fertilizer is confirmed, particularly for the BBFs rich in organic carbon. Overall, compost and sewage sludge emerged as the most effective BBFs for enhancing soil health, particularly through their positive impacts on SOC, soil structure, water retention, and microbial activity, although their N efficiency and crop yields were lower than those achieved with mineral fertilizer or human urine.

4.3. Influence of human urine fertilization on Na accumulation and soil structure

The application of human urine adds high amounts of NaCl to the soil, while providing no organic matter. Other bio-based fertilizers, such as Fertigro (Gómez-Muñoz et al., 2023) or digestates originating from food waste (Song et al., 2021), may also contain high NaCl concentrations. However, those fertilizers also provide additional organic matter input that can outbalance the negative effects due to NaCl. The possible negative effect of repeated NaCl addition from human urine fertilization has been part of an ongoing discussion when it comes to negative effects of recycling human urine as fertilizer (Krause et al., 2021). While pharmaceuticals and pollutants can easily be removed from urine via filtration with activated carbon (Özel Duygan et al., 2021), NaCl remains in the urine. After 20 years of annual addition of stored human urine in our trial, the Na concentration has significantly increased leading to an increase in SAR in urine-fertilized soil compared to NPK treatment. Although urine typically contains more Na than animal slurries, due to the dietary salt intake (Martin et al., 2020), for instance 2.6 g L^{-1} in urine (Udert et al., 2006) compared to 0.8–1 g L^{-1} in cattle slurry (Rodrigues et al., 2021), the SAR observed in the human urine treatment was not significantly different from that in the sewage sludge and cattle slurry treatments. Furthermore, the values were several magnitudes below the threshold of 13 for sodic soils (Richards, 1954). The EC in the compost treatments was even twice as high compared to human urine, reflecting the overall ionic strength of compost as it provided more nutrient ions than urine. The EC of human urine was 100 times lower than a potential critical threshold of 4 dS m^{-1} (Sigurnjak et al., 2017). The negative impact of Na accumulation on soil structure should have affected clay dispersion, but there were no significant differences among treatments, suggesting the slightly higher SAR did not affect soil structure. The negative effect on soil bulk density seems rather affected by the omission of external C inputs than by the NaCl addition, which was further corroborated by the positive correlation of SOC with soil physical indicators. Moreover, the positive water balance and the light soil texture of our soil (i.e., a sandy loam) enable leaching of soluble salts, which counteracted accumulation of NaCl. Therefore, the hypothesized potential risk of human urine due to NaCl, leading to salinization and negatively affecting soil physical properties, appears to be negligible under the tested pedo-climatic conditions. In contrast, other studies have shown an immediate increase in pH, SAR and EC with human urine application (Sangare et al., 2015), or a negative impact under already saline soil conditions (Boh et al., 2021). Moreover, the salinity and pH effect induced by human urine may vary with soil type (Rumeau et al., 2023). Higher clay contents can elevate the risk of Na accumulation, as demonstrated by Levy et al. (2014) who observed a stronger increase in SAR after irrigation with treated wastewater in sandy clay (15 % clay) compared to loamy sand (9 % clay). Fine texture soils are more susceptible than medium texture soils (sandy loam) and are prone to clay dispersion (Wang et al., 2024). In heavy soils with higher clay content and a negative water balance or prolonged dry conditions, the risk of NaCl accumulation might be higher. Therefore, long-term application of human urine might have a stronger effect on soil structure quality and base saturation in dry areas (Sangare et al., 2015) or clay-rich soils with extended dry seasons. For human urine fertilization, adding additional OM inputs could improve both base saturation, soil structure and nutrient retention. From an urban waste recycling perspective, recycling the entire human excreta fraction, including human urine and feces could contribute to a more sustainable fertilizer approach, including a more balanced nutrient budget (Reimer et al., 2025). For instance, a field experiment with cabbage showed that combining both human urine (as a nitrified product) and fecal compost had the potential to achieve comparable yields as urine-based fertilizer alone (Häfner et al., 2023). Furthermore, mixing human urine with fecal compost showed potential to mitigate salinity effects compared to urine alone in a pot study with okra (Sangare et al., 2015). Overall, our third hypothesis that the application of human urine, due to its high NaCl concentration and lack of C inputs, can negatively affect soil structural properties, does not seem to be true under our study conditions (sandy loam, temperate maritime climate), even if more studies are necessary under different climatic and pedological conditions.

4.4. Bio-based fertilizers, soil health and resilience

In their seminal paper on soil quality, Karlen et al. (1997) noted that critical soils functions could be defined with respect to issues such as capacity for biosolid application, among others. This highlights a central dilemma of recycling: organic residues – even animal manures, inevitably contain small amounts of unwanted substances, such as heavy metals, pharmaceuticals, microplastics, PFAS, and pathogens (Magid et al., 2020). This was elaborated further in a recent review raising the question: do contaminants compromise the use of recycled nutrients in organic agriculture (Bünemann et al., 2024), where it was highlighted that soils show great resilience and degrade or stabilize most pollutants.

We found that multiple substrate-induced respiration (MSIR, Table 7) was generally higher in compost and sewage sludge treatments, despite the compost treatment receiving nearly four times more C annually, while C, N and P-cycling enzymes activities were comparable among treatments, indicating no negative effects or over-compensation (Supplementary Fig. S1). Multifunctional analyses (Fig. 2; Supplementary Table S1) further confirmed that the benefits of urban waste fertilizers outweighed potential impacts of contaminants, demonstrating the intrinsic resilience of the soil system.

Previous CRUCIAL studies showed that long-term amendment of urban wastes equivalent to more than 100 years of application had minimal accumulation of potentially toxic elements (López-Rayo et al., 2016). Only Zn and Cu were slightly elevated in soil under compost and sewage sludge, while in oat grain, Cd remained below EU limits, and elevated Zn may even provide a nutritional benefit. Riber et al. (2014) only found short-term increases in antibiotic-resistant isolates of pseudomonas in the CRUCIAL trial with excessive amounts of sewage sludge, compost and farmyard manure. Thus, the introduced microbes were either not viable or lost resistance traits, suggesting a resilience of the soil microbiota and the soil ecosystem. Johansen et al. (2023) found that long-term fertilization with human urine, compost, and sewage sludge did not negatively affect nematode communities or soil food webs. Treatments with high organic matter input even supported grater abundance of bacteria and bacterial grazers. The Nematode Maturity Index values, a pollution indicator, did not differ significantly between treatments, confirming the absence of stress from urban waste amendments.

Our results suggest that recycling urban waste streams, such as compost, sewage sludge, and human urine, can effectively contribute to closing nutrient cycles and improving soil functionality without compromising soil resilience to contaminants, provided that appropriate source control, waste treatment and risk assessment are implemented. At the same time, it is important to continuously monitor the levels of contaminants and microplastic content in these BBFs to prevent potential negative impacts on soil health and food safety. For established BFFs guidelines, standards, and legal regulations are already in place to protect human health and the environment and assure the product quality. However, monitoring methods must be continuously adapted to emerging pollutants, and in some cases existing frameworks require refinement or new guidelines must be developed to cover novel substrates or BFF products (Krause et al., 2021).

The trade-off between productivity and long-term soil health benefits highlights the necessity of context-specific strategies and regulatory frameworks that consider contaminant thresholds, as well as differences in carbon recalcitrance, nitrogen efficiency, and site conditions. If BBFs are to play a larger role in future agriculture, they should enhance soil health and provide reliable nutrients levels. This requires a comprehensive approach, such as pre-treatments of biowastes to improve nutrient availability, optimizing nutrient recovery processes in waste management, and combining bio-based materials with mineral fertilizers in organo-mineral formulations to balance nutrient content and ensure consistent effectiveness (Álvarez Salas et al., 2024).

Our study was conducted on a sandy loam under temperate conditions, where there is normally leaching during winter. The effects of BBFs, especially human urine (with NaCl), may differ in clay-rich soils or arid regions where salt accumulation could be critical, and thus the results cannot merely be generalized to other regions.

5. Conclusion

While mineral fertilizers can improve some physical and chemical soil properties and sustain crop productivity, they do not offer the same long-term benefits to soil health as organic amendments. We therefore suggest that integrating organic and mineral fertilizers may represent the most effective approach to sustaining and enhancing soil health and crop productivity. Overall, the long-term application of compost and, to some extent, sewage sludge significantly improved several key soil health parameters, including SOC, CEC, bulk density, water retention, porosity, clay dispersibility and MSIR. Human urine reached comparable yields to NPK and thus shows strong potential as alternative nutrient resource for urban nutrient recycling. Within the tested pedo-climatic conditions, no relevant negative effects on soil functionality were observed after 20 years of repeated application compared to conventional mineral NPK fertilizer. Still, for some indicators such as bulk density and SAR, a negative trend was detectable with human urine, although values did not exceed any critical thresholds. For the sustainable use of human urine as a resource in agriculture, the additional supply of organic amendments would be beneficial and could mitigate potential negative effects.

The findings of our study underscore the potential benefits of bio-based fertilizers, particularly compost and sewage sludge, not only to increase SOC but also to improve soil physical, chemical and biological properties that are critical for sustainable crop production. Future research could assess the long-term application of human urine in contrasting soils and climates, as well as its mixture with organic amendments, e.g. compost. Another important perspective would be to evaluate the overall yield stability in relation to weather extremes and soil quality between the different bio-based fertilizers over the past 20 years and under future conditions.

CRediT authorship contribution statement

Veronika Hansen: Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Franziska Häfner:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation. **Bianca Messmer:** Writing – review & editing, Formal analysis, Data curation. **Luca Bragazza:** Writing – review & editing, Validation, Supervision. **Carsten Tilbæk Petersen:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Jakob Magid:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.110108.

Data availability

Data will be made available on request.

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