



Effect of manure co-digestion on methane production, carbon retention, and fertilizer value of digestate

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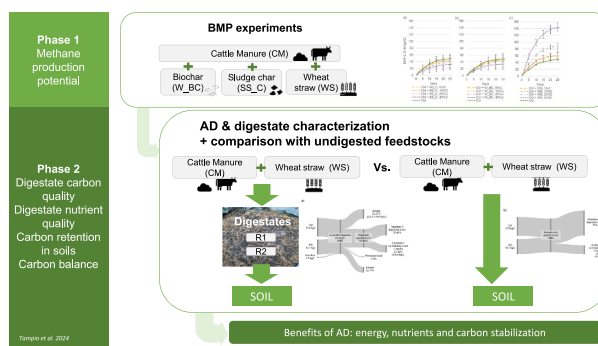
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HIGHLIGHTS

- Cattle manure was digested with carbon-rich co-feedstock materials, such as straw.
- Both methane production and digestate quality perspectives were studied.
- Anaerobic digestion improved the stabilization of carbon in the digestate.
- 22 % of initial carbon was modeled to contribute to soil carbon stocks.
- The use of digestion supports the buildup of soil organic carbon pool.

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic digestion can provide benefits not only from the perspective of renewable energy production but also in the form of fertilization effect and increased retention of C in soils after digestate application. This study consisted of two phases, where the first phase assessed the suitability of carbon-rich co-feedstocks for methane production via laboratory testing. The second phase assessed the balance and stability of C before and after anaerobic digestion by systematic digestate characterization, and by evaluating its carbon retention potential using a modeling approach. The results indicated that pyrolysis chars had a negligible effect on the methane production potential of cattle manure, while wheat straw expectedly increased methane production. Thus, a mixture of cattle manure and wheat straw was digested in pilot-scale leach-bed reactors and compared with undigested manure and straw. Although the total amount of C in the digestate was lower than in the untreated feedstocks, the digestion process stabilized C and was modeled to be more effective in retaining C in the soil than untreated cattle manure and wheat straw. In addition, digestion converted 23–27 % of the C into valuable methane, increasing the valorization of the total C in the feedstock. Considering anaerobic digestion processes as a strategy to optimize both carbon and nutrient valorization provides a more holistic approach to addressing climate change and improving soil health.

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1. Introduction

In securing food production, both soil health and the circular use of resources are increasingly important global topics. The loss of organic matter in agricultural soils poses a risk to the productivity of soils, challenging sustainable food production. It has been estimated that an increase in soil organic carbon (SOC) pool by 0.4 % each year would offset global anthropogenic carbon dioxide emissions (Minasny et al., 2017). Among other methods, the use of organic soil amendments can contribute to achieving the goal by increasing organic matter input and improving soil health. The management of SOC by utilizing organic biomasses is also closely linked to nutrient recycling, as nutrients and C can be simultaneously recirculated from the same biomasses.

The anaerobic digestion of organic side-streams, a process linked with the mitigation of greenhouse gas emission (Farghali et al., 2022), not only produces renewable energy but organic C and nutrient containing residue, digestate. Digestates have also been acknowledged as beneficial soil amendment and fertilizer products (Tambone et al., 2009; Wang and Lee, 2021). In anaerobic digestion, easily degradable organic matter is converted into biogas via microbiological transformations. The remaining digestate contains more complex and recalcitrant organic molecules such as lignocellulosic plant fibers than the raw material fed into the process (Tambone et al., 2009). This recalcitrant C, which has a longer residence time in soils, could also be used to increase SOC content in soils (Angst et al., 2023), and field trials have shown the potential of different digestates to contribute to the buildup of SOC (see e.g., Béghin-Tanneau et al. (2019)). However, the studies focusing on the C content and its stability in soils are sparse. Heikkinen et al. (2021) estimated that the anaerobic digestion of cattle slurry did not increase the amount of C in digestate-amended soils compared to the soil amended with untreated manure. Nyang'au et al. (2022) modeled the stability of digestate C in soils based on an experimental mineralization study and reported an increase in the slow turnover C pool after anaerobic digestion of cattle slurry and agri-biomass.

To introduce more recalcitrant C into the digestion process, different feedstock mixtures could also be explored. Traditionally, the co-digestion of different feedstocks is mainly used to enhance biogas production (Karki et al., 2021) and to utilize available local waste materials (Ervasti et al., 2022). In some cases, co-feedstocks' selection is based on their characteristics to balance digestate nutrient contents, or dilute harmful compounds, for example (Karki et al., 2021). However, the co-feedstocks are very rarely selected based on their contribution to the digestate C. For example, studies regarding the use of different C-rich feedstocks (e.g., biochars) in digesters have shown potential only in process stability and gas production (Lü et al., 2018; Pan et al., 2019; Wang and Lee, 2021). Nevertheless, the introduction of co-feedstocks should not compromise biogas production, but benefit the whole process.

This study's fundamental aim was to assess the characteristics and stability of C in the digestate from anaerobic digestion of cattle manure (CM). Additionally, the aim was to show the effects of optimizing the C content in the entire digestion process by considering both biogas production and digestate characteristics, nutrients, and C. The two phases of the study consisted of i) enhancing methane production potential of CM with C-rich co-feedstocks (pyrolysis chars and straw) and selecting the best-performing mixture for further studies; and ii) validating the digestate nutrient quality and characteristics of the digestate's C retention in soil and comparing it with untreated feedstocks.

2. Materials and methods

2.1. Materials

CM with peat/straw bedding material, collected from Natural Resources Institute Finland's dairy cattle farm (Jokioinen, Finland), was used as the main feedstock (Fig. 1).

The C-containing co-feedstocks used in the experiment were sewage-sludge-originating char (SS_C), willow biochar (W_BC), and winter wheat straw (WS). SS_C consisted of a pyrolyzed (565 °C, 75 min) mixture of sewage sludge (80 %) and waste wood (20 %) and was collected from Helsinki Region Environmental Services HSY's pilot pyrolysis plant. W_BC originated in the pyrolysis of willow wood (*Salix*) at a temperature of 450–500 °C, as described in Keskinen et al. (2021). WS was collected from the same farm as CM, and was ground using a cutting mill (Retsch SM 2000, Retsch GmbH, Germany) to a particle size of 2–3 mm.

In the BMP experiments, the inoculum originated in a mesophilic full-scale biogas reactor treating municipal biowaste (Forssa, Finland). The percolate liquid of farm-scale biogas plant treating grass silage and clover (Laukaa, Finland) was used as the inoculum in the pilot-scale experiment.

2.2. Biochemical methane potential tests

The BMP and residual methane potential (RMP, see Supplementary Material) were determined using an AMPTS II system (BPC Instruments Ab, Sweden). In co-digestion tests, the share of feedstocks was set based on their elemental C (see 2.4.). The co-feedstocks were added to the CM in four different ratios to correspond to 5, 10, 20, or 40 % of the C content of the mixture (Table 1). The amount of inoculum in each bottle was 360 g, and the substrate/inoculum volatile solids (VS) ratio was 1.

The tested materials and inoculum were added to 500 mL borosilicate test bottles. NaHCO₃ (3 g/L) was used as a pH buffer, and distilled water was added to the bottles to reach a total liquid amount of 400 g. The headspace of the bottles was flushed with N₂ to achieve anaerobic conditions. From the produced biogas, CO₂ was trapped using 3 M NaOH, after which the methane volumes were measured based on water displacement. The samples were incubated for 26 days in mesophilic conditions (37 °C), and the content of the bottles was mechanically mixed for 1 min per hour at 84 rpm. All batch tests were performed as triplicates.

2.3. Pilot-scale solid-state anaerobic digestion

The pilot-scale experiment was performed in mesophilic (37 °C) conditions using two parallel 1 m³ leach-bed reactors (R1 and R2, Metener Oy, Finland) as described in Pyykkönen et al. (2023). The reactors were filled with the CM and WS feedstocks in multiple layers, with WS as the bottom and top layer. A C ratio of 40 % of WS (based on the BMP experiments) was used as the basis of feedstock loading volume, which corresponded to 15.7 % of WS mass in the reactors.

The circulation of percolate liquid was intensified during the experiment to circulate 10 L every 48, 24, 12, 6, 4, and 2 h respectively, depending on the stage of the digestion process. Due to an equipment malfunction, after 65 days of operation, the circulation in R2 was switched to less frequent feeding, circulating 10 L every 24 h with breaks over the weekends. Tap water (in total 207 and 229 L in R1 and R2) was added to the percolation liquid tanks to maintain a constant liquid volume within the tanks.

The temperature, pH, volume of biogas and methane content were measured daily. At the end of the experiment, the reactors were drained of the liquid within the biomass, and both digestate and percolate liquid were sampled. The duration of the experiment was 139 days.

2.4. Chemical analyses

From fresh feedstocks and digestate samples the total solids (TS) and the volatile solids (VS) were analyzed according to SFS 3008 (Finnish Standard Association, Helsinki, 1990). The pH was measured using a pH analyzer (VWR pH 110, VWR International). Total nitrogen (TN) and soluble ammonium N (NH₄-N) were analyzed by the Kjeldahl method using FOSS Kjeltect 8400 (FOSS, Denmark).

From dried samples, the content of C, hydrogen (H), N, and sulfur (S) was determined using a Leco CHN628 elemental analyzer (Leco Corporation, USA), and oxygen (O) was calculated based on mass balance. The concentrations of trace elements (Ca, Fe, K, Mg, Na, P, and S) were analyzed with an ICP-OES (Perkin Elmer Optima 8300) after HNO₃ digestion.

A neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed with filtering apparatus Fibertec™ 8000 (FOSS Analytical A7S, Hillerød, Denmark). The NDF was analyzed according to ISO 16472:2006, and a detergent solution was made according to Van Soest et al. (1991). A heat-stable alpha amylase was used. The ADF and acid detergent lignin (ADL) were determined according to the EN ISO 13906:2008. The detergent solution was made according to Robertson and Van Soest (1981). In ADL determination, the FT 121 Fibertec Cold Extraction Unit (FOSS, Hillerød, Denmark) apparatus was used. The hemicellulose content was calculated from the difference between the NDF and ADF, and cellulose content from the difference between the ADF and lignin. All the results are reported on an ash-free basis.

Sequential extraction of acid soluble (A), water-soluble (W), ethanol-soluble (E), and non-soluble (N), i.e., AWEN fractions, was executed for the oven dried digestate samples to determine the composition of the organic C fraction. The extraction was carried out as described by Heikkinen et al. (2021). The results were corrected with the ash content of the sample.

From the pilot experiment, the concentration of gas components (CH₄, CO₂, O₂, and H₂S) was measured by a hand-operated gas analyzer (Optima 7 Biogas, MRU Labs).

2.5. Modeling the additionality of soil organic carbon

The soil C model Yasso07 (Tuomi et al., 2011, 2008) was used for the estimation of the additionality of SOC stock using the studied feedstocks (CM and WS) and digestates (R1 and R2).

The C input data for the model were derived from sequential AWEN extraction, providing four different C pools based on their solubility. In addition to the initial chemical composition of the raw material, the temperature and precipitation affect the decomposition rates of the organic material (Palosuo et al., 2015). Monthly climate data (10 × 10 km gridded), provided by the Finnish Meteorological Institute, were applied (Venäläinen et al., 2005). The actual mean annual temperatures, precipitation, and temperature amplitudes calculated from 1990 to 2018 for Southern Finland were utilized. The annual temperature used was 4.2 °C, annual precipitation 635.8 mm, and amplitude 13.3 °C. As a result, the decomposition of the soil enrichment materials and the share of C that remained in the mineral agricultural soil was assessed for a 100-year timespan.

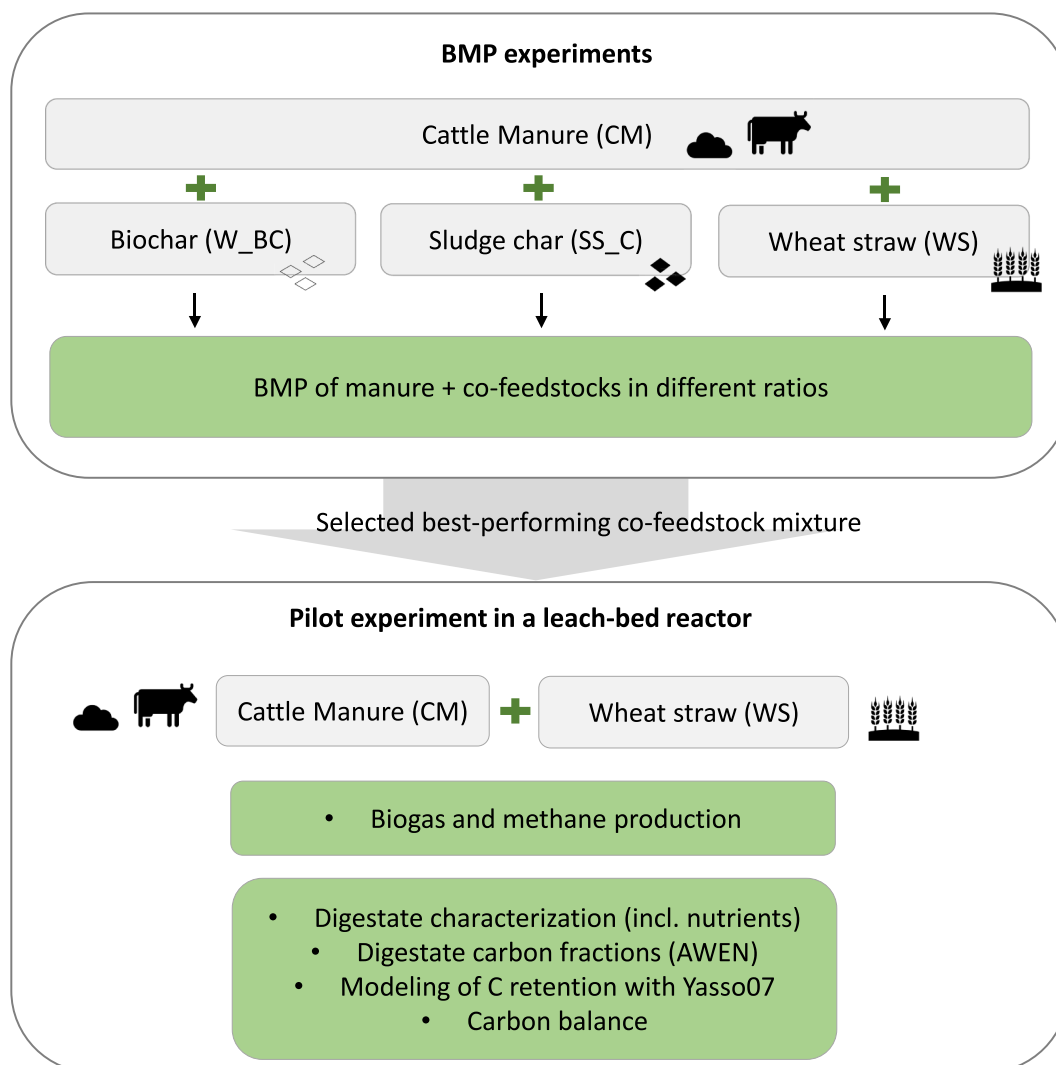


Fig. 1. Experimental design and materials used in the study.

2.6. Calculations

Methane yields were converted into normal conditions (0 °C, 101.32 kPa) according to the ideal gas law. In the BMP experiments, the methane production of the inoculum was subtracted from the results containing both substrate and inoculum to achieve the methane production of the feedstock.

A *t*-test was performed with the statistical software R (R Core Team, 2022) to analyze if the BMP (as ml of methane in each test bottle on day 26) in each co-digestion test had statistical significance compared with the main feedstock (CM) alone. The Bonferroni correction was used to adjust the *p*-values to control the false discoveries.

The mass balance was calculated as in Pyykkönen et al. (2023), but using Avogadro's law to transform CH₄ and CO₂ volumes into moles in normal conditions. For the percolate liquid (at the beginning and end of the experiment), the C content was unavailable, so its quantity was estimated based on VS content (assuming C = 55 % VS). The mass balance was based on the mass of feedstocks available in the Supplementary Material.

3. Results

3.1. Material characteristics

3.1.1. The effect of co-feedstocks on methane production

The use of both chars (SS_C and W_BC) as co-feedstocks for CM did not increase methane production, and trends in methane production dynamics were similar between SS_C and W_BC addition (Fig. 2). There was a negative effect on gas production compared with CM alone, when the SS_C addition rate was 5–40 % and W_BC 5, 20 or 40 % of the total C-content in the test bottles. The rate of 10 % of W_BC resulted in similar cumulative gas production to the control (CM alone). However, the effects of char additions on the gas production of CM were not statistically significant. WS as co-feedstock increased methane production of CM in all the tested C-ratios, while in the C-ratios 20 and 40 % the difference was significant (*p* < 0.05; see Supplementary Material).

Due to the highest methane production potential, and synergies in agricultural context, the WS co-feedstock with a C-ratio of 40 % was selected for the pilot-scale digestion experiment. The 139-day pilot-scale experiment with CM and WS (in 60/40C ratio) yielded methane 138 and 115 L CH₄/kgVS in reactors R1 and R2 respectively (see Supplementary Material). In the reactors, the cumulative gas production curves differed, and gas production slowed after day 70 and ceased around day 100 in R2, which was due to slightly different packing of the reactors and amounts of tap water added.

3.2. Digestate quality and effect on carbon retention in soils

3.2.1. Fertilizer quality

Despite the differences in the digestion process between reactors R1 and R2, concentrations of N, P, and K in both digestates were similar and reflected the nutrient content of the feedstocks (Table 2). The introduction of WS as co-feedstock (15.7 % WS in the reactor) did not dilute N and P concentrations, as the majority of the N and P in the digestate originated in the CM. P content was thus similar in both CM and WS (0.6–0.9 g/kg). However, WS contained a higher concentration of K

(15–20 g/kg) than CM, which increased K content in the digestates (~5 g/kg).

3.2.2. Effect of co-digestion on carbon retention in soils

The AWEN fractionation of the feedstocks and digestates from the pilot experiment showed that the anaerobic digestion increased the share of more stable fractions of C in the digestates. Of the tested feedstocks, CM had a higher share (29 %) of the ethanol and non-soluble C-fractions, while in WS, the share of the same fractions was 23 % (Fig. 3). When mixing these feedstocks, most of the stable C-fractions therefore originated in the CM. The digestion process concentrated the more stable C-fractions in the digestate, and the share of ethanol and non-soluble fractions increased from 26 % to 37–40 %, while the easily soluble C-fractions (namely acid and water-soluble fractions) were transformed into biogas. In addition, part of the easily soluble fractions was probably transferred to the percolate liquid as volatile fatty acids and other soluble compounds, for example.

Based on the Yasso07 modeling study, the higher stability of C after digestion resulted in increased C retention in soils. When applying digestates, 48–50 % of C remained in soils after five years, while only 43 and 39 % of C were retained after applying CM and WS respectively (Fig. 4). Throughout the modeled time period, up to 25 and 100 years, more C retained in soil when digested material was applied compared to undigested CM and WS. However, after 25 years, only around 20–27 % of the added C was left in the soil and after 100 years, only 3–4 %.

3.3. Carbon balance of co-digestion

The C mass balance was calculated to assess the faith of C during the co-digestion of CM and WS (Fig. 5a). According to the mass balance, the digestates R1 and R2 contained 53 and 63 % of the total C fed to the pilot reactors. 27 % and 23 % of C was captured as biogas (CH₄ and CO₂). The percolate liquid contained 2–3 % of C, which in an industrial plant would be utilized as the inoculant of the next digestion batch. However, there was 17 and 12 % loss in the C balance, probably due to volatilized C compounds (e.g., as volatile fatty acids) during the material and sample handling, as well as inaccuracy in the analyses. The C balance can be compared with the conventional use case of both CM and WS, which would end up in soils as soil amendments, thus similarly contributing to soil C stocks (Fig. 5b).

4. Discussion

This study sought to assess anaerobic co-digestion of CM, examining not only the biogas potential but also the impact on the digestate's C and nutrient quality. Specific attention was paid to C quality and balance throughout the process, with a focus on exploring opportunities for value generation through the optimization of C utilization within the process chain. The digestate was compared with undigested CM and WS.

4.1. Impact of feedstock selection on anaerobic digestion performance

Based on the initial co-feedstock screening, WS was selected for the pilot-scale reactor experiments to analyze the effects of co-digestion on the methane production potential, and further assess the C balance and digestate quality of selected digestion process. When co-digested with

Table 1

The share of co-feedstocks (SS_C = sewage sludge-based char, W_BC = willow biochar, wheat straw = WS) in the feedstock mixtures. Co-feedstocks were added in different ratios of C to the mixture (5, 10, 20, 40 %), which resulted in varying ratios of fresh matter (FM), volatile solids (VS), and total solids (TS).

% of co-feedstock in the mixture as	SS_C				W_BC				WS				
	C	5	10	20	40	5	10	20	40	5	10	20	40
FM		2	4.1	8.7	20.2	0.6	1.3	3	7.5	1.3	2.6	5.7	13.9
VS		1.9	4	8.5	19.9	2.2	4.5	9.5	22	5.7	11.1	21.9	42.7
TS		8.3	16	29.9	53.3	3	6.1	12.7	28	5.6	11	21.7	42.6

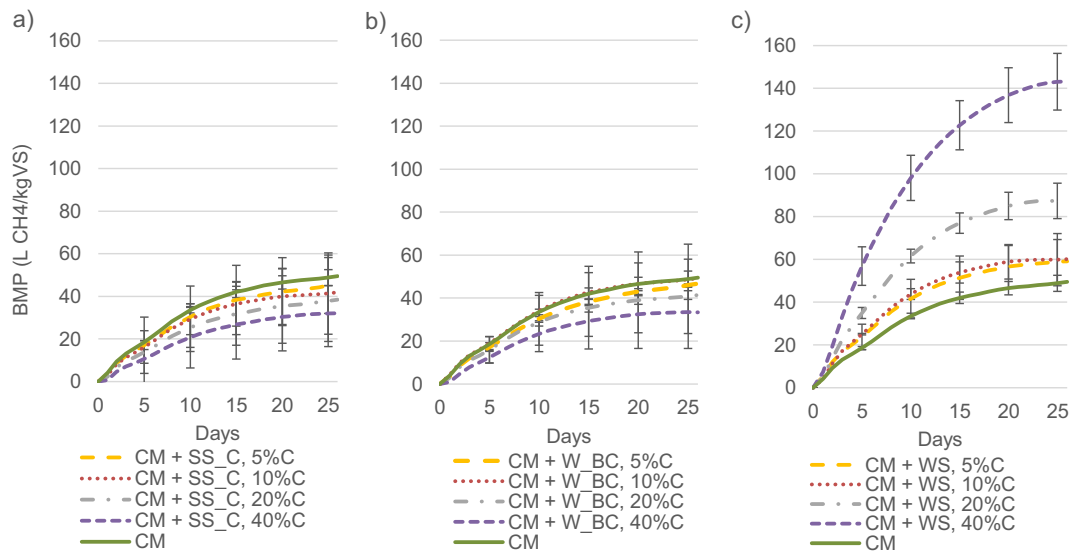


Fig. 2. The biochemical methane potential (BMP) of cattle manure (CM) with tested co-feedstocks and C-ratios. a) Sewage-sludge char (SS_C) as co-feedstock, b) willow biochar (W_BC) as co-feedstock, c) wheat straw (WS) as co-feedstock. The percentages indicate the ratio of C in which the co-feedstocks were added.

Table 2

Characteristics of the main and co-feedstocks used in the BMP and pilot experiments, as well as digestates from the pilot experiment. TS and VS were analyzed with an automatic gravimetric device; for the BMP experiment startup, TS and VS were also analyzed with an oven-drying method (the results are presented in parentheses).

		Main feedstock		Co-feedstocks			Digestates		
		CM (BMP)	CM (Pilot)	WS (BMP)	WS (Pilot)	C_SS	BC_W	R1	R2
TS	%	21.9 (20.5)	23.9	94.3 (93.9)	91.4	98.3 (92.1)	96.8 (97.7)	12.5	12.9
VS	%TS	92.7 (91.7)	91.3	92.3 (92.3)	93.2	29.8 (20.0)	96.3 (66.3)	83.5	85.4
C	%TS	48.4	48.0	46.4	46.3	29.8	89.7	46.0	46.1
H	%TS	5.4	5.9	5.7	6.2	0.3	1.5	4.8	5.3
N	%TS	2.1	2.2	0.6	0.6	2.4	1.0	2.0	2.0
S	%TS	0.3	0.3	0.2	0.2	1.6	0.02	0.3	0.3
O	%TS	36.4	34.9	39.5	39.9	-2.2 ¹	4.2	30.3	31.8
ash	%TS	7.3	8.7	7.7	6.9	68.1	3.7	16.5	14.6
Cellulose	g/kg TS	312.2	268.9	433.6	417.6	na	na	265.5	256.7
Hemicellulose	g/kg TS	322.2	271.8	312.2	333.5	na	na	191	228.3
Lignin	g/kg TS	130.4	152.5	64.2	56.7	na	na	155.8	133.1
Fe	g/kg TS	0.7	0.8	0.05	0.04	246	0.2	1.4	2.1
N Kjeldahl	g/kg FM	na	na	na	na	na	na	3.0	2.8
NH4-N	g/kg FM	2.1	5.5	0.5	0.4	0.4	0	1	0.8
P	g/kg FM	0.8	0.8	0.9	0.6	52.8	1.9	0.4	0.3
K	g/kg FM	3.6	3.7	19.5	14.7	2.2	5.9	4.8	5.0
CH ₄ production potential	L/kgVS _{added}	50	na	191	na	0	0	34 ²	51 ²

na = not analyzed.

¹ Negative value indicates incomplete combustion (550 °C, 2 h) in the analysis.

² Result from RMP test.

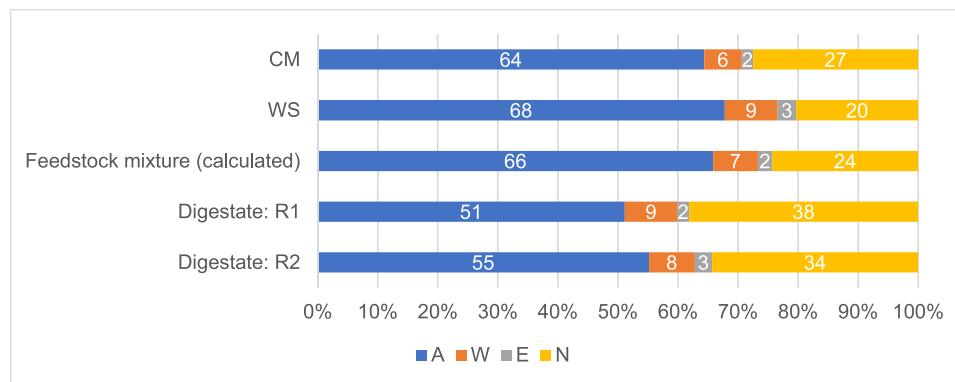


Fig. 3. AWEN extractions (A = acid, W = water, E = ethanol, N = non-soluble) of feedstock materials (cattle manure (CM), wheat straw (WS)), the feedstock mixture (calculated from the C-balance), and the digestates R1 and R2.

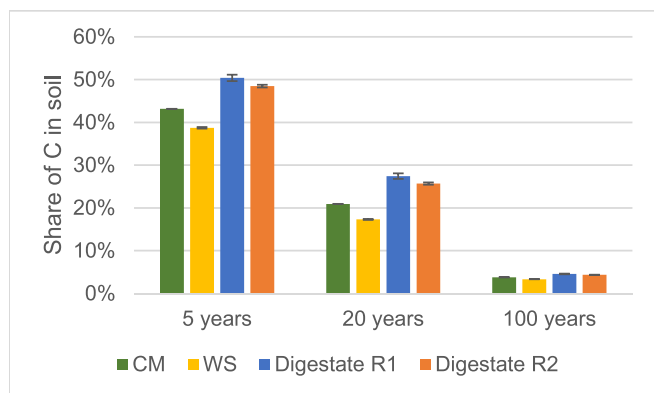


Fig. 4. Share of C in soils after 5, 20, and 100 years after feedstock (CM, cattle manure; WS, wheat straw) or digestate application. Error bars refer to the deviation between parallel samples in AWEN extractions.

CM, WS resulted in the highest methane production potential among the studied co-feedstocks. The studied C-ratio of 20 % corresponded to a WS fresh mass of 5.7 %, which led to a 76 % increase in methane production, while a larger share of WS (40 % of C, 14 % of fresh mass) increased methane production by 189 % (Fig. 2). The increase was mainly connected with the higher share of hemicellulose and cellulose in the WS than in CM. Previously, similar results have been reported with co-digestion of CM and WS (5 % of fresh mass), where shredded straw increased methane production by 29 % (Xavier et al., 2015). Although it may seem that the utilization of WS as a co-feedstock is beneficial in increasing shares, its potential as co-feedstock thus greatly depends on the characteristics of the materials and the BMP of the CM. In addition, the digester type (i.e., restrictions in feedstock TS content) and the availability of WS can limit the feasibility of using high shares of WS as co-feedstock.

The use of co-feedstocks was motivated by the hypothesis that the materials contained organic C forms more stable against the decomposition than CM, which would contribute to the C retention in the digestates. For char samples, it was known that the C after pyrolysis was slowly degradable and more stable when applied to soils (Heikkinen et al., 2021; Joseph et al., 2021). Of the tested chars, SS_C, originating in the pyrolysis of sewage sludge, had lower C-content than in wood-based W_BC biochar (30 vs. 90 % of TS, Table 2), which is typical of sewage-sludge-originating chars (Gopinath et al., 2021), and due to high concentrations of other elements, such as Fe in the sludge. In addition to chemical differences, the physical properties of the chars used can vary significantly, as the micrometer scale porosity of sewage-sludge-based chars (<0.2) is reported to be markedly lower than that of willow-based biochar (0.6, Rasa et al., 2018; Turunen et al., 2021).

Despite the differences in characteristics, the addition of W_BC and SS_C as co-feedstocks resulted in similar methane production potential (Fig. 2), which was negligible compared to the gas production of CM (Fig. 2). This contradicted previous studies in which char additions were observed to increase methane production (Lü et al., 2018; Wang and Lee, 2021). Unlike WS, chars do not contain degradable organic matter that could contribute to the gas production, but their positive effect is connected, for example, with increased buffer capacity, the introduction of trace elements, and microbial colonization due to their porous structure (reviewed in Lü et al., 2018). The reason for the negligible effect could be the amount of added chars and the inhibitive components they contain, for example, aromatic hydrocarbons (Zhao et al., 2021). However, the added amount of chars was unlikely to be the cause of the negligible effect on methane production because similar biochar concentrations of around 8–16 g/L (compared with 2–30 g/L with SS_C, 0.7–11 g/L with W_BC in the present study), have previously been successfully tested (reviewed in Pan et al., 2019). Furthermore, large char additions (>10 % as mass) may not even be practical. First, char,

especially biochar, is often expensive, and the feasibility of large biochar additions is questionable. Second, the addition of large amounts of char, which itself does not produce methane, occupies reactor volume decreasing the amount of other feedstocks to be digested. This can also lead to a decline in biogas plants incomes from gate fees. To be feasible for the biogas plant operator, char addition as a co-feedstock should therefore have measurable positive effects on the methane yield that outweigh negative ones. Another option for gaining the benefits of C sequestration in soils is to apply the digestate and biochar to soils separately.

4.2. Carbon transformations during and after processing

The CM and WS were co-digested in the pilot-scale anaerobic digesters and the production of biogas and the digestate C content and quality were analyzed. In a conventional use case, both CM and WS would end in soils without any treatment and almost all of the organic matter would be microbiologically degraded. According to the C balance, the conventional use of CM and WS would induce slightly higher share of stable C applied to soils (26 %) compared with the utilization of anaerobic co-digestion (21–23 %, Fig. 5). However, the Yasso07 modeling showed that the digestion process resulted in longer retention of the C fractions in the soil (Fig. 4) than in untreated CM and WS. This supports the use of anaerobic digestion as the processing step for CM and WS to assist the buildup of organic C content in soils. In addition, the conventional case fails to capture the volatilizable organic fraction as biogas and gain benefits through the production of renewable energy. In this study, 23–27 % of the C was captured in the biogas. The biogas and its biomethane fraction can be further utilized as a source of energy, while carbon dioxide could also be further valorized into energy or other value-added products by means of methanation (Cordova et al., 2022) to maximize C utilization, for example.

The resulting digestate contained approximately 53–63 % of the C of the feedstocks (CM and WS). According to the AWEN extractions, around 40 % of the digestate C was fairly recalcitrant toward further degradation in soils (i.e., ethanol and non-soluble C fractions). Around 21–23 % of the feedstock's C thus have the potential to enhance the C accumulation and buildup in soils (Fig. 5). Similarly, Nyang'au et al. (2022) reported a study which combined C mineralization data and four-pool C modeling, finding that 12–15 % of feedstock C could contribute to the long-term C sequestration. However, according to the present Yasso07 modeling, it was evident that C in all the studied organic amendments (digestates, CM, and WS) was gradually degrading in soils. The model has been shown to predict the decomposition of digested material, for example, well (Heikkinen et al., 2021). According to modeling, after five years, around 50 % of the applied digestate C will remain in the soil, while after 25 years, only one fourth of the applied C is left. After 100 years, 3–4 % of the added C remained in soils, suggesting still a limited and relatively short-term impact on the SOC pools. However, as such organic amendments are often regularly applied each year, their application has the potential to gradually increase soil C stocks over the long term.

The results also indicated that the share of easily soluble C-fractions (acid and water-soluble fractions) was correlated with the digestates' residual methane potential. The higher share of soluble C fractions led to higher RMP with digestate R2 (see Supplementary Material), which is consistent with the expectation that the easily soluble organic fraction is responsible for the RMP of the digestate. Further on, the easily soluble C fraction degrading in the soils after digestate use could be recovered with a more efficient digestion process and a longer retention time in the reactor (Nyang'au et al., 2022). Both tested digestates had similar RMP (34 and 51 L CH₄/kgVS), which has previously been reached in a similar reactor set-up (horse manure and faba bean co-digestion: 50 L CH₄/kgVS, Pyykkönen et al., 2023).

There was also a notable difference in the digestate C quality (AWEN fractions and RMP) between the digestates from the replicate reactors

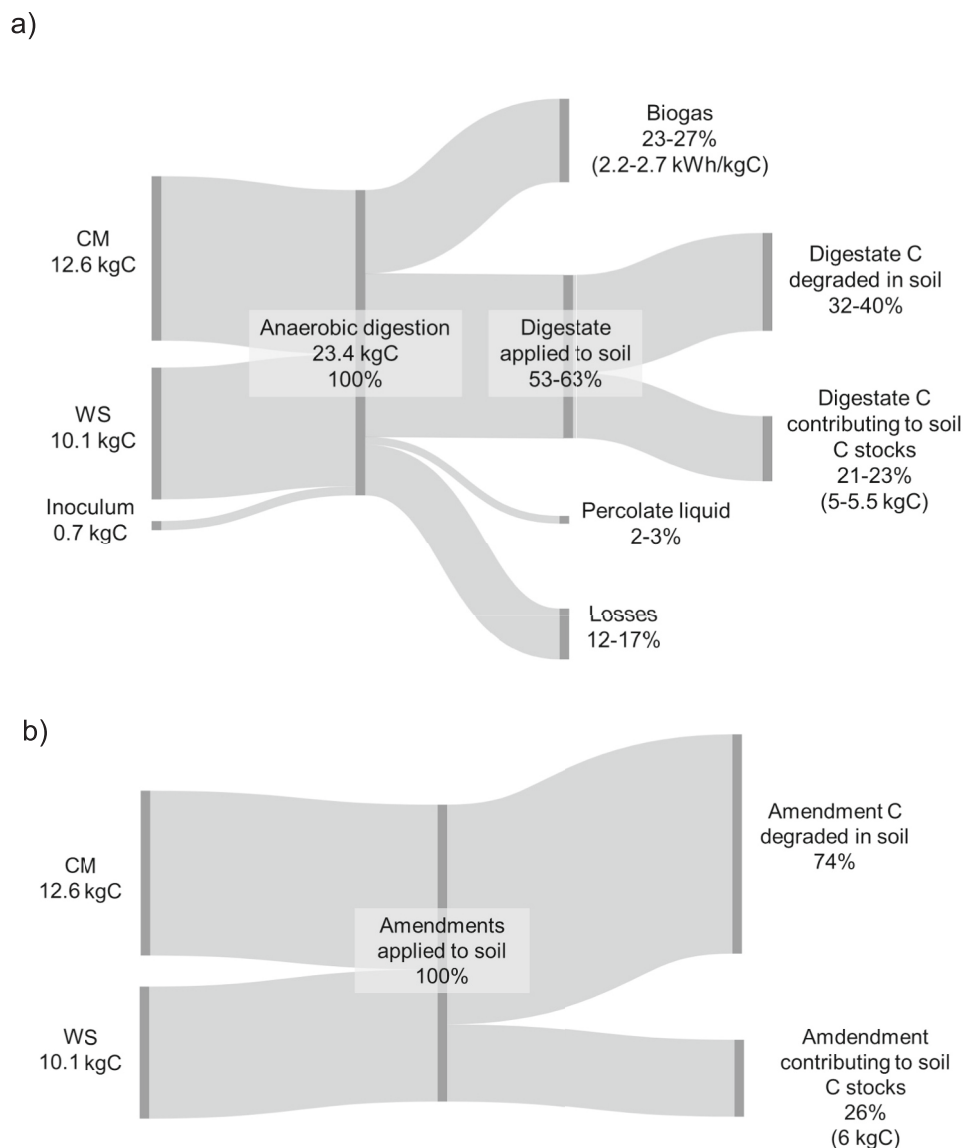


Fig. 5. C balance of a) anaerobic co-digestion of cattle manure (CM) and wheat straw (WS) in a pilot-scale leach-bed reactor, and b) the C balance in a conventional case, where CM and WS are applied as soil amendments without treatment. The amount contributing to soil C stocks is calculated based on AWEN extraction results of ethanol and non-soluble C-fractions.

R1 and R2. It was linked with the changes in process performance (Fig. 3, Supplementary Material), where the reactor R1 produced more methane decreasing the soluble C-fraction in the digestate, as the easily degradable soluble C was transformed into biogas. In R2, the gas production was lower, and the C in the digestate contained more easily soluble C-fractions, which means that the feedstocks were not fully degraded. The cause of the difference between reactors R1 and R2 was traced to the packing of the reactors and use of water to enable circulation of percolate liquid, where R2 was probably more rapidly concentrated with water.

4.3. Digestate fertilizing value

Anaerobic digestion is linked not only to C but also to the recycling and utilization nutrients, for example, N, P, and K. As a co-feedstock, the addition of WS played a minor role in the agronomic quality of the

digestate due to its low share in the feedstock mixture. The combined effect of WS and the digestion process itself had a slightly diluting effect on the N and P content of the digestates compared with raw CM. The dilution effect was mainly due to the reactor configuration, in which some of the soluble fraction will be in the percolate liquid and is therefore not considered in the digestate. However, WS contained a high concentration of K, which increased the digestate's K content, which can be a beneficial aspect in fertilization. For the digestion process, the slight diluting effect of co-feedstocks can also be beneficial for optimizing the C/N ratio. The C/N ratio of the feedstock mixture was 32, where the majority of N originated from the CM. The ratio was in the range of optimal C/N ratios (26–34) for manure co-digestion processes (Ma et al., 2020). Thus, in a review by (Ajayi-Banji and Rahman, 2022) it was implied that even lower C/N ratios were also applicable in solid-state digestion processes due to the microbes' lower energy requirements in a reactor with less microbial mobility.

5. Conclusions

Conventionally, both CM and WS end up in soils as fertilizers or soil amendments without any treatment. This study demonstrated the anaerobic digestion of CM and WS to be advantageous, offering benefits not only for renewable energy production and fertilization but also for C retention and the accumulation of SOC, compared to undigested CM and WS. The positive evaluation stems from the enhanced C quality in digestates, especially the more efficient short-term C retention in soils indicated by the Yasso07 modeling. This positive aspect is considered to outweigh any potential minor adverse effects on digestate nutrient content and the loss of organic matter in biogas that may arise from the digestion. It is crucial for anaerobic digestion studies and projects to consistently consider not only the potential for renewable energy production but also the nutrient and C content of the digestate. A comprehensive approach optimizes the entire process chain and maximizes the benefits of achieving climate change mitigation goals and improvement of soil health.

CRedit authorship contribution statement

Elina Tampio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ilmari Laaksonen:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Karoliina Rimhanen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Niina Honkala:** Writing – review & editing, Investigation, Data curation. **Johanna Laakso:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Helena Soinne:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Kimmo Rasa:** Writing – review & editing, Methodology, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.172083>.

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