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Biochar

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Recovery and composition of biochar after feeding to cattle

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Abstract

To address the urgent need to mitigate agricultural greenhouse gas emissions, research is investigating innovative strategies, including the application of biochar in various agricultural practices. Feeding biochar to cattle is an interesting strategy that not only aims to improve animal health and productivity, but can also have a cascading effect on soil improvement and CO_2 sequestration. Analysing the recovery efficiency of digested biochar and its structural integrity can provide insight into the potential of post-digestion biochar application. Here biochar quantification in dung is investigated for the first time using three different methodologies, namely thermal analysis, elemental analysis, and dichromate oxidation. Results indicate that a relative quantification within ± 1% biochar is possible. The majority of biochar (70–90%) fed to dairy cows survived digestion. The analysis further reveals selective preservation of the most stable condensed aromatic fractions of biochar during digestion, similar to short-term ageing in soil. The remaining digested biochar has an H/C ratio of 0.22 and an O/C ratio of 0.05, meeting the criteria for highly stable biochar. Our findings suggest that the digested biochar is highly suitable for long-term carbon sequestration when applied to soil via manure, offering a promising strategy for compensating agricultural greenhouse gas emissions.

Highlights

- Relative quantification of digested biochar in dung is achieved, with dichromate oxidation being the most accurate method.
- The majority of highly stable fed biochar survives digestion in cattle.
- Digested biochar retains its high stability through the selective survival of the condensed aromatic structure.

Keywords Biochar, Cow, Digestion, CO_2 sequestration, Agriculture

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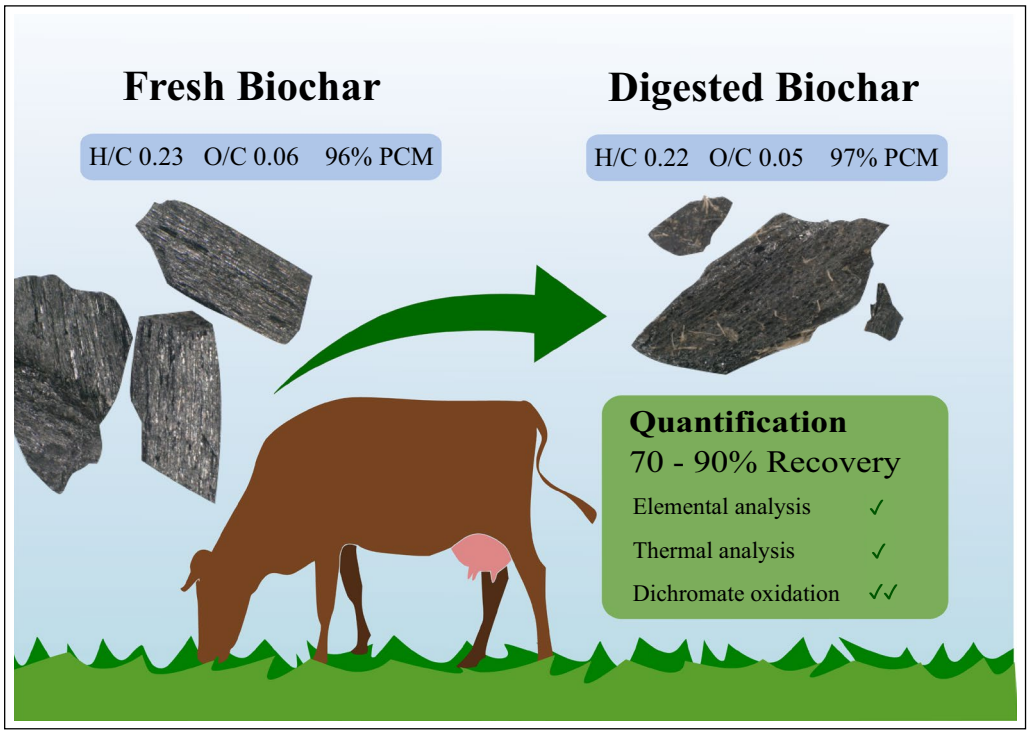
Graphical Abstract



1 Introduction

The global agricultural sector faces significant challenges in mitigating its greenhouse gas emissions while maintaining soil health and fertility. One promising strategy to address these challenges involves the use of biochar—a carbon-rich material produced from the pyrolysis of specific organic biomass—as a soil amendment (EBC 2022). Biochar is widely recognised for its potential to improve soil properties, such as soil aeration and nutrient and water retention, through its porous structure and adsorption capacity (Igalavithana et al. 2018; Schmidt et al. 2021). Furthermore, biochar's ability to store carbon in the soil makes it a valuable tool for sequestering atmospheric CO_2 (Ogawa et al. 2006). Its high stability and resistance to oxidation and microbial decomposition enable biochar to function as a long-term terrestrial carbon sink (Bird 2015; Zimmerman et al. 2013). The most stable carbon in biochar is primarily present in highly condensed aromatic structures, making it less susceptible to breakdown. This condensed aromatic fraction of biochar is commonly referred to as pyrogenic carbonaceous material (PCM) (Bird 2015) or black carbon (Bird et al. 1997; Preston et al. 2006; Schmidt et al. 2000; Scott 2010). The initial feedstock material and the production conditions for biochar, such as temperature and heating time, are decisive for biochar properties (Calvelo Pereira et al. 2011), and different applications potentially require different biochar characteristics (Igalavithana et al. 2018). For long-term soil carbon sequestration, high stability and pyrolysis yield are desirable, as indicated by a high PCM content and low H/C and O/C ratios (Rodrigues et al. 2023).

Innovative approaches to the application of biochar are continuously being explored to optimise its benefits and functional longevity in agricultural systems. An emerging area of interest is the incorporation of biochar into animal feed, which leads to a cascade of functionalities (Joseph et al. 2015; Schmidt et al. 2019). Feeding biochar to livestock, particularly ruminants, has been hypothesised to have various benefits, including improved digestive health and enhanced nutrient uptake (Schmidt et al. 2019). The digested biochar can play a role in long-term fertilisation through the slow release of nutrients stored in a nutrient-rich organic coating (Hagemann et al. 2017). Aside from direct carbon storage in biochar, indigestible biochar particles in dung may stabilise nitrogen and reduce the emissions of ammonia and other greenhouse gases, including methane (Kammann et al. 2017).

The majority of previous research on biochar and animal feeding has concentrated on the direct impacts of

Fig. 1 Microscopy pictures of **A** digested biochar after washing with acetone and water, and **B** digested biochar after washing with acetone, water, and NaOH, Blue bars lower right corner indicate size scale of 100 µm

biochar on animal health and productivity (Schmidt et al. 2019). Critical questions regarding the extent to which biochar retains its structural and functional integrity through the digestive process and the implications for its subsequent use for carbon sequestration have in only been addressed in a few studies (Joseph et al. 2015; Romero et al. 2021). These studies have focused on the quality aspects of biochar, including the costs and benefits of integrating biochar with animal husbandry, and analysing the impact of incorporating digested biochar on manure and soil properties. Joseph et al. (2015) examined digested biochar on properties such as aromaticity, functional groups, and surface properties. They observed minimal alterations in the quality of the high-temperature wood biochar that had undergone digestion, as well as an ageing effect in soil over the course of their three-year study. However, they did not present a mass balance for biochar digestion. Thus, the objective of this study is to address the aforementioned knowledge gap by quantifying the amount of biochar that survives digestion in cows. Additionally, we assess the degree of ageing of the specific biochar applied here during digestion. One of the primary mechanisms of biochar ageing in soils is oxidation, either chemical or biological through microorganisms (Hardy et al. 2017; Joseph et al. 2010; Murtaza et al. 2021). It is anticipated that the oxidative environment and rich microbiome in the digestive system of cattle induce comparable ageing effects on biochar. This may result in alterations to its structural and functional properties and therefore its CO_2 sequestration potential (Joseph et al. 2015). The properties investigated here include elemental composition (H/C, O/C, and C/N ratios), PCM fraction, and thermal stability. The corresponding tested quantification methodologies, namely elemental analysis (EA), dichromate oxidation (DO), and dynamic thermal analysis (TA), reflect the degree of biochar carbonisation, and therefore provide insights into its stability (Bird 2015; Bird et al. 1997; Calvelo Pereira et al. 2011; Hardy et al. 2022). Although these methods have previously been employed for the quantification and characterisation of biochar in soil (Bird 2015; Lebron et al. 2023), this study applies them for the first time for quantification in dung samples, with the objective of evaluating their applicability for this particular material. By evaluating the survival and quality of biochar post-digestion, this study provides insights into the potential of feed-integrated biochar to contribute to climate change mitigation.

2 Methods

2.1 Feeding trial and sample preparation

The analysis presented here is based on a feeding trial with dairy cows that explored the effect of biochar as a feed additive on animal health and performance (Dittmann et al. 2024). The trial was a cross-over study with eight dairy cows, which received biochar mixed into their total mixed ration (TMR, consisting mainly of grass silage, maize silage, sugar beet pulp, and concentrates, corresponding to the recommended ration for dairy cows in Switzerland [Münger et al. 2021]). The content of biochar in the TMR was approximately 1% on a dry matter basis, following producers' recommendations and rates provided in Schmidt et al. (2019). In addition to the TMR, which was offered to the cows *ad libitum*, all animals received the same quantity of concentrate pellets. The feeding experiment was carried out in two trial periods, each lasting 35 days. In the first period, four cows received the TMR with biochar (trial period 1), while four cows received the TMR without biochar. In the second period, the treatments were swapped, and the cows that did not receive biochar in the first period received biochar in the second period (trial period 2) and vice versa. Thus, each cow served as her own control. Each trial period started with a four-week adaptation to the diet, followed by a collection period during which the intake of the cows and the amount of excreted faeces was quantified. Representative samples of feed and dung were taken on a daily basis for five consecutive days. Samples were collected in a pool container and frozen immediately after collection. Samples of dung and TMR were oven-dried at 60 °C for 48 h before further analyses. Further details on the feeding trial are reported in Dittmann et al. (2024).

The dried dung samples of all cows, control as well as dung containing biochar, were mortared and then homogenised and ground to powder using a ball mill for 3 min. The same procedure was performed on the TMR feed of both trial periods. The biochar fed to the cows was from an EBC (EBC 2022) certified producer (APD Auen Pflegedienst, Flaach, Switzerland) using a slow pyrolysis unit (Biomacon, Germany). The source material for the biochar was wood chips from different soft- and hardwooded plants. The main characteristics of the applied biochar were a bulk density of 209 kg/m^3, ash content of 10.6%, particle size distribution of 1%, 23%, 26%, and 50% (for size fractions < 63, 63 – 630, 630 – 2000, and > 2000 µm, respectively), pH ($CaCL_2$) of 9.4, and H/C and O/C ratios of 0.23 and 0.06, respectively.

2.2 Biochar pre paration

Biochar pieces larger than 1 mm were isolated manually from the cow dung and validated by microscopy. In order to obtain clean particles free of dung for further qualitative analysis, we evaluated two different washing procedures. One batch was washed in acetone for 3 h, followed by washing three times in deionised water, and then the biochar pieces were dried at 90 °C. The other batch was additionally treated by stirring overnight in 0.05 M NaOH, followed by 1 h in 0.05 M HCl and 1 h in 0.05 M NaOH (Tsechansky et al. 2014). The biochar pieces were then rinsed with deionised water three times, dried at 90 °C, and separated by size > 4 mm and below. The same washing procedure was applied to fresh, undigested biochar to ensure consistency. Figure 1 exemplifies the resulting particles, indicating a better clean-up with NaOH than with acetone alone. The NaOH-procedure also removed impurities in the thermograms better (Fig. S1) and led to a 4.6% higher C content and 0.04 lower H/C ratio, on average. We therefore followed that procedure for all analyses of biochar in this study.

All biochar samples were homogenised and ground to powder using a ball mill for 3 min. The pH was measured after stirring overnight in a 0.01 M $CaCl_2$ solution in a 1:5 mixture.

2.3 Dynamic thermal analysis

Samples were scanned for their thermal stability using dynamic thermal analysis (TA), including differential scanning calorimetry (DSC), with STA 449 F3 Jupiter (NETZSCH, Germany) after calibration with zinc, tin, indium, bismuth, aluminium, and silver. All samples were diluted with Al_2O_3 and homogenised using a ball mill for 3 min. A total of 20 mg of each sample powder was weighed into an open Al pan and heated under a flow of 60 ml/min synthetic air. The mass loss was detected, and the heat flow rate was calculated based on the difference in temperature between the sample and the empty reference pan. The released CO_2 gas was measured using an LI-820 CO_2 gas analyser (Licor, USA). Peak temperature (°C), height (mW/mg), area (J/mg) and total heat of reaction (J/mg) were determined from DSC thermograms with the Netzsch Proteus Thermal Analysis software.

2.4 Elemental analysis

The elemental composition (C, H, N, and O) of the sample powder was determined by an elemental analyser EA3100 (Eurovector, Italy). Each measurement was calibrated with an acetanilide standard for C, H, and N, and acetanilide and benzoic acid for oxygen. To determine organic carbon (C_{org}) three biochar samples were treated with 6M HCl overnight to remove all carbonate before EA measurement. As carbonate values were ≤ 1% of total C, total C was hereinafter assumed to be all organic and is reported as C. Elemental ratios (H/C, O/C, C/N) were calculated based on atomic weight EA was measured for

1–2 mg of all dung samples, biochar, TMR, as well as pyrogenic carbonaceous material (PCM) remaining in dung samples after dichromate oxidation.

2.5 Dichromate oxidation

Dichromate oxidation (DO) was performed on all samples following Bird (2015) in order to obtain pure PCM. All biochar samples were oxidised after treatment with 6 M HCl. 250 mg of dung samples and 100–200 mg of biochar and feed samples were sonicated at 60 °C for 60 h in 40 ml 0.1 M $K_2Cr_2O_7$/2 M H_2SO_4. If $K_2Cr_2O_7$ was exhausted before the end of the pre-determined oxidation period, observable as green solution, additional acidic dichromate solution was added. The remaining solids were separated from the solution by centrifugation at 4600 rpm for 10 min. The samples were washed three times in distilled water to remove the remaining $K_2Cr_2O_7$ and dried at 30 °C. The samples were weighed before and after oxidation, and the remaining C content was measured.

2.6 Surface area

200–300 mg biochar samples were dried overnight at 60°C under vacuum. The surface area of the biochar samples was then measured by multipoint BET analysis (NOVA2000e, Quantachrome, USA) using 5 measurement points (0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 P/P_0) and the adsorption gas CO_2. The results were selected based on a positive slope and an r value ≥ 0.998. A t-test was performed to assess whether there was a significant difference between fresh and digested biochar.

2.7 Biochar quantification

We applied three different quantification approaches to the analysis of digested biochar based on the above methods (TA, EA and DO) and first tested these methods against a reference series in which we added known concentrations of 3% biochar to the control dung sample from each cow.

While EA and DO could be applied directly (see below), TA required method development because the shape of the thermograms depends on the measurement conditions and provides different types of information. We tested three different heating procedures and two types of integrating thermograms. The heating procedures tested were as follows: A) 20 °C/min from room temperature to 700°C; B) 5 °C/min from room temperature to 600°C; C) 20 °C/min from room temperature to 275 °C, steady temperature for 2 h, followed by heating at 5°C/min from 275 °C to 600 °C. Procedure B was the most viable for the quantification and characterisation of biochar using thermal analysis. Procedure A exhibited stronger variability shown by displaying partially negative biochar values (Fig. S2). The results of procedure C aligned strongly with those of procedure B. Both procedures yielded similar results in terms of peak shape, height, and area for the biochar peak (Fig. S3); however, procedure C requires more analysis time. Tested integration approaches included i) calculating the difference in the biochar peak (370 °C–540 °C) between dung with biochar and the control dung without biochar, and calculating its share of the whole thermogram of burned material (190 °C–540 °C), and ii) calculating the peak height of the biochar peak. Quantification via peak areas yielded more accurate results than quantification via peak heights (Table S1). We therefore used heating procedure B in combination with DSC peak areas for quantification. The average biochar content as determined by this approach was 4.1 ± 0.4% measured biochar in the reference series compared to the 3% that were actually added (Table S2). The analytical error of the calculated biochar percentage was determined to be 0.4% biochar content by repeating the analysis of dung and dung with biochar samples five times.

Using EA, the biochar in dung was quantified by calculating the amount of biochar needed to obtain the difference in C content between dung with and without biochar using the following formula:

$$\% = \frac{100 * (Cb - C)}{(B - C)}$$

where B is the carbon content of pure biochar, C is the carbon content of the control dung, and Cb is the carbon

Table 1 Properties (carbon content, elemental composition, pyrogenic carbonaceous material (PCM), pH, and surface area) of dung samples, fresh biochar, and digested biochar extracted from dung in trial period 1 (P1) and trial period 2 (P2)

Sample	C%	H/C	O/C	C/N	PCM%	pH	Surface area (m^2/g)
Dung control	40.83 ± 0.22	1.67 ± 0.04	0.57 ± 0.00	15.62 ± 0.02			
Dung with biochar	42.07 ± 0.14	1.59 ± 0.03	0.54 ± 0.01	16.27 ± 0.01			
Fresh biochar	82.97 ± 0.32	0.23 ± 0.01	0.06 ± 0.09	238.29 ± 0.01	96.50 ± 0.67	9.4	132.5 ± 2.4
P1 biochar	85.37 ± 0.19	0.25 ± 0.01	0.05 ± 0.01	145.88 ± 0.01	97.16	8.8	135.6 ± 7.9
P2 biochar	84.62 ± 1.26	0.19 ± 0.09	0.05 ± 0.09	124.79 ± 0.02	97.10	8.8	118.9 ± 10.5

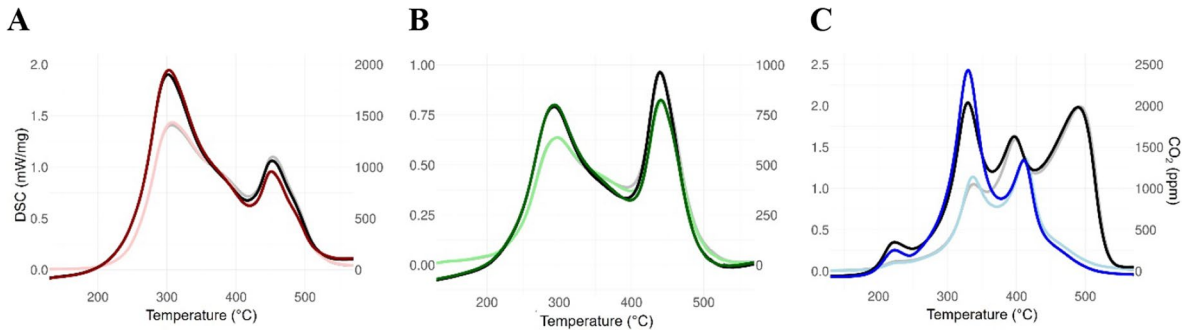


Fig. 2 Differential scanning calorimetry (DSC) (dark) and CO_2 gas (light) patterns of **A** the average of all dung samples diluted to 40% with (black) and without (red) biochar, **B** the average of all TMR feed samples diluted to 20% with (black) and without (green) biochar, and **C** the average of all dung samples oxidized with dichromate and diluted to 40% with (black) and without (blue) biochar (heating rate 5°C/min)

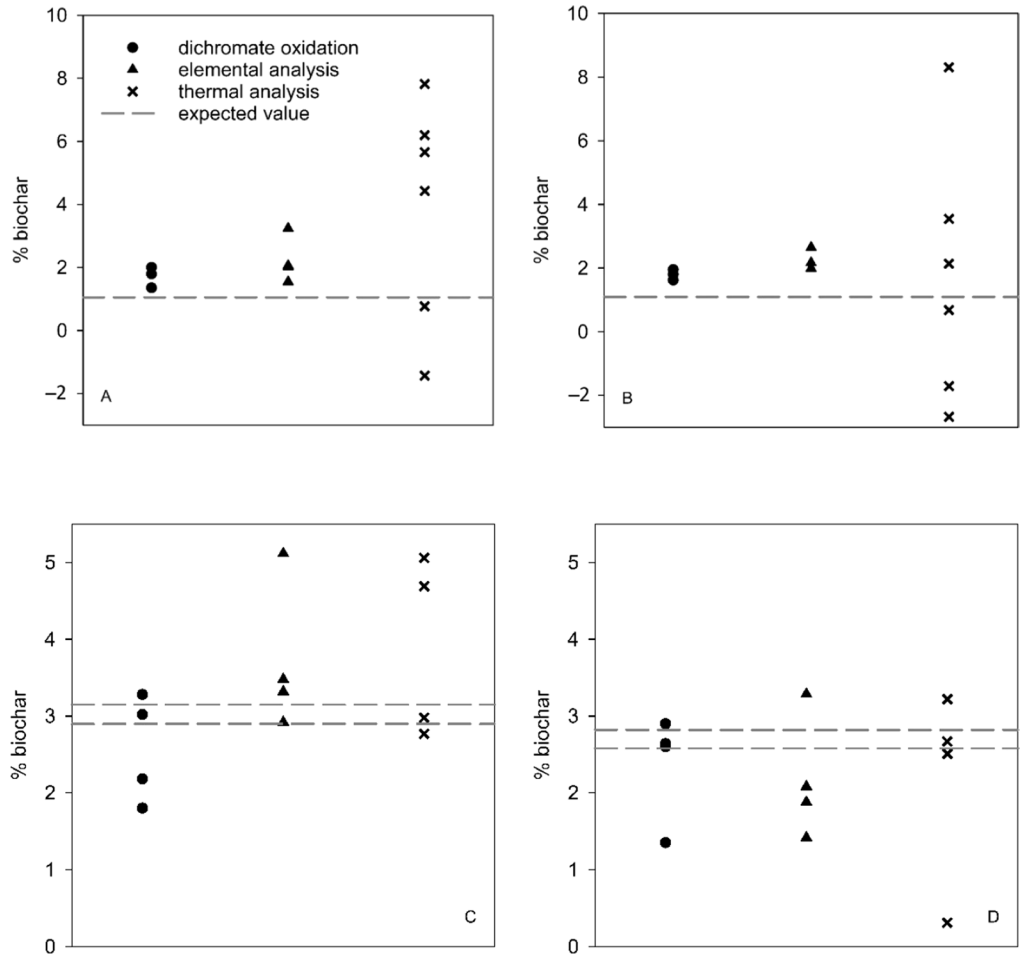


Fig. 3 Expected vs. measured contents of biochar in TMR feed (**A**, **B**) and dung (**C**, **D**). **A** and **B** Expected percent biochar (dashed line, based on known biochar concentration in the feed-biochar mixture) and corresponding results for dichromate oxidation, elemental analysis and thermal analysis (DSC) for TMR feed in trial periods 1 (**A**) and 2 (**B**), respectively. Each symbol represents an analytical replicate of the TMR feed provided in trial period 1 and 2. **C** and **D** Expected percent biochar (two dashed lines, indicating ± one standard deviation of the calculated amount based on the feed intake) and corresponding results for dichromate oxidation, elemental analysis and thermal analysis (DSC) for dung retrieved in trial periods 1 and 2, respectively. Each symbol represents the result for an individual cow.

content of dung with biochar. The reference series with 3% added biochar showed similar results to the quantification with TA, and an average of 4.0% ± 0.9 biochar was obtained (Table S2). The analytical error of EA (n = 4 per sample) was 0.2% C content in dung and 0.6% C content in biochar.

The third quantification method was the calculation of the weight percentage of PCM from the remaining material after dichromate oxidation DO. The oxidation of pure biochar (see Table 1) was taken into account to calculate the expected biochar content from the remaining PCM. The percentage of biochar in dung was calculated from the difference in the remaining mass of the control dung to dung with biochar under the assumption that this difference consists of PCM. The same reference series with 3% biochar in four control dung samples revealed great accuracy, with 2.9% ± 0.3% detected biochar (Table S2). The TMR samples and one dung sample were oxidised and measured three times to calculate the analytical errors of 0.25% for PCM in TMR and 0.09% for PCM in dung with biochar, respectively.

In this study, we compared the results of TA, EA, and DO against the expected values of biochar in dung and TMR feed from the experiment. The expected values of biochar in the dung are based on a weight balance with a known intake of biochar per weight of total dung per cow. This was under the assumption that 100% biochar would remain after digestion. The mean expected biochar content of the dung was 3.0% for the four cows in the first trial period and 2.7% for the four cows in the second period. The anticipated values for the TMR feed were calculated on the basis of the weight percentage of the biochar incorporated into the diet and were 1.05% for period 1 and 1.09% for period 2. These numbers diverge slightly from the actual biochar intake, which was 0.9% for trial period 1 and 0.84% for trial period 2, due to the additional intake of concentrates, which did not contain biochar. A t-test was applied to test the statistical significance of the differences between fresh, digested, and oxidised biochar. All results are presented as mean ± calculated error.

3 Results

3.1 Biochar quantification

3.1.1 Thermal analysis

The characteristic DSC pattern of dung exhibited two distinct thermal peaks (Fig. 2). The most pronounced peak was observed at 301 ± 3 °C with an onset temperature near 200°C. A second peak with a maxima at 453 ± 2 °C and an onset temperature at approximately 400 °C, was identified in the control dung samples. This peak exhibited a strong overlap with the thermal response of biochar, with maxima at 458 ± 4 °C and an onset of 350 °C.

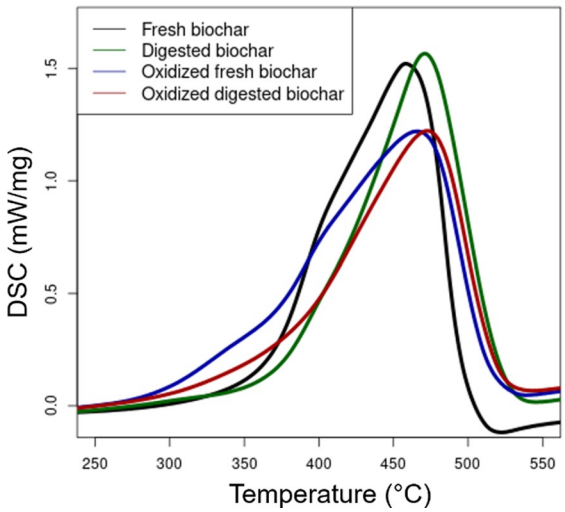


Fig. 4 Averaged differential scanning calorimetry (DSC) thermograms of biochar diluted to 10% undigested (black), digested (green), undigested and oxidised (blue), and digested and oxidised (red) (heating rate 5°C/min) (oxidation with dichromate, see methods)

Table 2 Carbon content and elemental composition of pyrogenic carbonaceous material (PCM) resulting from chemical oxidation of fresh and digested biochar

Sample	C%	H/C	O/C	C/N
PCM fresh biochar	83.09 ± 0.32	0.27 ± 0.04	0.09 ± 0.01	157.24 ± 0.02
PCM digested biochar	75.74 ± 1.99	0.25 ± 0.02	0.16 ± 0.01	126.51 ± 5.53

This peak had the strongest variability between the samples and was highly dependent on the concentration of the sample. An additional, less pronounced peak was detected within the range 350–400 °C, manifesting as a shoulder at lower sample concentrations.

The quantification of biochar in the TMR feed through thermal analysis varied strongly (Fig. 3A, B). The average values of 3.9% for trial period 1 and 1.7% for trial period 2 were much higher than the expected values of 1.05% and 1.09%, respectively. For dung, all samples showed biochar values in reasonable correspondence within the trial periods (Fig. 3C, B). The difference between the trial periods was more pronounced than the difference in expected values, with an average of 3.9% digested biochar for period 1 and an average of 2.2% for period 2. Overall, of all three tested quantification methods, TA quantification had the highest uncertainty.

3.1.2 Elemental analysis

Quantification with EA showed high variability between the individual dung samples. However, a consistent

difference was detected in carbon content between the control dung samples and the dung containing digested biochar (1.2 ± 0.09% higher C content), as well as in the lower H/C and O/C ratio, and a higher C/N ratio (Table 1). The quantification resulted in an average of 3.7% digested biochar for trial period 1 and an average of 2.2% for trial period 2. For the TME feed, both calculated biochar percentages were 1% higher than the expected values with 2.21% and 2.19%, respectively (Fig. 3A, B).

3.1.3 Dichromate oxidation

The results from DO show that, on average, 96.3% of the initial biochar remained as PCM after the applied oxidation procedure. DO did not successfully oxidise the complete dung material. An average of 9.5% material remained after oxidation of the control samples and 11.9% for samples containing dung with biochar (Table S3). Taking the percentage of biochar oxidation into account, the expected biochar content was quantified based on the weight loss difference. The calculated values were in good agreement with the expected values, with an average of 2.6% biochar for cows in period 1 and an average of 2.4% biochar for cows in period 2 (Fig. 3C, 3D). As in TA and EA, the biochar values in the TMR feed (1.71% and 1.79% for the first and second trial periods, respectively) were higher than the expected values (1.05% and 1.09%, respectively). The remaining control material after DO showed a DSC pattern in which the first two dung peaks remained with a right shift of 20–40°C, while the peak in the 420–500°C range, previously overlapping with biochar, disappeared completely (Fig. 2C).

Results of all three quantification methods are summarized in Table S5.

3.2 Biochar quality

The characteristic DSC pattern of biochar exhibits one distinct peak, with peak maxima at 458 ± 4 °C and an onset temperature at approximately 350 °C (Fig. 4). A slight shoulder was visible at a lower temperature of 400 °C, which was variably pronounced in different samples. After digestion, the temperature range of the thermal response of biochar remained mostly the same, with a slight broadening. There was a significant ($p < 0.001$) 24 °C shift of the upper peak edge to higher temperatures, while the shoulder at 400 °C was less pronounced. As shown in Fig. 4, the DSC peaks of oxidised biochar exhibited a similar shift as the digested biochar, with a 23 °C higher ($p < 0.001$) endset temperatures (Table S4). A new shoulder at approximately 340 °C led to an additional significant broadening of 27 °C ($p < 0.01$) at the lower temperature edge of oxidised biochar DSC peaks. Compared to fresh and digested biochar, the thermal energy response of oxidised biochar was lower with the same weight being burned.

The initial C content of the fresh biochar was 82.3% before feeding. After digestion, the biochar C content was 84.9%, with a variation of 0.8% between individual cows. O/C ratios and the average H/C ratio of digested biochars were the same as for fresh biochar, while C/N ratios were generally lower for digested biochar (Table 1). Interestingly, a difference between digested biochar collected from the two trial periods was detected for the C/N, as well as the H/C ratios, with averages of C/N 145.9 and H/C 0.25 for period 1 and C/N 124.8 and H/C 0.19 for period 2.

DO revealed overall very high PCM values in the applied biochar: 96.5% for fresh biochar, and a slightly higher 97.1% for biochar after digestion (Table 2). For both fresh and digested biochar, the H/C ratios increased slightly after DO, indicating a lower aromaticity. Both O/C ratios increased after DO, rising by a factor of 1.6 for fresh biochar and by a factor of 3 for digested biochar, indicating an increased oxidation of the remaining biochar.

The separation of coal pieces by size at approximately 4 mm showed no systematically different results in the TA, surface area, or EA. The observed variations are more likely attributed to the unequal purity of the biochar samples. The surface area did not change significantly during digestion ($p = 0.869$ for trial period 1 and $p = 0.629$ for trial period 2; Table 1).

4 Discussion

4.1 Biochar quantification

Previous reports on soil studies indicate that various techniques used in the same and in different laboratories to quantify biochar and PCM can yield widely divergent estimates, even when applied to the same standard material (Bird 2015). As this effect may also occur for dung samples, reference series were measured to validate each technique used in this study.

The three methods described here reliably quantified known concentrations of biochar in dung to within ± 1%, but all had specific shortcomings. In dynamic TA, the thermal response of biochar overlapped with the combustion of other organic materials in dung, a challenge also noted for quantification in soil (Hardy et al. 2022). For both TA and EA, accurate quantification without a reference sample containing no biochar is not possible because these methods cannot clearly separate dung from biochar. The DO method used in this study also works without a reference. However, it did not fully oxidise the dung, but could potentially be optimised to decompose all dung material. Quantification with differential scanning calorimetry from dynamic thermal

analysis (TA) appeared to be the least reliable quantification method tested in this study. It is important to note that peak height and peak temperature varied significantly across the three experimental methodologies A–C as tested beforehand whereas the onset temperatures of these peaks were more consistent. Given these observations, we recommend that future comparative analyses and studies prioritise the use of onset temperatures over peak maxima for enhanced reliability and consistency. This suggestion aligns with findings previously reported for the dynamic thermal analysis of soils (Lebron et al. 2023). The primary source of inaccuracy in the TA measurements applied in this study is expected to be the pyrolysis of organic material from the TMR feed and the dung samples during the heating process. This effect appeared to be more pronounced in the case of the TMR, which showed a discernible negative correlation observed between the intensity of the DSC thermal responses at 300 °C and that of a thermally more stable material with a peak around 400 °C. This resulted in a high degree of variability and a significant overestimation of biochar in the TMR feed, as illustrated in Fig. 3. The TMR feed supposedly contains a greater proportion of organic material susceptible to pyrolysis during heating. This conclusion regarding the presence of pyrolysis artefacts during thermal analysis is supported by the disappearance of the peak in the temperature range of 450–500°C, which overlaps with the thermal response of biochar in the remaining control dung sample after DO (Fig. 2c). By contrast, dung samples containing biochar exhibited a proportional increase in the thermally stable DSC peak (450–500 °C) after DO. This finding suggests that prior to the TA measurement, there is no chemically stable material (e.g. biochar/char) in the control dung samples that could cause a thermal response at this high temperature, and that the organic dung material prone to pyrolysis during TA is oxidised during the dichromate oxidation. Furthermore, a detection limit issue appeared to be significant for measurements with Procedure A, where the slightly lower expected concentration of biochar in period 2 led to negative measured biochar contents. The fast heating and the higher concentrations used for this procedure favoured pyrolysis during the measurement and therefore falsified the results. By using Procedure B, the TA reference series showed positive results and an overestimation with 4.1 ± 0.4% detected biochar compared to the initial 3% added biochar. Even with higher variation, the average calculated values for digested biochar are in close proximity to the expected values. However, similar to the reference series, for the digested biochar in trial period 1, there were measured values higher than possible according to the weight balance (expected values). Other studies have also reported an overestimation and false-positive results using DSC-TA techniques for biochar in soil (Bird 2015).

Quantification with elemental analysis EA showed a slightly higher variability for the reference series compared to DO, as well as a biochar overestimation by 1% in absolute numbers (33% relative). The measured biochar in dung lies in close proximity to the expected values; however, the difference between the trial periods is more pronounced. In the TMR feed, double the amount of biochar was detected using elemental analysis than expected from the mixing ratio. Since the TMR samples containing biochar were neither digested nor treated in any other way, the higher detected biochar percentage is a sign of inaccuracy either in sample collection and preparation or in the quantification methods.

By using relative quantification, that is, including a reference, acidic dichromate oxidation DO showed the highest accuracy and precision in the reference series, with an average percentage of 2.9± 0.3% measured biochar, and the lowest variability within the cow samples of trial periods 1 and 2. As shown in Fig. 3, the calculated biochar content in both trial periods overlapped with the expected values, while the calculated biochar in TMR was higher than expected, indicating that DO is better suited to quantify biochar in dung than in feed samples. However, similar to soils (Bird 2015), an overlap of the PCM continuum is expected with the organic materials in dung; therefore, clear separation is difficult. An optimisation should retain most PCM while minimising interference from all other dung material. A longer oxidation time, higher temperature, and pre-treatment to remove non-organic material might lead to a clear separation of PCM from dung, leading to a direct quantification method; however, a higher percentage of biochar would be oxidised in the process.

Initially, 1.05% biochar for period 1 and 1.09% biochar for period 2 were mixed with TMR feed and fed to the cows in a mixture with concentrated feed. A difference between the trial periods was initiated in the actual intake of the TMR, which—in relation to the constant amount of concentrates—was lower in period 2 than in period 1. The lower intake of TMR, and therefore biochar, in period 2 can be explained by the cows' progress in lactation, which results in a lower total feed intake. This led to intakes of 0.9% biochar of the total feed for cows in trial period 1 and 0.84% biochar intake for cows in trial period 2. This initially different biochar intake led to detectable different biochar percentages between trial periods in all applied quantification methods. TA measurements appeared to be most affected by differences on this scale of concentrations. For DO, the measured differences lie in close proximity to the expected 0.3% biochar difference in dung.

Despite the shortcomings discussed above, the measured and calculated percentages of digested biochar in dung are in close proximity to the expected values of 100% biochar retention by all three quantification methods (86% average retention according to DO, 102% according to EA, and 106% according to TA). Taking into account the overestimation of biochar in the reference series of up to 33% for EA and TA, these results suggest that the majority, but less than 100%, of the fed biochar persists during digestion in cows. This persistence is expected to depend highly on the quality of the initially fed biochar.

4.2 Biochar quality

Biochars vary in their chemical composition and stability due to varying degrees of aromatic condensation and different proportions of PCM, inorganic ashes, and partially or completely unpyrolysed organic material (Bird 2015). H/C ratios and O/C ratios reflect the degree of aromatic condensation of biochar to some extent and are generally considered to mirror its persistence in the environment (Budai et al. 2013; Rodrigues et al. 2023). The resistance to chemical oxidation with dichromate is negatively correlated with H/C and can also be applied as an indicator of the degree of aromatic condensation and stability of biochar (Hardy et al. 2017). Considering its H/C and O/C ratios, the biochar used in this work was in a range for very stable biochar and was thus suitable for long-term CO_2 sequestration and animal feeding (EBC 2022). The results of the DO support this conclusion with 96.5% chemically stable PCM in the applied biochar.

With ageing, a decrease in total C coupled with a strong increase in total O in biochar is expected, leading to higher H/C and O/C values (Hardy et al. 2017). Biochar digestion by cows was previously found to increase O containing functional groups on its surface (Joseph et al. 2015) similar to ageing in soil, where the introduction of functional groups increased negative charge on the surface, the O/C ratio and enhanced cation exchange capacity (Murtaza et al. 2021). In this study, however, the C content of the digested biochar was slightly higher than in the undigested biochar, and marginally lower O/C ratios were measured. The H/C ratio of digested biochar was, on average, the same as that of fresh biochar, and the PCM fraction was slightly higher in digested biochar. Hence, our data do not suggest pronounced oxidation or ageing of the biochar during digestion. A major difference between the biochar used here and that used by Joseph et al. (2015) was higher initial H/C and O/C ratios in their feeding trial. Additionally, the manual separation of biochar pieces > 1 mm from dung may have induced a selective factor that influences the quality results.

Dynamic thermal analysis is widely applied for studying biochar quality (Harvey et al. 2012; Liu et al. 2022). Hardy et al. (2017) divided the biochar's DSC thermogram into three peaks, where the first thermally less stable peak is significantly correlated with O-rich material. This less thermally stable fraction of biochar exhibited a negative correlation with ageing time in soil, whereas the main biochar peak remained largely unaffected (Hardy et al. 2017). In this study, the first thermally least stable peak was detectable as a shoulder. This shoulder decreased in digested biochar, indicating an ageing effect similar to the observed long-term ageing in the soil. The decrease in the less thermally stable fraction of biochar during digestion might result from a faster decomposition of the already oxidised fraction of biochar, leading to preferential enrichment of the O-poor, more thermally stable fraction. This supports the selective survival of the more stable highly condensed aromatic structure in biochar during digestion, resulting in a higher C content in EA, as suggested above. Consistently, the content of aromatic C, as well as the aromatic condensation, previously quantified using ^{13}C nuclear magnetic resonance spectroscopy, correlates positively with the proportion of thermally stable material (Leifeld 2007). An additional factor is the removal of the organic coating on the biochar surface by the NaOH washing procedure, which may have affected the O/C and C/N ratios and the surface properties (Hagemann et al. 2017). With regard to the assignment of thermal peaks to organic constituents, the most prominent peak observed at 301 ± 3 °C with an onset temperature close to 200 °C has been attributed to the degradation of hemicellulose, cellulose and other organic material. An additional, less pronounced peak detected in the 350–400 °C range, manifesting as a shoulder at lower sample concentrations, has previously been attributed to lignin degradation (Lebron et al. 2023; Ma et al. 2021). However, we refrain from attributing organic components to any of the observed thermal signals, as the thermal signals are influenced by too many other sample properties (Plante et al. 2009).In contrast to the process of digestion, the application of pure chemical oxidation with DO did not selectively reduce the thermally less stable fraction of biochar. Instead, an additional small fraction of even less thermally stable oxidised material was formed. This suggests that the oxidation process does not directly induce complete decomposition of the labile biochar material but rather that oxidation of high-quality biochar is a preliminary step for microbial or physical decomposition, as suggested by (Hammes et al. 2009). Moreover, despite the oxidation process, the measured total carbon content of fresh oxidised biochar remained constant (Tables 1, 2), whereas its O/C ratio showed a slight increase. This supports that most carbon is not lost as CO_2, but instead was

transformed into oxidised forms with induced O, thereby retaining the carbon atoms within the biochar structure. By contrast, the digested biochar lost carbon during the dichromate oxidation and exhibited an increased O/C ratio by a factor of three. This indicates that the preceding oxidation and alteration during digestion decomposed some carbonaceous material during chemical oxidation. Consequently, such altered fractions in digested biochar may also be susceptible to decomposition in soil. However, it should be noted that only 2.9% of digested biochar was decomposed during chemical oxidation, while the rest remained as highly stable PCM.

5 Summary and conclusion

All three methods described in this study reliably quantified biochar in dung with an accuracy of ≥ 1% in relation to a control material. A slight overestimation of biochar content is expected for elemental analysis EA and thermal analysis TA methods. In DSC-TA, in which the biochar percentage was estimated from the additional exothermal heat released by biochar combustion, quantification was the least accurate. In EA, the elemental composition of biochar and the difference in carbon content between control dung and dung with digested biochar were used to calculate the biochar percentage. Using acidic dichromate oxidation DO the weight percentage of pyrogenic carbonaceous material PCM was calculated from the difference in the remaining material from control dung to dung with biochar. The biochar percentage was calculated by accounting for the measured oxidation of biochar. Among all the tested methods, quantification through DO showed the best accuracy and precision. By optimising the DO method to completely decompose the dung material, a direct quantification of biochar in dung—that is, without the need to use control dung samples—could be feasible.

The findings indicate that the majority of the biochar fed in this study survived digestion in cows. A small O-rich fraction of the initial biochar was decomposed during digestion, while the majority of the biochar remained in the category of stable biochar, highly suitable for CO_2 sequestration in soil after manure application. In addition, all three methods provided insight into the characteristics and stability properties of digested biochar. The initial quality of the fed biochar was mostly retained during digestion, with a selective survival of the stable condensed aromatic structure in the biochar. The percentage of biochar remaining stable after digestion, which in this study was, on average, 98% of the biochar (with an overestimation of maximum 33%), is expected to depend highly on the quality and composition of initially fed biochar.

Supplementary Information

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Additional file 1.

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Author contributions
Material preparation, data collection, and analysis were performed by Iva Lucill Walz and Marie Dittmann. Jens Leifeld directed the project and supervised the work. All authors contributed to the study's conception and design. The first draft of the manuscript was written by Iva Lucill Walz, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets used or analysed in this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

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