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Influence of Biogas Digestates on the Composition of Soil Organic Matter

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Abstract

The increasing number of biogas plants with various digested organic materials and output of digestates calls for more detailed investigations of their suitability as soil amendments. In a trial in Northern Germany two replicate plots each were treated with fresh or prolonged fermented digestate to investigate the influence of digestates on the composition of soil organic matter (SOM). The relative proportions of relevant SOM compound classes were determined by pyrolysis-field ionization mass spectrometry (Py-FIMS) and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Using these two methods the influence of digestate on the SOM composition was illustrated by the statistical spectra evaluation by principal component analysis. In both digestate-treated plots, the relative proportions of the slowly degradable alkylaromatic compounds and lipids were larger. In contrast, easily degradable carbohydrates and amides and mostly heterocyclic N-compounds were less than in the control plots. However, these SOM differences due to digestates were less pronounced than differences in the composition of autochthonous SOM in the control plots. In one of the treatments N- and alkylaromatic compounds were more abundant in the aggregate core than on the aggregate surface. In contrast, aliphatic compounds were more abundant on the aggregate surface as disclosed by Py-FI and pyrolysis-gas chromatography-mass spectra (Py-GC/MS). In conclusion, increasing amounts of digestate should be critically monitored according to different digestate feedstock, soil types, ecological factors and crop growth/yield.

Keywords
Biogas; digestate; soil organic matter; mass spectrometry; aggregates

1. Introduction

In the development of renewable energy, increasing numbers of biogas plants for industrial utilization of anaerobic digestion of agro-industrial residues and domestic organic wastes, produce large amounts of highly diverse digestates [1]. Their suitability as alternative fertilizers needs to be tested (e.g., [2-4]). Generally, mineral fertilized has a lower microbial activity than soils that received organic amendments [5]. Soils treated with food waste digestate had lower microbial activity than soil that received cattle slurry as shown for grassland soil in an incubation experiment [6]. This probably was due to the low labile carbon (C) content in anaerobically produced digestates, depending on feedstock and methane yield [7, 8]. Therefore, the hypothesis that digestates alter the SOM composition must be studied.

The fate of organic N-compounds during biogas production was estimated by the difference between the total Kjeldahl N and NH_4^+-N, showing that the organic N content decreased during the anaerobic digestion of a municipal solid waste/bark mixture [7]. This degradation is explained by the mineralization of organic N to NH_4^+-N, the formation of struvite and ammonium carbonate, as well as partial volatilization of N [9]. This mineralization was probably incomplete and the molecular composition of remaining organic N compounds is largely unknown. Furthermore, a possible negative influence of lignin on biogas yield was reported [10], meaning that lignin compounds resist fermentation, remain in the digestate and affect the SOM composition after application to field soils.

Fourier transform-infrared spectroscopy (FT-IR) and thermogravimetric analysis coupled with mass spectrometry (TG-MS) revealed an increase of aromaticity degree and decreases in the contents of volatiles and aliphatic structures, such as fats and lipids during the digestion process of slaughterhouse waste [11], which were previously described as typical for anaerobic stabilization of organic matter [12]. Similarly, increased proportions of heavily degradable aromatic structures at the expense of aliphatic compounds and polysaccharides were disclosed by FT-IR and the fluorescence spectroscopy method excitation-emission matrix (EEM) during the digestion of pig slurry [13].

Another organic fraction, the volatile fatty acids, were analyzed with near-infrared spectroscopy (NIRS) in digestates of corn silage [14], corn silage with added livestock residues [15, 16], pig slurry [17] and mixtures of pig slurry and energy crops [18]. This showed that NIRS can rapidly analyze volatile fatty acids in livestock manure and digestates [19]. The use of this method for the examination of bulk digestate samples or digestate-treated soils has not yet been reported. Liquid chromatography/tandem mass spectrometry (LC/MS-MS) showed the degradation of polyphenolic compounds and toxic flavonoids during the methanogenesis of grape marc in the biogas plant reduced the toxicity of these compounds of the feedstock [20]. However, digestates may contain other potentially toxic substances and pose an environmental risk when used as fertilizers. Cross polarization magic angle spinning ^{13}C nuclear magnetic resonance spectroscopy (CP MAS ^{13}C NMR) confirmed the effects of the individual original feedstocks on the molecular composition of digestates [3]. Furthermore, the determination of various C molecular structures such as short-chain aliphatic C, long-chain aliphatic C, O-alkyl C, aromatic C and carbonyl C in diverse feedstock mixtures and their related digestates showed degradation of aromatic structures and carbohydrates such as cellulose and hemicelluloses during the anaerobic digestion process, thus, resulting in accumulation of recalcitrant aliphatic molecules [21]. This contradicts the above-cited studies by [11] and [13], indicating that it is impossible to predict the effects of digestates on the SOM composition.

Another promising approach to characterize compounds of complex matrices, e.g., municipal solid waste leachates, was described for sulfur compounds, N-compounds, alkylaromatic compounds, lipids, phenols and carbohydrates by using the two independent mass-spectrometric methods Curie-point pyrolysis-gas chromatography/mass spectrometry (Cp Py-GC/MS) and pyrolysis-field ionization mass spectrometry (Py-FIMS) [22, 23]. Py-FIMS of a biogas digestate-amended soil revealed significant changes in SOM composition such as lignin decomposition and increased proportions of N-containing compounds immediately after injection and following tillage [24].

Organic fertilizers, such as composts [25], farmyard manure [26], slurry [6] and sewage sludge [27] can be amended to agricultural soils and improve plant nutrition. In contrast, knowledge about the chemical composition of digestates, possible phytotoxic effects, as shown for biomass residues produced by hydrothermal carbonization [28], and digestate effects on soil organic matter (SOM) quality in agricultural soils is needed.

The fate of organic matter during the anaerobic digestion process, the chemical composition of digestates and the impact of digestate applications need to be studied by advanced chemical analytical methods considering the structural properties of soil, e.g., aggregation. The transportation by mass flow and diffusion inside the aggregates can alter the mechanical strength due to the impacts of hydrophobicity on shear resistance. Furthermore, enhanced problems of dispersion depending on the chemical composition of the digestate soil solution have been reported [29-31]. Increasing soil strength after digestate application could be generally linked with the retarded swelling due to the hydrophobicity of dried soil solution as a varnish coating or film outside the aggregates. This is expected to be most pronounced in the topsoil, or within aggregates, especially directly after the digestate application. The increased concentration of monovalent cations like sodium can result in an enhanced clay mobilization that due to repeated swelling and shrinkage results in clay enrichment at the outer skin of the aggregate. In contrast, the aggregate matrix contains more silt and sand [32, 33]. This clay enrichment would coincide with an aggregate strengthening but at the same time also with a separation of the intra- from the interaggregate pore space. Digestate effects on organic matter in these processes have not yet been studied at the molecular scale.

Thus, the present work aimed to investigate by two independent mass spectrometric methods (i) how digestates affected the SOM composition, (ii) how this effect differs among digestates of different origins, and (iii) the influence of digestates on the organic matter composition at an aggregate core and surface fraction compared to bulk aggregates.

2. Materials and Methods

2.1 Experimental Site

The predominant soil at the experimental site Hohenschulen (Schleswig-Holstein, Germany) was classified as Chromic Luvisol from glacial till; the texture class is strongly loamy sand [34] with 16% clay, 26% silt and 58% sand. The field was cultivated with winter barley in 2016, was fallow in winter 2016/17 and was cultivated in spring 2017 to start the field experiment with sugar beet. Hohenschulen has farmed the arable land since 1989 without plowing, the classical crop rotation consists of winter wheat, winter barley and winter rye. In 2017, sugar beet was cropped. Schematic representations of the test plots are shown in Figure 1 and in the Figure S1.

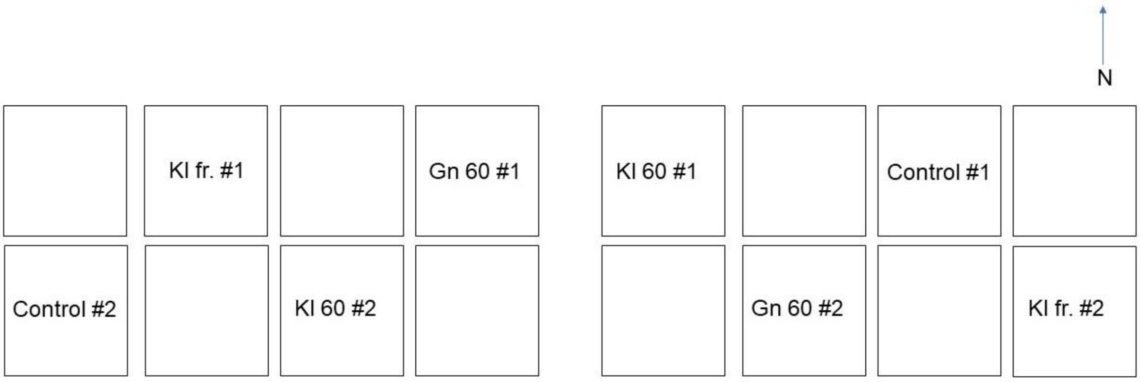

Figure 1 Scheme of the field occupancy with the treatments control (Control #1, Control #2) and the soils treated with additionally (for 60 days) fermented digestates from the biogas facility in Gnutz (Gn 60 #1, Gn 60 #2) and Kletkamp (Kl 60 #1, Kl 60 #2), respectively, and with fresh digestate (Kl fr. #1, Kl fr. #2) from the Kletkamp facility in the test trial Hohenschulen (*n* = 2).

Three different digestates were applied. These originated from Kletkamp (Kl) and Gnutz (Gn) biogas plants. The two biogas plants mainly digested maize silage and various locally available organic feedstocks such as liquid pig manure and other animal excrement or manures. A “fresh” digestate from the biogas plant Kletkamp (Kl fr.) and each one digestate from Kletkamp and Gnutz that after the normal production cycle (resulting in “fresh” digestate with certain residual biogas potential) were incubated in laboratory-scale fermenters at 38°C for a prolonged time of 60 days to determine the residual potential capacity for biogas release. The three digestates slightly differed in composition: Kl (fr.): 6.6% (w/w) dry matter, 4.2 kg total nitrogen m^{-3}, C:N ratio 7.0, 16.8 g P (kg dry matter)$^{-1}$, 59.8 g K (kg dry matter)$^{-1}$, 20.9 g Ca (kg dry matter)$^{-1}$, 6.5 g Mg (kg dry matter)$^{-1}$, 3.2 g Na (kg dry matter)$^{-1}$; Kl (60): 6.0% (w/w) dry matter, 4.4 kg total nitrogen m^{-3}, C:N ratio 5.7, 23.9 g P (kg dry matter)$^{-1}$, 63.4 g K (kg dry matter)$^{-1}$, 22.2 g Ca (kg dry matter)$^{-1}$, 10.3 g Mg (kg dry matter)$^{-1}$, 3.5 g Na (kg dry matter)$^{-1}$; Gn (60): 5.2% (w/w) dry matter, 3.7 kg total nitrogen m^{-3}, C:N ratio 5.5, 27.8 g P (kg dry matter)$^{-1}$, 72.1 g K (kg dry matter)$^{-1}$, 23.9 g Ca (kg dry matter)$^{-1}$, 14.7 g Mg (kg dry matter)$^{-1}$, 4.7 g Na (kg dry matter)$^{-1}$. These digestates were spread on the respective research plots.

On March 28, 2017, each plot of 3 × 3 m size received 40.5 liters of digestate (4.5 L m^{-2}) (control plot: 40.5 liters of water), corresponding to the practice-typical spreading of 45 m^3 liquid manure ha^{-1}. The digestates were applied manually using watering cans. Three different digestates were applied as shown in Figure 1. Immediately after application, plow-less tillage was carried out with a plot cultivator with a working depth of 12 cm to mix the digestate into the upper soil layers. Soil samples were taken from the plots on October 16, 2017, six months after the application of the digestate.

2.2 Preparation of the Aggregate Surface and Core Subfractions

The disturbed soil material from the sampling on October 16, 2017, stored in a fridge, was used for aggregate preparation. Polyhedral aggregates of 1.5 to 2 cm diameter were prepared manually using a scalpel. The aggregates were separated into subsamples of aggregate surface fraction, leaving aggregate core fractions behind. In detail, the aggregate surface fraction (1.5 to 2 mm-surface layer) was scraped and collected in a glass dish. The undamaged aggregate core was also collected in a second glass dish. All samples were then dried in an oven at 40°C.

2.3 Pyrolysis-Field Ionization Mass Spectrometry (Py-FIMS)

For Py-FIMS about 4 mg of the air-dried and extra finely ground (<0.125 mm) and homogenized soil and aggregate samples were degraded by pyrolysis in the ion source (emitter: 4.7 kV, counter electrode -5.5 kV) of a double-focusing Finnigan MAT 95. The samples were heated in a vacuum of 10^{-4} Pa from 50°C to 650°C in temperature steps of 10 K over 15 minutes. Between magnetic scans the emitter was flash heated to avoid residues of pyrolysis products. 65 spectra were recorded for the mass range *m/z* 15 to 900. Ion intensities were referred to 1 mg of the sample. The Py-FIMS method details, including assignment of marker signals (*m/z*) to relevant compound classes (carbohydrates, phenols + lignin monomers, lignin dimers, lipids (alkanes, alkenes, alkanoic acids, alkyl ester), alkylaromatic compounds, N-containing compounds (aromatic N-heterocyclic compounds and nitriles), sterols, nonaromatic amides (peptides, amino sugars) + free amino acids, suberin, and *n*-alkanoic acids from n-C_{16} to n-C_{34}), are described by [35, 36]. Furthermore, close relationships between microbial decomposition and thermal stability in Py-FIMS [36] were reflected by a separation of Py-FIMS thermograms into thermally labile (faster degradable) and stabile (slower degradable) proportions [37, 38]. Therefore, this criterion was used to discuss SOM stability. The thermostability was calculated after dividing the area under the thermograms into the thermally labile (release <420°C) and stabile proportions (≥420°C) using the formula: Thermostability = stabile proportions/(stabile + labile proportions).

2.4 Curie-Point Pyrolysis-Gas Chromatography/Mass Spectrometry (Cp Py-GC/MS)

For Cp Py-GC/MS 15 mg of samples were pyrolyzed at 500°C in a Curie-Point Pyrolyzer Fischer 1040 PSC. Pyrolysis products were separated using a gas chromatograph Varian 3800 (Varian, USA) equipped with a 25 m capillary column coated with a 0.25 µm film thickness of BPX 5 stationary phase (SGE, Australia) and with an inner diameter of 0.32 mm. The injector temperature was adjusted to 300°C. The carrier gas helium 5.0 was set up with a constant flow of 1 mL min^{-1}. Following split injection up to 45 s (splitless), the split ratio was 1:100 from 45 s up to 90 s and 1:5 starting at

90 s. The temperature program for the GC started at 100°C for 5 min, followed by heating at a rate of 5°C min^{-1} to 280°C for 40 min. The GC was connected to a double-focusing MAT 95 mass spectrometer (MS) (Finnigan, Bremen, Germany). Conditions for mass spectrometric detection in the electron impact mode were 4.7 kV accelerating voltage, 70 eV electron energy, 1.2 kV multiplier voltage, *m/z* 48–600 mass range, 0.3 s (mass $decade)^{-1}$ scan rate, and 0.6 s interscan time. Peaks were assigned by comparison of their mass spectra with the Wiley mass spectral library, software edition 6.0. The relative composition of the organic matter is based on the proportion of the peak areas (% of total peak area in chromatogram), representing the thermal degradation products of the organic matter through pyrolysis, summed up for characteristic compound classes that can be paralleled with compound classes from Py-FIMS. This methodological approach was described in detail by [39-42].

3. Results

The soil pH ranged from 6.5 to 6.7, the bulk density from 1.53 to 1.7 g cm^{-3} and the porosity from 35.8 to 42.2 vol.-%. The soil C-content in the Control #1 plot (12.3 g kg^{-1}) exceeded that in the digestate-treated plots Gn 60 #1 (10.4 g kg^{-1}), Kl 60 #1 (10.4 g kg^{-1}) and Kl fr. #1 (12.1 g kg^{-1}). Similarly, the Control #2 had more C (12.1 g kg^{-1}) than Gn 60 #2 (11.6 g kg^{-1}), but less than Kl 60 #2 (15.5 g kg^{-1}) and Kl fr. #2 (15.5 g kg^{-1}) (Table 1).

Table 1 C- and N-concentrations, C:N ratio, pH value, bulk density and porosity of the treatments Control #1 and Control #2, as well as the treated soils with prolonged fermented digestates from the biogas facility in Gnutz (Gn 60 #1, Gn 60 #2) and Kletkamp (Kl 60 #1, Kl 60 #2), respectively, and with fresh digestate (Kl fr. #1, Kl fr. #2) from the Kletkamp facility at the test trial Hohenschulen (*n* = 2).

Nr.	Treatment	C (g kg^{-1})	N_t (g kg^{-1})	C:N	pH ($CaCl_2$)	Bulk density (g cm^{-3})	Porosity (vol.%)
1	Control #1	12.3	1.2	10	6.68	1.57	40.7
2	Control #2	12.1	1.2	10	6.51	1.58	40.4
3	Gn 60 #1	10.4	1.2	10	6.59	1.7	35.8
4	Gn 60 #2	11.6	1.2	10	6.53	1.63	38.5
5	Kl 60 #1	10.4	1.1	9	6.61	1.54	42.0
6	Kl 60 #2	15.5	1.5	10	6.74	1.53	42.2
7	Kl fr. #1	12.1	1.2	10	6.53	1.64	38.2
8	Kl fr. #2	15.5	1.2	10	6.46	1.66	37.4

The Py-FI mass spectra of the control (Figure 2a) and the soil treated with fresh digestates (Figure 2b) are dominated by signals from carbohydrates (*m/z* 84, 96, 110), lignin monomers (*m/z* 194, 208, 220) and alkenes (*m/z* 252, 266, 280, 308, 322, 336, 364, 392). Thermograms of total ion intensity (TII) (inserts upper right) showed, that Control #1 (Figure 2a) had two maxima at the same TII level at 380°C and 420°C. These two maxima are higher in the thermogram of the digestate-treated soil (KI fr. #1), and slightly more pronounced for the maximum at 420°C (Figure 2b). The other samples' Py-FI mass spectra and thermograms were similar, and visual inspection was insufficient to disclose differences between samples. Therefore, principal component analysis (PCA) was applied as a more powerful mathematical spectra evaluation.

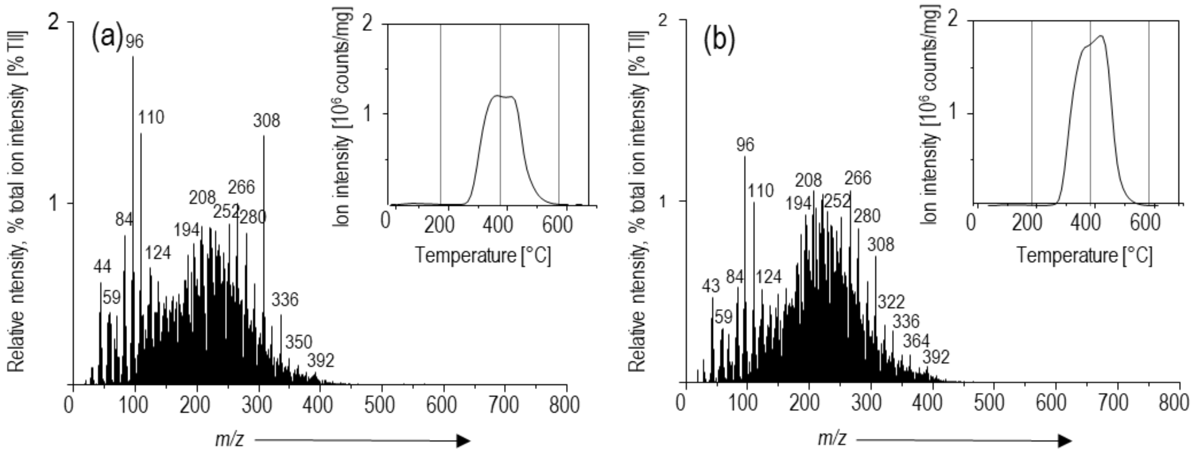

Figure 2 Pyrolysis-field ionization mass spectrum (Py-FIMS) of the treatments (a) control (Control #1) and (b) soil treated with fresh digestates (Kl fr. #1) from the biogas facility in Kletkamp at the test trial Hohenschulen.

The scoreplot of the principal component analysis (PCA) showed a clear separation of the samples from the two experimental plots, independent of the digestate origin and state of fermentation (Figure 3). This illustrates, that the composition of the autochthonous, digestate-unaffected SOM, formed by pedogenesis, historical land use and previous soil management dominated over the influence of all digestate treatments at each of the two experimental plot soils. Subordinate to this overarching site effect, the separation of each control from the corresponding digestate-treated soils, as well as the different treatments from each other, revealed a statistically significant influence of the digestate applications. In treatment 1, smaller relative proportions of carbohydrates in favor of alkylaromatic compounds were responsible for the separation of Control #1 from the digestate-treated soils (Table 2). In treatment 2, the separation of Control #2 from the corresponding digestate-treated soils is induced by larger relative proportions of lipids in the latter (load plot not shown) (Table 2). Moreover, the separation of treatment 1 from treatment 2 originates from higher relative proportions of carbohydrates, amides, N-compounds, phenols + lignin monomers and alkylaromatic compounds (Table 2, load plot not shown). On the contrary, separating the samples of treatment 2 from treatment 1 results in higher relative proportions of lignin dimers and lipids (Table 2, load plot not shown).

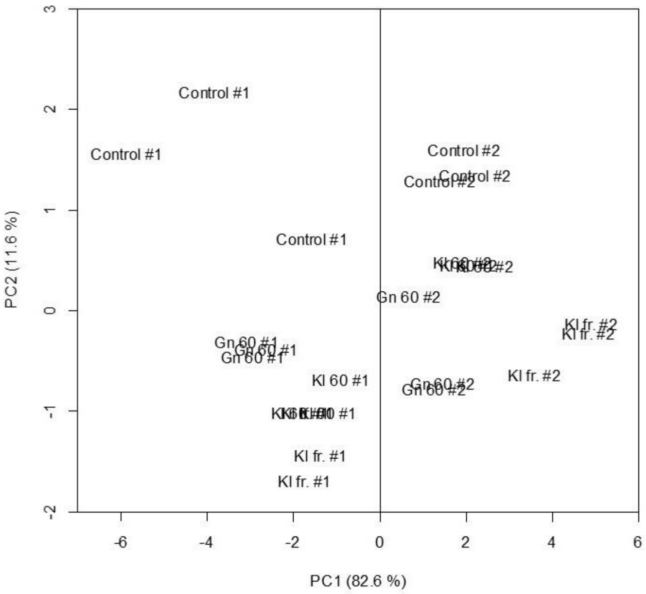

Figure 3 Scoreplot of the principal component analysis of the pyrolysis-field ionization mass spectrometry (Py-FIMS) signals of the treatments control (Control #1, Control #2) and the treated soils with prolonged fermented digestates from the biogas facility in Gnutz (Gn 60 #1, Gn 60 #2) and Kletkamp (Kl 60 #1, Kl 60 #2), respectively, and with fresh digestate (Kl fr. #1, Kl fr. #2) from the Kletkamp facility at the test trial Hohenschulen (*n* = 2).

Table 2 Abundance of compound classes determined by pyrolysis-field ionization mass spectrometry in % TII (total ion intensity) in the digestate treated soils of the treatments Control #1 and Control #2, as well as the treated soils with prolonged fermented digestates from the biogas facility in Gnutz (Gn 60 #1, Gn 60 #2) and Kletkamp (Kl 60 #1, Kl 60 #2), respectively, and with fresh digestate (Kl fr. #1, Kl fr. #2) from the Kletkamp facility at the experimental site Hohenschulen (*n* = 2).

	Carbohydrates	Phenols + lignin monomers	Lignin dimers	Lipids[1]	Alkylaromatics	N-compounds[2]	Amides[3]	Suberin	Free fatty acids[4]
Control #1	10.3	17.2	3.1	7.3	16.4	4.9	7.3	n.d.	0.3
Control #2	6.9	14.3	4.2	8.9	15.1	3.1	5.5	0.1	0.8
Gn 60 #1	9.0	17.3	3.1	8.2	17.6	4.4	6.6	n.d.	0.3
Gn 60 #2	6.6	15.1	3.8	9.2	16.8	3.3	5.5	0.1	0.6
Kl 60 #1	7.8	17.0	3.4	8.5	17.5	3.8	6.0	n.d.	0.5
Kl 60 #2	6.1	14.8	3.8	8.9	15.5	2.7	5.1	0.2	1.2
Kl fr. #1	7.7	17.4	3.2	8.4	17.9	3.7	5.9	n.d.	0.5
Kl fr. #2	5.4	13.0	5.1	10.2	16.4	2.6	4.5	0.1	0.7

[1] Lipids: alkanes, alkenes, alkanoic acids, alkyl ester;

[2] N-containing compounds: aromatic N-heterocyclic compounds and nitriles;

[3] Amides: nonaromatic amides (peptides, amino sugars) and free amino acids;

[4] Free fatty acids: *n*-alkanoic acids from *n*-C_{16} to *n*-C_{34}, n.d.: not determined.

The relative proportions of SOM compound classes (Table 2) showed that phenols + lignin monomers and alkylaromatic compounds were most abundant in all samples at approximately the same proportions. Whereas in the samples of treatment 1 the proportions of the phenols + lignin monomers were nearly unchanged, in treatment 2 the proportions of this compound class were slightly higher than in Control #2, except for Kl fr. #2. In treatment 1, the lignin dimers remained unchanged and in treatment 2 they were lower in the treated soils, except for a higher proportion in the soil Kl fr. #2. The proportions of alkylaromatic compounds and lipids in both treatments exceeded those of their corresponding controls. Conversely, the relative proportions of carbohydrates, amides and N-compounds (except Gn60 #2) were smaller in the digestate-treated soils.

The data evaluation of Py-FIMS from aggregate core and surface fraction samples showed a clear separation for samples that received additionally fermented digestate from the biogas facility Kletkamp (Kl 60 #1) by computing a PCA with the 465 *m/z* values. This indicates differences in the organic matter composition of the aggregate core fraction (Kl 60 #1i) and the corresponding aggregate surface fraction (Kl 60 #1o) and the bulk aggregates (Kl 60 #1w) (Figure 4). Such a clear separation was not obtained for the aggregate samples from plot Kl 60 #2. Furthermore, the abundance of aliphatic compounds, N-compounds and alkylaromatic compounds as determined independently by Py-FIMS and Py-GC/MS (Figure 5), showed in good agreement that the aggregate surface fraction had higher proportions of aliphatic compounds and lower proportions of N- and alkylaromatic compounds, when compared to the aggregate core fraction.

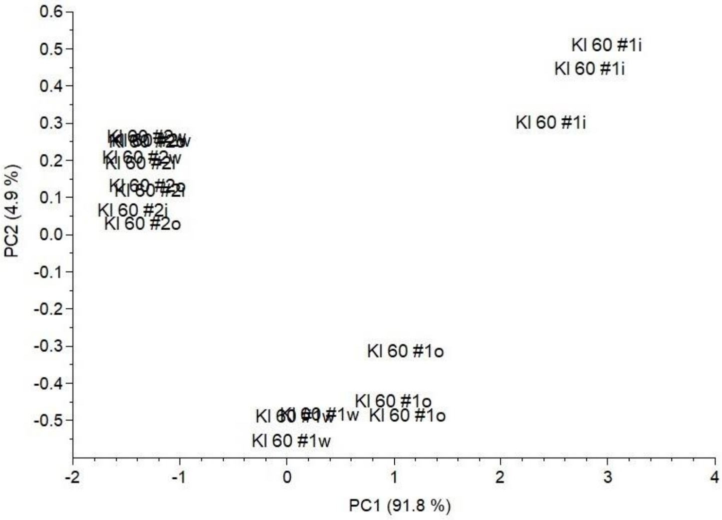

Figure 4 Principal component analysis of the 465 *m/z* pyrolysis-field ionization mass spectrometry (Py-FIMS) signal values with significant differences between the aggregate core fraction (Kl 60 #1i, Kl 60 #2i), aggregate surface fraction (Kl 60 #1o, Kl 60 #2o) and bulk (Kl 60 #1w, Kl 60 #2w) aggregate treatments of additionally fermented digestate from the biogas facility in Kletkamp at the test trial in Hohenschulen.

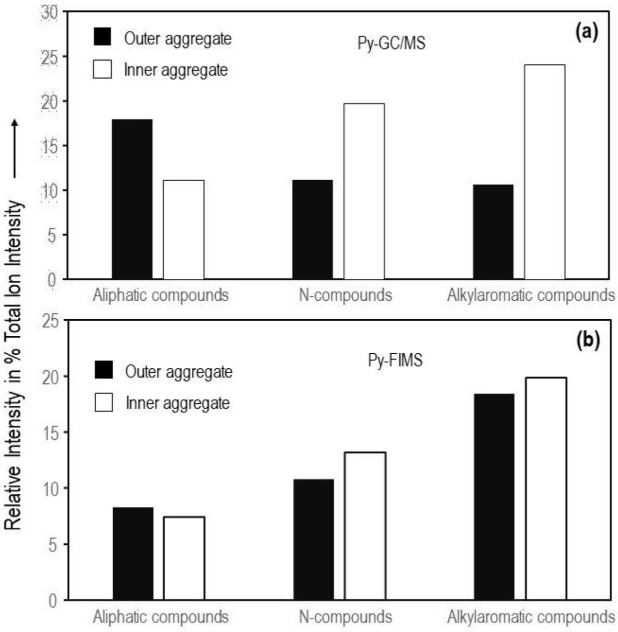

Figure 5 Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) (a) and pyrolysis-field ionization mass spectrometry (Py-FIMS) (b) of the aggregate core fraction (Kl 60 #1i) and aggregate surface fraction (Kl 60 #1o) in the treatments with prolonged fermented digestate from the biogas facility in Kletkamp at the experimental site in Hohenschulen. (Compound class proportions of total ion intensity in %).

4. Discussion

A possible explanation for the lower C-content, contrary to the input of additional organic matter can be an increased microbial activity, induced by the digestates (Table 1). This eventually resulted in increased degradation of the original SOM [43]. On the other hand, larger C-contents in Kl 60 #2 (15.5 g kg^{-1}) and Kl fr. #2 (15.5 g kg^{-1}) in comparison to Control #2 (12.1 g kg^{-1}) indicated, that organic matter enrichment exceeded the possible activation of aerobic microbes, eventually due to a generally lower level of microbial abundance and activity in plot #2 [43]. Furthermore, since it is known, that anaerobically produced digestates have a lower content of labile carbon (C) than organic and mineral fertilized soils [7, 8], the generally lower C-contents in the treated soils 1 (Control #1: 12.3 g kg^{-1} vs. Gn 60 #1: 10.4 g kg^{-1}; Kl 60 #1: 10.4 g kg^{-1}; Kl fr. #1: 10.4 g kg^{-1}) may have originated from a higher amount of degradable C in comparison to treatment 2. Here, the treated soil Gn 60 #2 (11.6 g kg^{-1}) had a lower C-content than Control #2 (12.1 g kg^{-1}). The higher C content in Kl 60 #2 (15.5 g kg^{-1}) and Kl fr. #2 (15.5 g kg^{-1}) indicated a reduced microbial activity in digestate-treated soils as described by [6].

If considering the most intensive signals of the Py-FI mass spectra, the digestate-treated soils under study agreed with a farmyard manure (FYM) treated soil [44] and a digestate-treated soil [24] (Figure 2). The agreement is particularly obvious for the abundance of various lignin monomer indicators among which *m/z* 208 was prominent in all three spectra. The predominant signals of carbohydrates in the soils under study, *m/z* 84, 96, 110 (Figure 2), were also present in an FYM treated soil [44] and a digestate-treated soil [24] with additionally intensive *m/z* 126 and 162. The indicator signals of alkenes *m/z* 252, 266, 280, 308, 322, 336, 364, 392 were also predominant in a digestate treated soil [22], whereas alkenes were not predominant in a FYM treated soil [42]. The digestate-treated soils under study (Figure 2) had fewer signals of peptides, which appeared more prominent in a FYM-treated soil [44] and digestate-treated soils [24].

Comparison of the TII-thermograms showed maxima for the labile proportions at approximately the same narrow pyrolysis temperature range in the digestate-treated soils under study (380°C), farmyard manure (FYM) treated soils [44] (390°C) and digestate treated soils (390°C) [24]. When compared to the labile portions, differences in the pyrolysis temperature were obvious at the maxima of the stabile proportions and the intensity. The maximum for the stabile proportions was at 420°C. It exceeded the maximum for labile proportions in the digestate-treated soils under study (Figure 2), whereas in farmyard manure (FYM) treated soils [44] the maximum was at 480°C and at the same level as the labile proportions. The maximum for the stabile proportions in the digestate-treated soils by [24] was at 490°C and lower than that of the labile proportions. Thus, the present Py-FIMS investigation indicates some general agreement but also some differences to previously investigated soils that received organic amendments, even those receiving digestates.

The separation of the Py-FIMS thermograms into thermally labile and stabile proportions is based on findings concerning microbial decomposition in dissolved organic matter [38], thus allowing to distinguish between slower and faster degradable organic matter portions [39]. Consequently, the thermogram of the digestate-treated soil (KI fr. #1), especially the slightly higher maximum at 420°C, indicated that overall rather slowly degradable compounds had been relatively enriched in the SOM by the input of digestate-organic matter. This can be interpreted by preferential microbial decomposition of labile organic matter originally abundant in biogas feedstock but also in soil, eventually fuelled by the easily available major nutrients in the digestates such as N-containing ions, potassium and phosphates [45].

A direct comparison of the data in Table 2 with results from a short-term digestate application study [24] showed the same enrichment trend for alkylaromatic compounds but the opposite trend for all other compound classes in the digestate-amended soils. The decomposition of labile carbohydrates explains lower carbohydrate proportions in present digestate-treated soils [46]. This is surprising if one considers reports of reduced microbial activity in anaerobically produced digestates [7, 8]. Conversely, the higher proportions of alkylaromatic compounds and lipids in the digestate-treated soils indicated an overall higher stability of SOM and resistance against microbial decomposition. The high proportions of alkylaromatic compounds in the soils under study agreed with those in six particle-size fractions of pig slurry [47]. In that study, higher proportions of lignin dimers (7.0 to 15.4%) relative to lignin monomers (4.5 to 12.3%) were reversed to the digestate-treated soils (lignin dimers: 3.1 to 5.1% vs. lignin monomers + phenols: 13.0 to 17.4%) (Table 2). These more detailed insights at the molecular level complement soil C stock predictions based on different crop residue recycling streams and compound recalcitrance [48].

The disagreeing results of the Py-FIMS from aggregate core and surface fraction samples, i.e., the nearly identical organic matter composition of the aggregate core and surface fraction and whole aggregate of Kl 60 #2 versus a different organic matter composition in separated aggregate core and surface fraction and the aggregate bulk samples of Kl 60 #1 (Figure 4) are difficult to explain. First of all, it may be that the manual separation of aggregate spheres has been incomplete in the one (Kl 60 #2) and more complete in the other sample (Kl 60 #1). Furthermore, it has been reported that different soils reflect the chemical properties of the aggregate surface and core fractions differently (e.g., [49, 50]). This would mean that the disagreeing results may arise from differences in soil properties at the aggregate scale. On the other hand, [51] reported that the distribution of dung-derived organic matter between aggregate surface and core spaces was a time-dependent process, indicating that the SOM composition in the samples, with or without differences between aggregate surface and core fractions, may be related with the period between digestate application and soil sampling.

The larger proportions of N-compounds and alkylaromatic compounds, probably originating from digestates (Table 2), in the aggregate core fraction at the expense of aliphatic compounds in the aggregate surface fractions in plots from Kl 60 #1 (Figure 5), correspond with the finding that soluble C from dung was only initially preferentially attached to aggregate surfaces followed by incorporation into aggregate core spaces [51]. However, these authors also reported that the amount of dung sequestered in macroaggregates accounted for only 32–66% of that in the whole soil. Reference [52] described soil C enrichments in aggregate core fractions and microbially more processed organic matter at aggregate surfaces, confirmed by enrichments of relatively recalcitrant N-compounds and alkylaromatic compounds in the aggregate core fraction of the Kl 60 #1 sample but more aliphatic compounds outside. The high proportions of lipids in the aggregate surface fraction can increase hydrophobicity, thus leading to pore clogging and restricted soil solution organic matter transfers into aggregate core spheres. Considering particle rearrangement and separation processes that lead to a finer texture outside aggregates [53], more mineral-stabilized organic matter may be at the aggregate surface fraction. By contrast, the compounds enriched in the aggregate core likely have a stronger inherent recalcitrance due to their chemical structure than labile products of microbial metabolism and aliphatic compounds, which may be stabilized only by associations with clay minerals at the aggregate surface fraction irrespective of contact with plant roots. Furthermore, the high proportions of greatly processed microbial degradation products or remnants of microbial transformations, such as largely heterocyclic N-compounds and alkylaromatic compounds in the aggregate core fraction, possibly can be explained by still active anaerobic Archaea from the biogas reactor [54]. These Archaea communities may survive not at the temporarily drier aggregate surface but in the wetter core spaces. This speculation, however, needs to be tested by microbiological investigations.

5. Conclusions

1. The chosen methodological approach with the two independent mass spectrometric methods Py-FIMS and Py-GC/MS was well suited to detect and release relative proportions of compound classes that reflect the influence of biogas digestates on the composition of SOM.
2. Since the composition of the autochthone SOM dominated the influence of all digestate treatments on each of the two experimental plots, it can be concluded that digestate application has a little short-term effect on SOM chemistry and storage. Hypothetically, this can be explained by promoting the SOM mineralization with additional inputs of easily available nutrients like nitrate, ammonium and phosphate, counteracting any additional SOM formation.
3. Because of the clear SOM differences in aggregate core and surface in one digestate-treated plot but not in the other we conclude that small-scale differences in aggregate-affecting soil properties, like, e.g., texture, as well as the time-dependent processes of SOM incorporation into aggregate cores likely govern the distribution of the organic matter in aggregates.

4. In summary, considering the increasing digestate application in agricultural practice which here yielded disagreeing results for materials of different origins and different field plots, it appears to be more challenging than traditional soil amendments such as farmyard manure or slurry applications for which long-term and consistent experimental data are at hand. Therefore, digestate application needs to be evaluated critically for different soil types, feedstocks of digestates or fermentation durations, and possible adverse effects on soil organisms, fundamental soil fertility features and field crop performance, and needs eventually regulated by governance.

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Author Contributions

Gerald Jandl: Analyzed the samples with Py-GC/MS, evaluated the data and wrote the manuscript. Peter Leinweber: Wrote the manuscript. Kai-Uwe Eckhardt: Analyzed the samples with Py-FIMS and evaluated the data. Rainer Horn: Supervised the project. Richard Schroeder: Carried out the design and took the samples.

Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: Division of the individual plots in a diagonal arrangement (upper left), incorporation of the fermentation residues directly after application (below left), localization of the experimental areas in the field (upper right), trial area after cultivation (below right).

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