



Pyrolysis-MS and FT-IR analysis of fresh and decomposed dairy manure

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Abstract

The ability to predict manure nitrogen mineralization is essential for optimizing crop growth while preventing N losses to the environment. However, estimating mineralizable manure N is problematic because of the wide variety of organic manure N forms, as well as the lack of a rapid standardized method. Fourier-transformed infrared spectroscopy (FT-IR) is a promising technology, since it can detect the absorbance of proteins and primary amines, in addition to being instantaneous and non-destructive. Likewise, analytical pyrolysis-mass spectrometry (Py-GC/MS) has been used to study lignin and protein composition of forages. We carried out a 10-week laboratory incubation of manure-amended soil in order to test the sensitivity of FT-IR and Py-GC/MS to detect changes in manure during decomposition in soil. Four different dairy or beef manures were included. The manures (0.15 mg manure-N g⁻¹ soil) were placed inside mesh bags during the soil incubation to allow for the recovery and analysis of fresh as well as decomposed manure. Infrared spectroscopy of the fresh and incubated manures showed that this technique is sensitive to changes in manure organic N after soil application. Bands associated with primary amines decreased during the incubation, while bands associated with proteins increased during manure decomposition. Bands associated with fatty acids tended to decrease during the incubation, possibly due to utilization as C sources. The spectroscopic data also showed that lignin-specific signals increase during manure decomposition, suggesting that the relative amount of lignin in manure increases as it decomposes in soil. The changes in peak areas of pyrolyzates were related to changes in manure constituents during decomposition. The Py-GC/MS data showed qualitative changes in manure lignin during decomposition. The relative amounts of nitrogen containing pyrolyzates, such as phenylacetonitrile and methylindole, changed during the decomposition, but the changes were not consistent across manures. In summary, this experiment showed that the use of manure bags is valuable in discerning between the N cycling dynamics of manure and soil, and that infrared spectroscopy, as well as Py-GC/MS show potential as analytical tools to study manure decomposition in soil.

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1. Introduction

Animal manure can be an important amendment in sustainable agricultural systems by providing nutrients for crops as well as increasing soil organic matter. The beneficial effects of manure can be seen for decades after a regime of manure application [1]. Recently, the negative

environmental impacts of manure mismanagement have received notoriety. Manure NO₃ is prone to leaching and can be an important source of pollution to groundwater [2,3]. To achieve a balance of agronomic benefit and minimal environmental pollution, it is necessary to have accurate estimates of the potentially available nutrients in manures and use this knowledge to make correct management decisions.

Animal manure is made up of a diverse set of components including partially digested feed, cells and bedding, each with different mineralization properties ranging from recalcitrant lignin to labile N pools [4]. Because of this,

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manures were stored in a desiccator at room temperature. The homogenization with dry ice resulted in fine manure solids, so the manures did not need to be ground after lyophilization. Lyophilized samples were scanned in the mid-infrared from 4000 to 400 cm^{-1} (2500–25,000 nm) on a DigiLab FTS 7000 FTIR spectrometer, using KBr as background reference.

2.2. Py-GC/MS

Duplicate subsamples (1 mg each) of lyophilized manure were pyrolyzed at 600 °C for 30 s using a AS-2500 Pyrolysis Autosampler (Chemical Data systems, Oxford, PA) fitted with a quartz pyrolysis chamber surrounded by a platinum coil. The pyrolyzer was interfaced at 200 °C with a GCQ Benchtop Ion Trap GC/MS (Thermo Finnigan, San Jose, CA). The operating conditions were as follows: splitless injection, injector temperature at 300 °C, J&W DB-5 ms column (30 m \times 0.25 mm i.d., 0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA). The temperature

program started at 50 °C (held for 10 min) and increased at 5 °C min^{-1} to 300 °C (held for 10 min). The He carrier gas was set at a constant velocity of 40 cm s^{-1} . The transfer line temperature was 300 °C, and mass spectral scan from 40 to 650 m/z , recorded (1 scan s^{-1}) at 70 eV. The peak identification was carried out by comparison of each peak's mass spectrum to library spectra [21].

2.3. Principal components analysis

We used the proc PRINCOMP procedure of SAS Version 8.02 (Cary, NC, USA) in order to illustrate the changes in the FT-IR spectra and Py-GC/MS during manure decomposition. All spectral data were converted to percent of total absorbance, and all Py-GC/MS data was converted to percent of total peak height. All principal components were computed from the covariance matrix, since all the data used for each principal components analysis (PCA) had the same scale. A total of 35 pyrolyzates were used to carry out the PCA analysis (Table 1). The PROC CORR of SAS Version

Table 1
Identification of Py-GC/MS products of manures and feeds

	Pyrolyzate	R_t (min)	Ions (m/z) ²
1	Unknown 1	2.8	43, 61, 75, 45, 41
2	Toluene	4.5	91, 92, 65, 39, 63, 51
3	Furfural	6.1	96, 95, 67, 42
4	Unknown 2	7.5	43, 55, 60, 91, 106, 81
5	Unknown 3	8.6	43, 78, 104, 42, 55
6	Unknown 4	11.6	41, 55, 70, 42, 43
7	Phenol	15.3	94, 66, 39, 65
8	Unknown 5	15.8	67, 91, 79, 80, 77, 93
9	Unknown 6	16.8	84, 112, 41, 55, 42, 91
10	Unknown 7	17.8	77, 108, 79, 107, 41, 89
11	Guaiacol + methylphenol ^a	18.8	129, 124, 81 and 107, 108, 77, 79, 80, 51
12	Phenylacetonitrile	20.0	90, 117, 89, 116
13	Unknown 8	20.2	126, 43, 55, 83, 97, 41
14	A dimethylphenol	21.3	107, 122, 77, 103
15	Methylguaiacol	22.2	138, 123, 95, 67
16	Unknown 9	23.0	43, 41, 57, 55, 42
17	Vinylphenol	24.0	51, 65, 91, 120
18	Ethylguaiacol	25.2	137, 152, 122, 91, 77
19	Vinylguaiacol	26.3	150, 135, 77, 107
20	Eugenol	27.4	164, 77, 103, 149, 133
21	Syringol	27.4	154, 65, 93, 139, 96, 111
22	Methyl indole	28.2	130, 131, 77, 103, 51
23	<i>cis</i> Isoeugenol	28.4	125, 180, 91, 41, 69
24	Vanillin	28.7	151, 152, 109, 53, 65, 137
25	Methylsyringol + <i>trans</i> isoeugenol ^a	29.9	168, 107, 153, 125 and 164, 149, 77, 103, 131, 121
26	Unknown 10	30.6	55, 41, 69, 67, 83, 97
27	Unknown 11	30.8	41, 57, 43, 71, 85
28	Ethylsyringol	31.7	167, 182, 79, 107, 137, 151, 95
29	Vinylsyringol	32.8	180, 165, 137, 91, 77, 122
30	Allylsyringol	33.6	194, 77, 119, 179, 131, 103, 147
31	<i>cis</i> Propenylsyringol	34.8	194, 119, 179, 77, 131, 151
32	<i>trans</i> Propenylsyringol	36.0	194, 119, 179, 77, 131, 151
33	Unknown 12	36.3	41, 55, 111, 70, 69
34	Unknown 13	38.7	67, 95, 81, 41, 55, 123
35	Unknown 14	39.5	67, 81, 95, 79, 41

All are unconfirmed library search results. Unknowns are unresolved molecules or mixtures of unknown molecules.

^a Unresolved mixture of methylsyringol and *trans* isoeugenol.

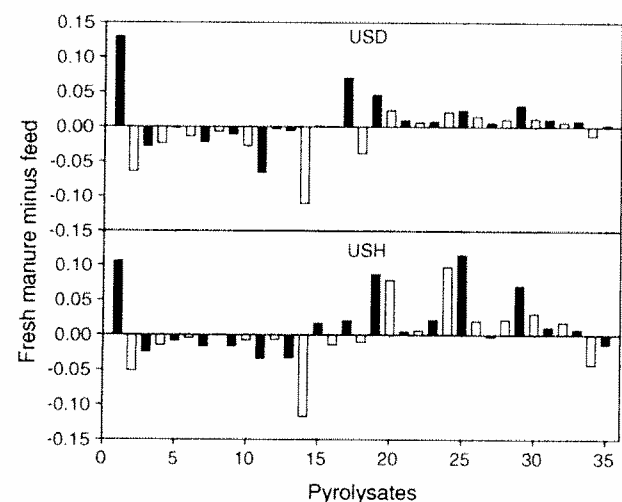


Fig. 8. Subtracted pyrolysates between fresh manure (time 0) and feed. The x-axis numbers correspond to the first column in Table 1.

pyrolysates increase during digestion. Furfurals are derived from carbohydrates (14) and the Py-GC/MS data seems to contradict the FT-IR data, which shows slight increases in carbohydrate bands during conversion from feed to manure. However, it is important to note that the band at 1739 cm^{-1} could come not just from carbohydrate, but also from lignin esters and hemicellulose. Nitrile pyrolysates are derived from proteins (14). Phenylacetone nitrile declined during digestion, indicating uptake or modification by the animal. Similarly to the furfural, the phenylacetone nitrile data seems to contradict the FT-IR results, where the protein band increased during digestion. Vinylguaiacol may be derived from lignin or protein, and it increased during digestion in both the USD and the USH treatments.

3.6. Changes in manure pyrolysates during decomposition in soil

Fig. 9 illustrates the pyrograms of the fresh (week 0) and decomposed manures from the USD treatment. Many peaks

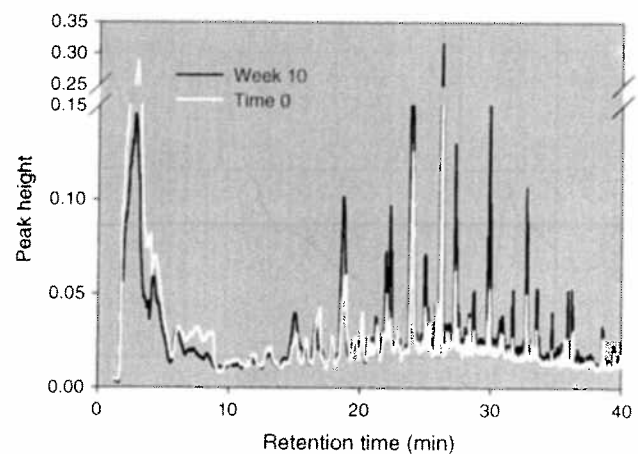


Fig. 9. Pyrogram of the fresh USD manure (time 0), and manure incubated for 10 weeks in soil.

are comprised of lignin pyrolysates, such as phenol, guaiacol + methylphenol, a dimethylphenol, methylguaiacol, vinylphenol, ethylguaiacol, vinylguaiacol, eugenol, syringol, *cis* isoeugenol, vanillin, methylsyringol + *trans* isoeugenol, ethylsyringol, vinylsyringol, allylsyringol, *cis* propenylsyringol and *trans* propenylsyringol [20]. As with digestion, the concentration of many lignin products increased during manure decomposition (Fig. 10). For most lignin-derived pyrolysates, the changes were not consistent across manures. Exceptions are syringol and dimethylphenol, which increased in all the manures during decomposition. The changes in relative abundance of the different lignin products suggest a change in the lignin composition of the manures. As with the digestion results, the carbohydrate-derived furfural decrease contradicts the FT-IR data where a slight increase in the carbohydrate band was observed. We hypothesize that the Py-GC/MS is better suited than the FT-IR to detect changes in carbohydrate content in manure, since carbohydrates are readily available for microbial utilization in soil and are bound to decrease during manure decomposition. Phenylacetone nitrile, a peak of protein origin,

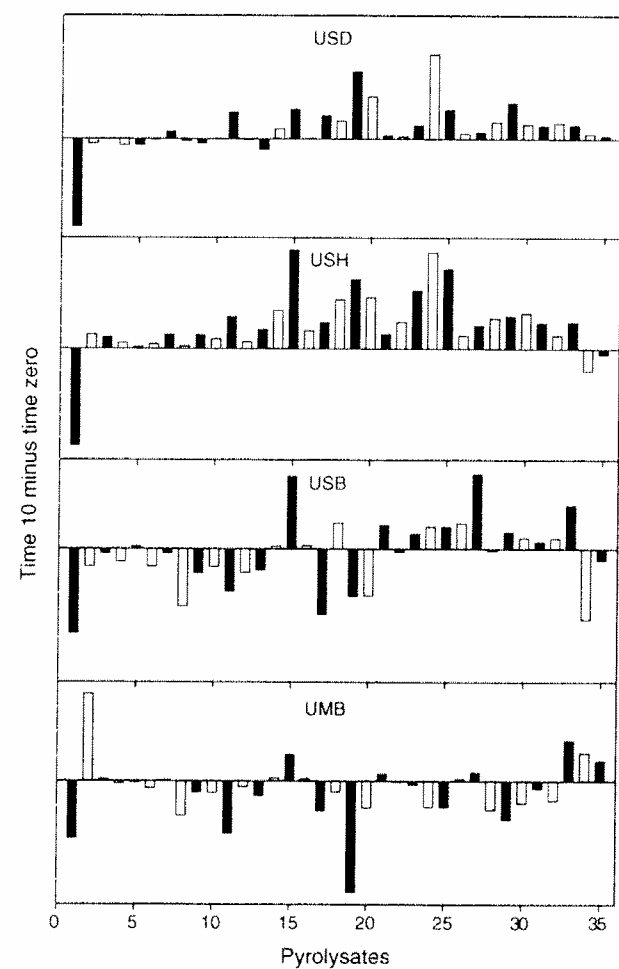


Fig. 10. Subtracted pyrolysates between fresh manure (time 0) and manure incubated for 10 weeks in soil. The x-axis numbers correspond to the first column in Table 1.

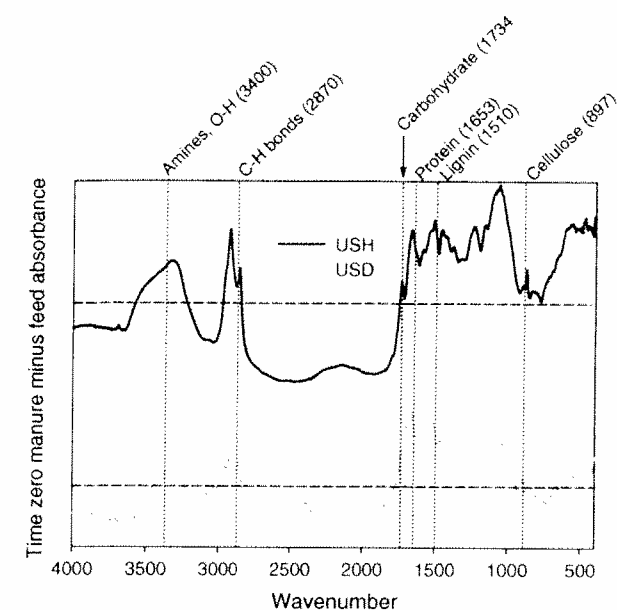


Fig. 3. Subtracted spectra of the manures and feeds. Two manures are shown: USD and USH.

(amides) increase relative to C–H bonds, cellulose and esterified carbohydrates during feed digestion in the animal. Besides protein and lignin bands, esterified carbohydrate bands decrease during the digestion relative to cellulose bands. The band at 2870 cm^{-1} , associated with C–H bonds, decreases relative to most other wavelengths included in both manures. In both USD and USH, fresh manure absorbed more at 1083 cm^{-1} than the corresponding feed. This band includes aromatic C–C groups, which could be

Table 2
Ratio of absorbance at selected spectral bands between feed spectra and fresh manure

Ratio ^a	USD feed	USD manure	USH feed	USH manure
Amines, –OH:C–H bonds	1.19	1.17	1.14	1.18
Amines, –OH:carbohydrates	1.29	1.22	1.08	1.12
Amines, –OH:amides in proteins	1.12	0.99	1.03	0.97
Amines, –OH:lignin 1	1.09	0.99	1.01	0.99
Amines, –OH:lignin 2	1.20	1.06	1.07	0.97
Amines, –OH:cellulose	1.40	1.40	1.30	1.35
C–H bonds:carbohydrates	1.09	1.04	0.95	0.95
C–H bonds:amides in proteins	0.94	0.84	0.90	0.82
C–H bonds:lignin 1	0.92	0.84	0.89	0.84
C–H bonds:lignin 2	1.01	0.90	0.94	0.82
C–H bonds:cellulose	1.17	1.19	1.15	1.14
Carbohydrates:amides in proteins	0.87	0.81	0.95	0.87
Carbohydrates:lignin 1	0.85	0.81	0.93	0.89
Carbohydrates:lignin 2	0.93	0.87	0.99	0.87
Carbohydrates:cellulose	1.08	1.15	1.20	1.20
Amides in proteins:lignin 1	0.98	1.00	0.98	1.02
Amides in proteins:lignin 2	1.07	1.07	1.04	1.00
Amides in proteins:cellulose	1.25	1.41	1.27	1.39
Lignin 1:lignin 2	1.10	1.07	1.06	0.98
Lignin 1:cellulose	1.27	1.42	1.29	1.36
Lignin 2:cellulose	1.16	1.32	1.22	1.39

^a Amines + OH band = 3400 cm^{-1} , C–H bonds band = 2870 cm^{-1} , esterified carbohydrates band = 1734 cm^{-1} , amides in proteins band = 1653 cm^{-1} , lignin 1 band = 1600 cm^{-1} , lignin 2 band = 1510 cm^{-1} and cellulose band = 897 cm^{-1} [11].

attributed to lignins or proteins. Also, fresh manures have higher absorbance than feeds at 1734 cm^{-1} , specific for esterified carbohydrates, and at 2870 cm^{-1} , specific for C–H groups. Lignins have esters, so it is difficult to interpret whether the 1734 cm^{-1} band belongs to the relatively easy to digest carbohydrates, or from a recalcitrant lignin. However, given the finding that absorption at 1734 cm^{-1} increases after digestion, we speculate that some of the absorption at this wavelength is due to lignin. Straws are rich in CH groups, suggesting that this type of material is relatively resistant to digestion. In addition, fatty acids and amino acids are also CH-rich, suggesting that these two substances increase in concentration during digestion.

3.3. Changes in manure FT-IR spectra during decomposition in soil

Fig. 4 shows the series of spectra according to the weeks of incubation for the USD manure. Only the USD manure is included, since it illustrates what happened to the rest of the manures. Bands associated with primary amines ($1340\text{--}1020\text{ cm}^{-1}$), lignin (1510 cm^{-1}), as well as amides associated with proteins (1653 cm^{-1}) increase as the USD manure decomposes in soil. The increase in the protein band in manure during decomposition may be due to microbial growth and N immobilization within the manure as C is mineralized and lost. Bands associated with fatty acids, such as C–H (2870 cm^{-1}) and carboxylic acids ($3000\text{--}2500\text{ cm}^{-1}$) tend to decrease during the decomposition, possibly due to utilization as C sources. The spectroscopic data also shows that lignin-specific signals ($1600\text{--}1590$ and 1510 cm^{-1}), change during manure decomposition,