Supporting information

Chemical structures and characteristics of animal manures and composites during composting

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Abbreviations Used

Germination index (GI); Fourier transform infrared (FT-IR); nuclear magnetic resonance (NMR); cross-polarization/total sideband suppression (CP/TOSS); cross-polarization/total sideband suppression with dipolar dephasing (CP/TOSS/DD).
Materials and methods

Sample preparation and samplings

For composting, the manures were put in a cool and ventilated place to allow them to dry naturally until their moisture contents reached about 60% (w/w). Then 12 kg each of three manures were composted separately in impervious plastic boxes (44 L volume, 53cm length x 38cm width x 22cm height), with three replicates for each manure. All the boxes were maintained in a temperature adjustable incubator for 70 days. The incubator was opened to ventilate for 15 minutes, and temperature recorded at the same time every day. The incubator temperature was adjusted to 1°C below that of manure composts to reduce heat loss. During the first month, the manure composts were turned and mixed every day to provide aeration, and water was immediately added to maintain a moisture content of 60% (w/w). After one month, they were turned and mixed every other day to promote aerobic decomposition. The triplicate samples of each manure or compost were collected at day 0, 3, 7, 16, 24, 30, 36, 44, 56 and 70. A portion of the samples were air dried and ground through a 100-mesh sieve for NMR, FT-IR and elemental analysis, and the remaining portion stored in a refrigerator at -20 °C for other analyses

Chemical analyses

The elemental compositions (%C, %N, %H, and %O) were determined by an Elementar Vario EL cube elemental analyzer through dry combustion at 1150 °C [1].

FT-IR spectroscopy

The FT-IR analyses were conducted on a Nicolet 8700 IR spectrometer. Each
sample was prepared by grinding 3 mg of the sample with 300 mg of oven-dried KBr in a vibrating puck mill and then compressing about 150 mg of the mixture into a translucent pellet under a hydraulic compressor. The pellet was immediately placed on a sample holder, and a spectrum ranging from 4,000 to 400 cm\(^{-1}\) was recorded under the conditions of 4 cm\(^{-1}\) wavenumber resolution and 32 scans. Pure KBr spectra were used as the background [2]. The major peaks (intensity and wavenumber) were found by using OMNIC software.

**Statistical analysis**

The FT-IR absorbance spectra were processed with OMNIC 8 spectroscopy software. The spectra were normalized by setting the highest value of each spectrum to 1 absorbance unit.

**Results**

**Elemental compositions during composting**

The changes in elemental compositions of the three manures and composts at different composting phases are shown in Table 1. Carbon was most abundant in all of the three manures. Swine manure had an obviously a higher carbon content (37.5%) than those of cattle manure (30.8%) and chicken manure (27.0%). Also chicken manure contained a higher nitrogen content (5.3%) than those of swine manure (4.7%) and cattle manure (2.9%). Swine manure had an obviously higher hydrogen content (6.4%) than those of cattle manure (3.8%) and chicken manure (4.2%). Moreover, chicken manure contained a higher oxygen content (37.6%) than those of swine manure (34.0%) and cattle manure (32.1%). Consequently, the C/N ratios of chicken
manure (5.1) and swine manure (8.0) were lower than that of cattle manure (10.7).

S1 Table 1 Elemental compositions and C/N, C/H and C/O ratios of three manures and composts

| Time[d] | C%  | N%  | H%  | O%  | C/N | C/H | C/O |
|---------|-----|-----|-----|-----|-----|-----|-----|
| 0       | 37.5| 4.7 | 6.4 | 34.0| 8.0 | 5.9 | 1.1 |
| 3       | 36.8| 4.8 | 6.4 | 35.3| 7.7 | 5.7 | 1.0 |
| 7       | 36.1| 4.7 | 6.5 | 35.7| 7.7 | 5.6 | 1.0 |
| 16      | 32.7| 4.7 | 6.2 | 34.6| 7.0 | 5.2 | 1.0 |
| 24      | 33.9| 4.5 | 6.3 | 35.8| 7.6 | 5.4 | 1.0 |
| 30      | 30.8| 4.2 | 6.1 | 34.4| 7.4 | 5.1 | 0.9 |
| 36      | 29.7| 3.8 | 5.8 | 35.3| 7.9 | 5.1 | 0.8 |
| 44      | 29.4| 3.9 | 5.7 | 33.0| 7.6 | 5.2 | 0.9 |
| 56      | 28.6| 4.3 | 5.4 | 31.3| 6.7 | 5.4 | 0.9 |
| 70      | 28.6| 4.4 | 5.3 | 32.8| 6.5 | 5.4 | 0.9 |

Swine manure and composts:

| Time[d] | C%  | N%  | H%  | O%  | C/N | C/H | C/O |
|---------|-----|-----|-----|-----|-----|-----|-----|
| 0       | 30.8| 2.9 | 3.8 | 32.1| 10.7| 8.2 | 1.0 |
| 3       | 30.8| 2.7 | 5.0 | 34.8| 11.3| 6.2 | 0.9 |
| 7       | 28.6| 2.5 | 4.6 | 34.6| 11.6| 6.2 | 0.8 |
| 16      | 26.4| 2.5 | 4.3 | 34.0| 10.5| 6.1 | 0.8 |
| 24      | 26.4| 2.8 | 4.3 | 32.6| 9.6 | 6.2 | 0.8 |
| 30      | 29.4| 2.7 | 4.6 | 34.8| 10.9| 6.4 | 0.8 |
| 36      | 24.6| 2.8 | 4.0 | 31.5| 8.9 | 6.2 | 0.8 |
| 44      | 25.2| 2.9 | 3.8 | 27.7| 8.7 | 6.6 | 0.9 |
| 56      | 24.5| 2.7 | 3.8 | 27.6| 9.1 | 6.5 | 0.9 |
| 70      | 24.4| 2.7 | 3.8 | 26.8| 8.9 | 6.4 | 0.9 |

Cattle manure and composts:

| Time[d] | C%  | N%  | H%  | O%  | C/N | C/H | C/O |
|---------|-----|-----|-----|-----|-----|-----|-----|
| 0       | 27.0| 5.3 | 4.2 | 37.6| 5.1 | 6.5 | 0.7 |
| 3       | 26.5| 5.0 | 4.1 | 38.5| 5.3 | 6.4 | 0.7 |
| 7       | 25.8| 4.7 | 4.1 | 37.9| 5.6 | 6.4 | 0.7 |
| 16      | 23.3| 3.7 | 3.7 | 37.2| 6.3 | 6.2 | 0.6 |
| 24      | 22.4| 3.0 | 3.5 | 37.2| 7.4 | 6.4 | 0.6 |
| 30      | 20.2| 2.3 | 3.2 | 35.5| 8.7 | 6.4 | 0.6 |
| 36      | 19.4| 2.5 | 3.2 | 31.6| 7.8 | 6.1 | 0.6 |
| 44      | 19.5| 2.6 | 3.2 | 29.6| 7.5 | 6.2 | 0.7 |
| 56      | 18.5| 2.5 | 2.9 | 30.8| 7.4 | 6.5 | 0.6 |
| 70      | 19.5| 2.9 | 3.2 | 30.3| 6.8 | 6.0 | 0.6 |

Chicken manure and composts:

| Time[d] | C%  | N%  | H%  | O%  | C/N | C/H | C/O |
|---------|-----|-----|-----|-----|-----|-----|-----|
| 0       | 37.5| 4.7 | 6.4 | 34.0| 8.0 | 5.9 | 1.1 |
| 3       | 36.8| 4.8 | 6.4 | 35.3| 7.7 | 5.7 | 1.0 |
| 7       | 36.1| 4.7 | 6.5 | 35.7| 7.7 | 5.6 | 1.0 |
| 16      | 32.7| 4.7 | 6.2 | 34.6| 7.0 | 5.2 | 1.0 |
| 24      | 33.9| 4.5 | 6.3 | 35.8| 7.6 | 5.4 | 1.0 |
| 30      | 30.8| 4.2 | 6.1 | 34.4| 7.4 | 5.1 | 0.9 |
| 36      | 29.7| 3.8 | 5.8 | 35.3| 7.9 | 5.1 | 0.8 |
| 44      | 29.4| 3.9 | 5.7 | 33.0| 7.6 | 5.2 | 0.9 |
| 56      | 28.6| 4.3 | 5.4 | 31.3| 6.7 | 5.4 | 0.9 |
| 70      | 28.6| 4.4 | 5.3 | 32.8| 6.5 | 5.4 | 0.9 |

During the 70 days of composting, the carbon contents of the three manure composts gradually decreased. At the end of composting, the carbon content of swine manure composts decreased to 28.6%, still greater than those of the day-70 cattle manure compost (24.4%) and chicken manure compost (19.5%). The nitrogen content
of cattle manure comports slightly decreased in the first 16 days, increased to 2.8% and remained almost constant in the subsequent time. The nitrogen content of chicken manure comports rapidly decreased in the first 30 days and then slightly increased afterwards. However, the nitrogen content of swine manure comports was almost constant in the first 16 days, then decreased from day 24 to day 36, and increased in the remaining days. The hydrogen content of all the manure comports generally decreased. The oxygen content of cattle manure comports decreased in the first 24 days, increased to 34.8% at day 30, and then decreased until the end of composting. The oxygen content of chicken manure comports decreased to 29.6% in the first 44 days, and subsequently remained almost constant. But the oxygen content of swine manure comports was almost constant in the first 36 days, and decreased to 32.8% at the end of composting. The C/N ratio of chicken manure comports had an increasing trend whereas those of swine and cattle manure comports decreased during the composting. The C/H ratios generally decreased and the C/O ratios did not change much for all the three manure comports. All the differences described above were significant based on Duncan’s multiple range tests at P < 0.05.

**FT-IR analysis**

**S1 Fig FT-IR spectra of swine, cattle and chicken manure comports during composting**

The FT-IR spectra of the cattle, swine and chicken manure comports at different stages (0, 7, 24, 44, and 70 days) exhibited similar spectral patterns (S1 Fig 1), except the differences in the intensity of certain bands. The main absorbance bands were those around 3300-3400 cm⁻¹, attributed to the intra-molecular and inter-molecular
hydrogen-bonded OH groups in phenol, carbohydrate and carboxylic acid compounds, as well as N-H stretching in amines and amides [6]. The two distinct small peaks at 2930 and 2854 cm\(^{-1}\) were ascribed to the stretching of aliphatic CH\(_3\) and CH\(_2\), respectively [7]. A pronounced peak at 1650 cm\(^{-1}\) was attributed to the C=O stretching of amide groups (amide I) and aromatic C=C vibrations, as well as to symmetric stretching of COO\(^{-}\) groups. A small peak at 1540 cm\(^{-1}\) was assigned to vibrations in aromatic rings [8] and a distinct peak at 1430 cm\(^{-1}\) was due to the C-O asymmetric stretching, O-H deformation and C-O-H deformation of the carboxyl groups and symmetric stretching of the COO\(^{-}\) ions [6]. The small peak at 1230 cm\(^{-1}\) was attributed to C-O stretching and O-H deformation of carboxyls, phenols, and aromatic ethers; and a big band at 1030 cm\(^{-1}\) is frequently assigned to C-H in-plane deformation vibration, C-O stretching of carbohydrates, polysaccharides or polysaccharides-like substances [9-11].

Spectral differences were observed among different manure composts. Compared with the spectra of the swine and cattle manure composts (S1 Fig(a) and (b)), the band around 3400 cm\(^{-1}\) became less intense and shifted toward a lower wavenumber at 3300 cm\(^{-1}\) in the spectra of the chicken manure composts (S1 Fig (c)). A small sharp peak at 2520 cm\(^{-1}\) was observed only in the spectra of chicken manure composts, which can be attributed to S-H stretch of aromatic or nonaromatic mercaptans and sulfides. Spectral differences were also observed in the 1540–1430 cm\(^{-1}\) region. The bands at 1540 cm\(^{-1}\) were distinct in the spectra of swine manure composts but smaller in the spectra of cattle and chicken manure composts. However, the bands at 1430
cm$^{-1}$ were stronger in the spectra of chicken manure composts but weaker in the spectra of cattle and swine manure composts. The bands at 870, 765 and 612 cm$^{-1}$ were distinct in the spectra of chicken manure composts but weaker in those of cattle and swine manure composts. These bands are preferentially assigned to vibrations in proton-substituted aromatic rings.

The FT-IR spectra did not differ noticeably among different composting stages. Only slight reduction of peaks in the aliphatic region at 2930 and 2854 cm$^{-1}$ for cattle manure composts during the thermophilic phase was observed (S1 Fig (b)), possibly due to preferential biodegradation of aliphatic structures [12]. In addition a decrease of peak intensity in the aromatic region at 1650 cm$^{-1}$ for chicken manure composts (S1 Fig (c)) and an increase of peak intensity in the polysaccharide region at 1030 cm$^{-1}$ in swine (S1 Fig (a)) and chicken manure composts were observed as the composting proceeded.

**Discussion**

**Evolution of elemental composition during composting**

The elemental composition evolution of manures and composts during composting in the present study was similar to that of most other studies [13, 14], showing the decreased trends of C and N contents. However, Sánchez-Monedero et al. [15] and Huang et al. [16] observed the increase trend of N in humic acids as composting proceeded. They attributed the increase of N to incorporation of N in humic acids by condensation of lignin with proteins and formation of refractory, complex structures between nitrogen and the humic 'core'. The decreased trend of C/N
ratios for swine and cattle manure comports during our composting was also similar to that of other studies [13, 17-19]. Nevertheless, the change of C/N ratio for chicken manure comports in many studies was in disparity, with some showing the increase trend [13, 20] in agreement with our results but others indicating the decrease trend [17, 21]. Antil et al. [21] ascribed the decrease of C/N ratio to the decreased carbon content and increased N content in their study. The chicken manures and comports in the present study had greater N and pH but lower C/N ratio than swine and cattle manures and comports. The high pH in chicken manure comports contributed to faster losses of N relative to C via NH\textsubscript{3} volatilization [22] and thus as composting proceeded, the C/N ratio of chicken comports increased.
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Figure Captions

S1 Fig FT-IR spectra of swine, cattle and chicken manure comports during composting