

Effect of the carbon:nitrogen relationship on the chemical and microbial composition of composts and wormcomposts made with pig manure

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Abstract: Wormcomposting is a useful alternative to recover the organic carbon (OC) and nitrogen (N) and to reduce the pathogenic bacteria in animal manure; but, little is known about the best initial carbon:nitrogen relationship (CRN) to maximize the nutritional value or to minimize the microbial load of the final wormcompost product. The objective of the study was to evaluate the changes over time of OC and total N (TN) content and the total aerobic bacteria (TAB), coliforms (Coli) and fungi and yeast (F and Y) counts in composts and wormcomposts prepared with pig manure using different initial CNR. The initial analyzed CNR for composts were: 22, 26, 34 and 46, and for wormcomposts: 15, 31, 46 and 70. Samples were taken for chemical analysis (1, 28 and 140 days) and microbiological analysis (1, 7, 28, 56, 84 and 140 days). The data was analyzed using the MIXED procedure with repeated observations over time. In composts and wormcomposts, the OC and CNR diminished and the TN increased over time ($p < 0.01$); only the composts with the highest initial CNR (46) showed the lowest TN and highest CNR at 140 days. The TAB and Coli and F and Y counts showed diminishing cubic responses over time and were similar at 140 days in composts and wormcomposts with different initial CNR ($p < 0.01$). In summary, composts and wormcomposts with a wide range of initial CNR were very effective in converting pig manure in a high quality organic fertilizer with a lower load of potential pathogenic bacteria

Keywords: compost; wormcompost; pig manure; chemical composition; microbial counts

1. Introduction

Composting and wormcomposting are useful alternatives to recover the organic carbon (OC) and nitrogen (N) and to reduce the pathogenic bacteria in animal manures [1,2,3]. Different factors need to be taken into account before starting the compost process to obtain a final product with high fertilizer quality [4]. One of these factors is the carbon:nitrogen relationship (CNR) since the microbes that transform the organic residues into an organic fertilizer require 25–35 parts of C per each part of N [4,5,6]. High or low CNR at the beginning of the process causes higher C, as CO₂, and N (as NH₄⁺) losses, respectively, and lower quality fertilizer. It has been also shown that during the first three months of composting, high initial CNR promotes higher microbial biomass and respiration

compared to low CNR, and these differences are reversed after 12 months of composting [5]. It is also known that the initial CNR has a direct effect on the CNR changes during the composting process and these, in turn, have a significant influence on the bacterial composition during the process [5,6]. On the other hand, it has been reported that the chemical and microbial composition of wormcomposts is affected by the properties of the initial organic materials used, such as mixtures of vegetable residues or animal manure [1,2,7], but little is known about the optimal initial conditions, like the CNR, to maximize the nutritional value or to minimize the microbial load of the final wormcompost product. Therefore, the objective of the study was to evaluate the changes over time of OC and total N (TN) content, and the total aerobic bacteria (TAB), coliforms (Coli) and fungi and yeast (F and Y) counts in composts and wormcomposts prepared with pig manure using different initial CNR.

2. Materials and Methods

2.1. Compost and wormcomposts preparation

The pig manure used in the study was collected from the dunghill of a whole-cycle pig farm in which solid manure is daily removed by shovel from the pens. The manure was homogeneously mixed before the start of the study. The manure was mixed with corn stover and water in different amounts as shown in Table 1 to produce 50 kg heaps inside a greenhouse with concrete floor and plastic ceiling. The initial analyzed composts CNR (CP/CNR) were: 22, 26, 34 and 46 (22-, 26-, 34- and 46-CP/CNR, based on the initial OM and TN content of the mixture). A black plastic was placed on the concrete floor before placing the mixture and the heap was covered with the same plastic during the thermophilic phase to reduce the losses of heat, organic matter and water. During the first 37 days of the study the heaps were subjected to a thermophilic process. The temperatures of the heaps were registered every two days and are presented in Figure 1. The heaps were turned every week and water was added at the same time to keep an adequate moisture of the mixture. After the thermophilic phase, the heaps were kept uncovered but water was added every two weeks to moisturize the mixture. The study was finished at 140 days after the initial day.

Table 1. Initial carbon:nitrogen relationships in composts and wormcompost made with pig manure, corn stover and water.

Carbon:nitrogen relationship ¹			
	Pig manure	Corn stover	Water
Composts			
22	20	7.5	22.5
26	15	15	20
34	10	20	20
46	5	27.5	17.5
Wormcomposts			
15	15	10	25
31	15	15	20
46	10	20	20
70	10	25	15

¹Analyzed values, calculated using the initial organic carbon and total nitrogen content of the mixtures.

The initial composts CNR to produce the wormcomposts (WC/CNR) were: 15, 31, 46 and 70 (15-, 31-, 46- and 70-WC/CNR). The initial composts were also subjected to a thermophilic phase and all

the procedures were similar to those outlined before for composts. After the thermophilic phase, the heaps were inoculated with the earthworm *Eisenia fetida* to produce the wormcomposts. The wormcomposts were protected from sun light with a black plastic cover all the time, and water was added twice a week or as needed to keep an adequate moisture.

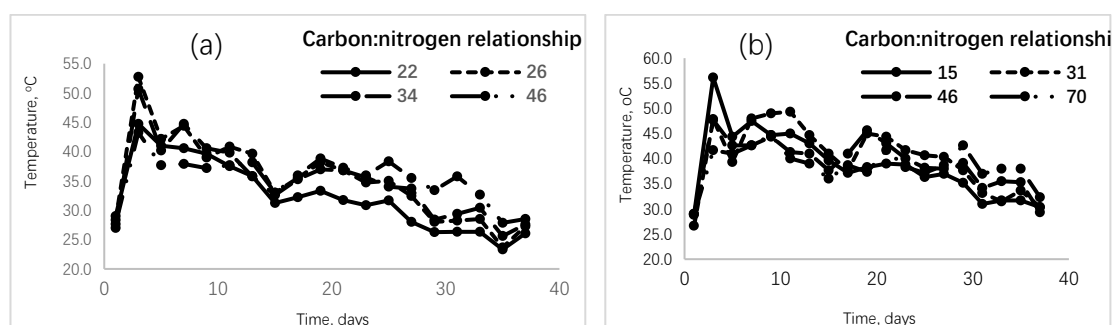


Figure 1. Temperatures (°C) of the (a) composts and (b) wormcomposts during the first 37 days of the thermophilic phase.

2.2. Sampling and laboratory determinations

In composts and wormcomposts, samples for chemical analysis were taken on days 1, 28 and 140 and samples for microbiological analysis were taken on days 1, 7, 28, 56, 84 and 140 of the study using sterile plastic bags. In the laboratory, samples for chemical analysis were lyophilized and ground using a 2 mm mesh. Determinations of dry matter (DM), ashes, total nitrogen (TN) and ammonia-nitrogen (N-NH⁴⁺) were carried out following standard procedures. Results of DM and ashes were used to estimate the organic matter (OM) and organic carbon (OC) content. Results of OC and TN were used to estimate the CNR. In samples for microbiological analysis, the total aerobic bacteria (TAB) counts were determined using the standard plate count method using decimal dilutions up to 10⁻⁶ and incubating at 37 °C for 24 hours. The counts of viable coliforms (Coli) were performed by plating serial 10-fold dilutions onto Triptosa agar plates and were incubated for 24 h, under aerobic conditions.

2.3. Statistical analysis

The data was analyzed using the MIXED procedure with repeated observations over time using the day of sampling as random effect. There were 5-6 replications for composts and 3 replications for wormcomposts. Percentage results were transformed to log₁₀ before the analysis. Results of microbial counts were expressed as log₍₁₀₎ of colony-forming units per gram (log₍₁₀₎CFU g⁻¹).

3. Results

3.1. Temperatures during the thermophilic phase

3.1.1. Composts

In composts, the temperatures ranged from 44.75 to 52.75 °C at 3 days after the initiation of the thermophilic phase in 22-, 26- and 34-CP/CNR (Figure 1a), remaining between 40.20 to 44.70 °C on days 5 and 7; after that, the temperatures fell from 40.6 down to 26.08 °C at 37 days. In contrast, a temperature of 43.25 was recorded at 3 days in 46-CP/CNR, and it fell rapidly after that.

3.1.2. Wormcomposts

In wormcomposts, the temperature varied from 56.17 (15-WC/CNR) to 41.67 °C (70-WC/CNR) at 3 days after the start of the study (Figure 1b), and they remained above 40 °C until 13 days after the initial day. And then dropped down to 30 °C at 37 days.

3.2. Changes in chemical composition

3.2.1. Composts

The OM, OC, N-NH⁴⁺ content and CNR decreased over time and differences due to the CNR were observed within each day of sampling (Interaction of initial CNR and Day of sampling, *p* < 0.01). The OM and OC content on days 1, 28 and 140, had a linear increment related to the increasing initial CNR. The N-NH⁴⁺ content on day 1, diminished due to the increasing initial CNR, but at days 28 and 140, no differences were found regarding the initial CNR. The CNR on days 28 and 140 had a linear elevation due to the increasing initial CNR (Table 2). The TN content increased over time (Table 2) and differences due to the initial CNR were observed within each day of sampling (Interaction of initial CNR and Day of sampling, *p* < 0.01). The TN on day 1 was lowest in 46-CP/CNR, but at days 28 and 140, the TN showed a linear decreasing response due to the initial CNR.

Table 2. Chemical and microbial composition of composts and wormcomposts.

Days	Carbon:nitrogen relationship							
	Composts ¹				Wormcomposts ¹			
	22	26	34	46	15	31	46	70
	Organic carbon, % ²							
1	41.04 ^{ag}	45.03 ^b	45.67 ^b	47.70 ^c	40.78 ^a	45.22 ^{bc}	47.36 ^{bd}	49.33 ^d
28	34.88 ^d	38.02 ^e	39.55 ^{fg}	40.23 ^g	36.97 ^d	36.43 ^d	42.32 ^{ae}	44.02 ^{ce}
140	34.27 ^d	36.80 ^e	38.55 ^{ef}	42.19 ^a	30.94 ^f	32.88 ^{fg}	30.05 ^f	35.15 ^{dg}
	Total nitrogen, % ²							
1	2.13 ^a	1.84 ^{ad}	2.13 ^a	1.07 ^b	2.69 ^{af}	1.50 ^b	1.05 ^c	0.71 ^d
28	3.20 ^c	3.12 ^c	2.33 ^a	1.57 ^d	3.40 ^e	3.17 ^e	2.38 ^f	1.94 ^g
140	3.63 ^e	3.39 ^e	2.99 ^c	1.72 ^d	3.13 ^e	2.59 ^a	3.04 ^e	2.81 ^a
	Carbon:nitrogen relationship ²							
1	22.40 ^a	26.39 ^b	34.09 ^c	45.93 ^d	15.22 ^{aef}	31.01 ^b	45.95 ^c	70.43 ^d
28	10.91 ^e	12.23 ^e	18.37 ^f	26.38 ^b	10.90 ^a	11.55 ^{ae}	18.24 ^{ef}	22.80 ^f
140	9.49 ^e	11.04 ^e	13.27 ^e	25.78 ^b	9.90 ^a	12.83 ^{ae}	9.93 ^a	12.51 ^{ae}
	Total aerobic bacteria, CFU(log ₁₀)/g ²							
1	8.06 ^a	9.68 ^b	9.88 ^b	8.34 ^{ac}	8.42 ^a	9.69 ^{bc}	8.54 ^a	8.37 ^a
7	9.04 ^{bc}	8.71 ^c	9.82 ^b	10.09 ^b	9.44 ^{bc}	8.59 ^a	10.00 ^{bc}	10.29 ^c
28	8.95 ^c	9.42 ^b	9.33 ^b	8.88 ^c	8.63 ^a	9.08 ^{ab}	9.24 ^{ab}	8.67 ^a
56	6.98 ^d	7.07 ^d	7.22 ^d	7.53 ^d	6.73 ^{de}	6.92 ^{de}	7.72 ^e	7.13 ^e
84	5.74 ^e	5.90 ^e	5.91 ^e	6.29 ^e	6.08 ^{df}	6.17 ^{df}	6.04 ^{df}	5.72 ^f
140	6.15 ^e	6.23 ^e	6.11 ^e	6.34 ^e	5.89 ^f	6.25 ^{df}	6.16 ^{df}	6.34 ^{df}
	Coliforms, CFU(log ₁₀)/g ²							
1	7.65 ^{ab}	8.39 ^{ac}	8.68 ^{ac}	7.40 ^b	8.08 ^{ab}	8.31 ^{abc}	8.62 ^{ac}	7.68 ^b
7	8.66 ^a	7.59 ^b	9.20 ^c	9.00 ^c	7.70 ^b	7.96 ^b	9.13 ^c	9.14 ^c
28	7.21 ^b	7.84 ^{ab}	8.01 ^{ab}	7.66 ^{ab}	6.54 ^d	6.56 ^d	7.60 ^b	8.13 ^{ab}
56	5.45 ^d	5.32 ^d	5.40 ^d	6.68 ^e	5.16 ^{ef}	5.73 ^e	5.55 ^e	5.31 ^e

84	4.78 ^{df}	4.41 ^f	4.26 ^f	5.42 ^d	4.32 ^{ef}	4.32 ^f	4.47 ^{ef}	4.42 ^{ef}
140	3.91 ^f	3.94 ^f	3.66 ^f	4.51 ^f	4.90 ^{ef}	4.94 ^{ef}	3.99 ^f	4.26 ^f

¹ In composts and wormcomposts, the SEM for organic matter, total nitrogen, carbon:nitrogen relationship, total aerobic bacteria and coliforms were 0.559 and 1.062, 0.119 and 0.146, 1.552 and 3.052, 0.390 and 0.322 and 0.342 and , 0.373, respectively.

² Interaction of CNR and Day of sampling, ^{a-g} ($p < 0.01$).

3.2.2. Wormcomposts

The OM, OC, N-NH₄⁺ content and CNR decreased over time (Table 2) and differences due to the CNR were observed within each day of sampling (Interaction of initial CNR and Day of sampling, $p < 0.01$). The OM and OC on days 1, 28 and 140 raised regarding the increasing initial CNR. The N-NH₄⁺ on day 1 showed a linear decrease response related to the initial CNR; but on days 28 and 140 the N-NH₄⁺ was not affected by the initial CNR. The CNR on day 28 increased due to the increasing initial CNR but on day 140 no differences were found related to the initial CNR. The TN content increased over time (Table 2) and differences due to the CNR were observed within each day of sampling (Interaction of CNR and Day of sampling, $p < 0.01$). The TN on days 1 and 28, showed linear decreasing responses due to the initial CNR, but at day 140 the TN was similar in 15-, 46- and 70-WC/CNR.

3.3. Changes in microbial composition

3.3.1. Composts

The TAB and Coli counts showed diminishing cubic responses over time, but differences due to the initial CNR were observed within each day of sampling (Interaction of initial CNR and Day of sampling, $p < 0.01$). TAB were higher in 26- and 34-CP/CNR on day 1 and in 46-CP/CNR at day 7, but TAB counts were similar on days 28, 56, 84 and 140 regardless of the initial CNR. Coli were higher in 26- and 34-CP/CNR on day 1, in 34- and 46-CP/CNR on day 7, and in 46-CP/CNR at days 56, 84 and 140. In 22-, 26- and 34-CP/CNR similar Coli counts were seen on days 56, 84 and 140.

3.3.2. Wormcomposts

The TAB and Coli counts showed diminishing cubic responses over time, but differences due to the initial CNR were observed within each day of sampling (Interaction of initial CNR and Day of sampling, $p < 0.01$). TAB were higher in 31-WC/CNR on day 1 and in 46- and 70-WC/CNR at day 7, but after that, the TAB counts were similar on days 28, 56, 84 and 140 regardless of the initial CNR. Coli were similar on day 1 independently of the initial CNR, but were higher in 46- and 70-WC/CNR on day 7 and in 70-WC/CNR at day 28. On the subsequent days of sampling, the Coli counts were similar regardless of the initial CNR.

4. Discussion

4.1. Composts

The reductions in OC content and CNR and increases in TN content over time are in agreement with previous reports in which composts were made with pig manure [4,8]. According to the maturity indices established for composts of different sources, a CNR lower than 20, and preferable lower than 10, is desirable [4]. The higher CNR in 46-CP/CNR (25.78) at the end of the study was probably due to the lower temperature recorded in this treatment at the beginning of the thermophilic phase, and the rapid drop of this index after three days of the initiation of the study. It has been reported that high initial CNR lowers the composting process due to an excess of degradable substrate but a lower N content to sustain the of microbes [4,5]. In accordance with this, the opposite

response was observed on the TN at 140 days that was lower in 46-CP/CNR compared to the rest of the treatments. The TAB and Coli counts were reduced over time and were similar at the end of the study regardless of the initial CNR, which is in agreement with the lower coliform number, microbial communities and antibiotic resistant genes reported at the end of the composting process of pig and cow manure [1,3]. Important to note was that at the end of the study, the Coli were reduced by 49, 53 and 58 and 39 % in 22-, 26-, 34- and 46-CP/CNR. The lower Coli reductions at 140 days and the higher Coli counts on days 7, 56 and 84 in 46-CP/CNR may also be related to the lower temperature shown in this treatment at the beginning of the process.

4.2. Wormcomposts

The reductions in OC content and CNR and increases in TN content over time are in agreement with previous reports in which wormcomposts were made with pig [9,10] and cow manure [2,7]. At the end of the study, the CNR, as well as the N-NH⁴⁺ (not shown in Table 2), was inside the range recommended for stable composts. Unlike the temperatures observed during the thermophilic phase in the composts kept as composts, the temperature of the composts used to produce the wormcompost were higher, on average above 40 °C, and were kept for a longer period, from 3 to 11 days. Nevertheless, the temperatures in 46- and 70-WC/CNR were lower compared to those of the 15- and 31-WC/CNR. The TAB and Coli counts were reduced over time and were similar at the end of the study regardless of the initial CNR. In a previous research, it was found that the coliform population was reduced by 98 % after being digested by the earthworms, probably due to the action of digestive enzymes and microflora resident in the gut [1]. Reductions in fecal coliforms and *Escherichia coli* were also found in a vermifilter loaded with cow manure [11]. At the end of the present study, the Coli were reduced by 39, 41 and 54 and 44 % in 15-, 31-, 46- and 70-CP/CNR. The lower and higher Coli reductions were observed in 15- and 46-WC/CNR, respectively.

4.3. Comparison of the chemical and microbial composition of composts and wormcomposts

During the thermophilic phase, compost heaps kept as composts and compost heaps used to produce the wormcomposts were kept under the same conditions; hence, the reasons for the differences in the recorded temperatures are unknown to the authors. The average TN content and CNR at the end of the study were about the same in composts and wormcomposts, but the OC content was slightly higher in composts (39 %) compared to wormcomposts (32 %). It should be taken into account that part of the original C and N can be recovered in the worm biomass. It also seems that at the end of the study the average reductions in Coli counts were slightly higher in composts (50 %) compared to wormcomposts (45 %). This difference was probably due to the higher humidity kept on the wormcomposts (50-70 %) compared to the composts moisture. In summary, composts and wormcomposts with a wide range of initial CNR were very effective in converting pig manure in a high quality organic fertilizer with a lower load of potential pathogenic bacteria.

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