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Effects of earthworm casts and zeolite on the two-stage composting of green waste

Lu Zhang, Xiangyang Sun *

College of Forestry, Beijing Forestry University, Beijing 100083, PR China

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ABSTRACT

Because it helps protect the environment and encourages economic development, composting has become a viable method for organic waste disposal. The objective of this study was to investigate the effects of earthworm casts (EWCs) (at 0.0%, 0.30%, and 0.60%) and zeolite (clinoptilolite, CL) (at 0%, 15%, and 25%) on the two-stage composting of green waste. The combination of EWCs and CL improved the conditions of the composting process and the quality of the compost products in terms of the thermophilic phase, humification, nitrification, microbial numbers and enzyme activities, the degradation of cellulose and hemicellulose, and physico-chemical characteristics and nutrient contents of final composts. The compost matured in only 21 days with the optimized two-stage composting method rather than in the 90–270 days required for traditional composting. The optimal two-stage composting and the best quality compost were obtained with 0.30% EWCs and 25% CL.

1. Introduction

With the increase in urban green space, the quantity of green waste (GW) originating from parks, reserves, and domestic gardens has increased dramatically in recent years (Belyaeva and Haynes, 2009). Composting, in which the organic waste is biologically decomposed and stabilized under thermophilic conditions resulting from biologically produced heat, is considered as an environmentally sound and economically viable alternative for the management of organic residues (Gabhane et al., 2012; Zhang et al., 2013). The final compost product is a humus-like substance that can be used as a soil amendment or an organic fertilizer (Song et al., 2014).

In some cases, however, the physical and chemical properties of organic wastes may make the wastes unsuitable for composting. These properties may result in insufficient decomposition and poor sanitation of the compost product. In addition, a substantial portion of the nitrogen (N) in the waste may be lost to the atmosphere as a pollutant. The potential need for a lengthy composting process can also be an important problem (Khalil et al., 2008). Finally, the application of immature and unstable compost may inhibit plant growth because the microorganisms in the compost may compete with plants for oxygen or because of insufficient biodegradation of phytotoxic residues (Ko et al., 2008). These composting problems can be reduced or eliminated by using organic or inorganic fermentation additives and by optimizing composting conditions and thus providing an appropriate environment for microbial growth and activity (Shao et al., 2014). The research described in this report determined how the composting of GW is affected by two fermentation additives: earthworm casts (EWCs), which are organic, and zeolite, which is inorganic.

EWCs are finely divided, peat-like materials with high porosity that results in good drainage and water-retaining properties; EWCs have a large surface area and also contain many nutrients in forms that are readily taken up by microorganisms and plants (Lalander et al., 2013; Lazcano et al., 2008). In previous reports, EWCs greatly accelerated the decomposition of organic waste, and the effect was mainly attributed to the diverse microbial communities and enzymes in the EWCs (Sen and Chandra, 2009). Relative to other organic fermentation additives, EWCs contain less soluble salts, have greater cation exchange capacity, and contain more humic substances. As a consequence, the addition of EWCs to organic wastes will not result in salt stress and will tend to neutralize the pH during composting, resulting in an environment that favors microbial growth and activity (Liu and Price, 2011; Raphael and Velmourougane, 2011). EWCs contain exchangeable phosphorus, potassium, calcium, and magnesium, which can support microbial growth and also enhance the quality of compost product (Song

* Corresponding author at: College of Forestry, Beijing Forestry University, P.O. Box 111, Beijing 100083, PR China. Tel.: +86 01062338103, +86 13366950662; fax: +86 01062338103.

E-mail addresses: zhanglu1211@gmail.com (L. Zhang), xysunbjfu@gmail.com, sunxy@bjfu.edu.cn (X. Sun).

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et al., 2014). In addition, EWCs provide conditions that favor nitrification and result in the rapid conversion of ammonium into nitrate (Lazcano et al., 2008). Compost produced with EWCs as a fermentation additive can also improve plant growth (Lalander et al., 2013; Raphael and Velmourougane, 2011). However, little information is available on how EWCs influence GW composting.

Researchers have also suggested that inorganic fermentation additives can increase composting efficiency and the stability of compost products (Belyaeva and Haynes, 2009). Natural zeolite is a crystalline mineral with a unique structure capable of adsorbing gases, water, nutrients, heavy metals, and other materials (Venglovsky et al., 2005). Studies have shown that, when added during composting, zeolite can increase water and nutrient retention, reduce N volatilization, improve aeration, and enhance microbial activities (Kuráň et al., 2014; Steiner et al., 2010; Wang et al., 2014; Zorpas and Loizidou, 2008). However, information on how zeolite affects the physical, chemical, and microbiological characteristics during GW composting is very limited.

Recent research also indicates that traditional composting can be improved by using an innovative two-stage composting technology that includes a primary fermentation (PF) and a secondary fermentation (SF) (Zhang et al., 2013). Based on the study of two-stage composting by Zhang et al. (2013), the thermophilic phase is attained twice, and the production of a mature compost requires only 30 days rather than the 90–270 days required for traditional composting (Khalil et al., 2008).

The purpose of the current study was to investigate the influence of EWCs and zeolite, in various proportions, on the changes in the physical, chemical, and microbiological properties of the compost during the two-stage composting of GW. The results of this work will contribute to the improvement and the development of the two-stage composting technology.

2. Materials and methods

2.1. Collection of raw materials

GW, consisting mainly of fallen leaves and branch cuttings, was collected in Beijing, China during greening maintenance. EWCs were purchased from Duoyuduo Biological Technology Co. (Guangzhou, China); these were black, elliptical to oblong, and from 1.0 to 2.0 mm long. The EWCs were produced by the earthworm *Eisenia foetida* when fed GW-derived compost in a controlled, vermicomposting environment. Clinoptilolite (CL) is a kind of natural zeolite with a microporous arrangement of silica and alumina tetrahedra. Because of its structure, CL has high adsorptive and ion exchange properties. CL was purchased from Jufang Trading Co., Ltd. (Guangzhou, China), and the CL particles used in this study ranged in size from 2.0 to 2.5 mm (Zorpas and Loizidou, 2008). Bamboo vinegar, which was purchased from Beijing Kaiyin Organic Fertilizer Production Co. (China), should be added during composting because it can reduce N volatilization (Zhang et al., 2013). To accelerate the initial composting process, raw materials were inoculated with a microbial inoculum, which was a mixture of *Trichoderma* spp. (60%, v/v) and *Phanerochaete chrysosporium* Burdsall (40%, v/v). Tables 1 and 2 list the selected physico-chemical properties of EWCs, CL, and GW. The determination methods are described in Section 2.4.

2.2. Composting process

Before the composting began, the GW was reduced to a particle size of about 1 cm with a grinder. Water was added to the particles to obtain a moisture content of approximately 60%; additional water was added as needed throughout the whole composting process to maintain that moisture content. Then, the C/N ratio was

Table 1
Physico-chemical properties of earthworm casts (EWCs), clinoptilolite (CL), green waste (GW), and the final composts. Values are means (SD); $n = 3$. Treatments T1–T9 are described in Table 3.

Material or treatment	BD (g/cm^3)[a]	WHC (%)[a]	TPS (%)[a]	AP (%)[a]	WHP (%)[a]	pH[a]
EWCs	0.4202(0.033)	1.73(0.03)	71.52(0.44)	12.23(0.28)	59.29(0.31)	6.03(0.12)
CL	0.8022(0.021)	3.99(0.09)	–	–	–	6.14(0.24)
GW	0.7390(0.010)	–	–	–	–	8.03(0.23)
T1	0.2906(0.019)i	1.04(0.05)h	39.08(0.31)i	12.71(0.18)h	26.37(0.34)i	7.94(0.19)a
T2	0.3239(0.016)g	1.21(0.04)g	45.72(0.19)g	13.96(0.27)g	31.76(0.29)g	7.83(0.20)b
T3	0.3602(0.014)e	1.44(0.08)e	53.09(0.56)e	16.82(0.24)e	36.27(0.50)e	7.67(0.16)d
T4	0.3053(0.017)h	1.17(0.11)g	41.33(0.42)h	13.03(0.32)h	28.30(0.33)h	7.85(0.23)b
T5	0.4260(0.030)a	1.67(0.09)d	62.44(0.37)d	18.61(0.22)d	43.83(0.19)d	7.22(0.12)e
T6	0.4018(0.010)c	2.23(0.02)a	72.96(0.28)a	23.52(0.20)a	49.44(0.42)a	6.61(0.14)h
T7	0.3394(0.024)f	1.32(0.07)f	49.20(0.55)f	15.25(0.19)f	33.95(0.38)f	7.75(0.11)c
T8	0.3815(0.026)d	1.78(0.10)c	67.36(0.40)c	20.19(0.35)c	47.17(0.24)c	6.96(0.15)f
T9	0.4173(0.022)b	1.90(0.06)b	69.48(0.34)b	21.34(0.31)b	48.14(0.30)b	6.89(0.27)g
	EC (mS/cm)[a]	TOC (%)[a]	C/N ratio	NH_4^+–N (mg/kg dry wt)[b]	NO_3^-–N (mg/kg dry wt)[b]	NH_4^+–N/NO_3^-–N ratio
EWCs	1.29(0.06)	18.22(0.20)	12.15(0.08)	–	–	–
CL	0.53(0.03)	0.06(0.01)	0.06(0.02)	–	–	–
GW	2.08(0.02)	49.52(0.36)	39.66(0.28)	–	–	–
T1	1.15(0.04)i	39.10(0.22)a	17.22(0.11)a	240.2(3.8)a	242.6(5.2)i	0.99(0.01)a
T2	1.29(0.08)g	34.22(0.27)c	12.39(0.07)c	214.4(4.2)c	289.7(3.4)g	0.74(0.04)c
T3	1.46(0.10)e	31.09(0.18)e	10.40(0.20)e	205.5(6.1)d	373.6(2.9)e	0.55(0.02)e
T4	1.21(0.03)h	37.94(0.30)b	14.29(0.09)b	223.0(2.9)b	265.5(4.1)h	0.84(0.07)b
T5	1.58(0.01)d	28.12(0.16)f	8.13(0.04)f	201.8(3.3)d	429.4(2.8)d	0.47(0.03)f
T6	1.82(0.09)a	21.23(0.25)i	5.02(0.05)i	150.3(4.0)g	536.8(3.0)a	0.28(0.05)h
T7	1.37(0.12)f	32.02(0.17)d	10.92(0.13)d	217.1(2.2)c	319.3(4.3)f	0.68(0.09)d
T8	1.64(0.04)c	26.09(0.34)g	7.21(0.02)g	190.9(1.6)e	477.3(2.6)c	0.40(0.02)g
T9	1.75(0.05)b	24.58(0.11)h	6.76(0.15)h	182.6(3.7)f	507.2(1.9)b	0.36(0.01)g

BD = bulk density; WHC = water-holding capacity; TPS = total porosity; AP = aeration porosity; WHP = water-holding porosity; EC = electrical conductivity (25 °C); TOC = total organic carbon.
Means in a column followed by the same letter are not significantly different at $p \leqslant 0.05$ by LSD.
[a] Percentages are based on air-dry weight.
[b] Percentages are based on oven-dry weight.

Table 2
Contents of macro-nutrients (TKN, TP, TK, Ca, Mg, and S) and micro-nutrients (Fe, Cu, Mn, Zn, B, and Mo) in earthworm casts (EWCs), clinoptilolite (CL), green waste (GW), and the final composts. Values are means (SD); $n = 3$. Treatments T1–T9 are described in Table 3.

Material or treatment	Macro-nutrients					
	TKN (%)[a]	TP (%)[a]	TK (%)[b]	Ca (%)[b]	Mg (%)[b]	S (%)[b]
EWCs	4.77(0.05)	0.86(0.06)	2.01(0.08)	2.42(0.11)	1.41(0.09)	14.29(0.08)
CL	4.03(0.09)	1.21(0.03)	2.15(0.11)	1.68(0.02)	2.11(0.10)	0.90(0.05)
GW	1.25(0.01)	0.06(0.04)	0.33(0.02)	0.64(0.02)	0.38(0.02)	6.13(0.04)
T1	2.27(0.04)g	0.12(0.07)f	0.30(0.06)f	1.01(0.04)h	0.55(0.03)g	8.29(0.10)f
T2	2.76(0.02)e	0.24(0.02)e	0.52(0.04)e	1.17(0.03)fg	0.85(0.01)e	10.27(0.08)e
T3	2.99(0.07)d	0.35(0.01)d	0.65(0.01)d	1.26(0.06)e	1.01(0.05)c	10.65(0.05)d
T4	2.66(0.05)f	0.19(0.03)e	0.47(0.05)e	1.12(0.05)g	0.74(0.04)f	10.06(0.07)e
T5	3.46(0.06)c	0.41(0.02)c	0.74(0.03)c	1.38(0.02)d	1.06(0.06)c	11.28(0.06)c
T6	4.23(0.04)a	0.58(0.05)a	0.91(0.01)a	1.63(0.01)a	1.23(0.07)a	13.41(0.09)a
T7	2.94(0.08)d	0.30(0.06)d	0.61(0.03)d	1.22(0.07)ef	0.92(0.03)d	10.32(0.10)de
T8	3.62(0.02)b	0.44(0.03)bc	0.79(0.04)bc	1.45(0.02)c	1.15(0.02)b	11.76(0.02)c
T9	3.64(0.03)b	0.47(0.08)b	0.84(0.02)b	1.51(0.04)b	1.18(0.01)ab	12.54(0.03)b
	Micro-nutrients					
	Fe ($\times 10^{-3}$%)[b]	Cu ($\times 10^{-3}$%)[b]	Mn ($\times 10^{-3}$%)[b]	Zn ($\times 10^{-3}$%)[b]	B ($\times 10^{-3}$%)[b]	Mo ($\times 10^{-3}$%)[b]
EWCs	21.50(0.27)	0.07(0.04)	0.01(0.01)	0.08(0.03)	0.23(0.10)	0.33(0.11)
CL	12.06(0.13)	0.64(0.02)	0.09(0.03)	0.05(0.01)	0.49(0.05)	0.48(0.06)
GW	9.09(0.14)	0.10(0.01)	6.28(0.13)	0.84(0.08)	0.97(0.09)	0.42(0.09)
T1	10.12(0.22)i	0.24(0.04)g	9.62(0.20)g	1.02(0.09)f	1.59(0.17)g	0.87(0.07)g
T2	16.34(0.30)g	0.36(0.06)f	11.83(0.09)e	1.20(0.11)e	2.26(0.14)f	1.50(0.05)e
T3	21.01(0.18)e	0.49(0.03)d	12.54(0.15)d	1.29(0.15)d	2.38(0.11)de	1.58(0.06)d
T4	15.40(0.26)h	0.32(0.02)f	11.19(0.18)f	1.15(0.07)e	2.21(0.15)f	1.43(0.03)f
T5	22.96(0.16)d	0.58(0.07)c	13.05(0.12)c	1.36(0.06)c	2.42(0.18)cd	1.65(0.02)c
T6	26.44(0.17)a	0.79(0.03)a	15.33(0.11)a	1.52(0.13)a	2.61(0.10)a	1.85(0.09)a
T7	18.38(0.29)f	0.43(0.04)e	12.11(0.23)de	1.27(0.16)d	2.33(0.12)e	1.56(0.01)d
T8	24.09(0.20)c	0.63(0.01)bc	13.79(0.07)b	1.42(0.15)b	2.47(0.16)c	1.73(0.07)b
T9	25.12(0.10)b	0.67(0.05)b	14.02(0.16)b	1.47(0.09)ab	2.54(0.13)b	1.76(0.04)b

TKN = total Kjeldahl nitrogen; TP = total phosphorus; TK = total potassium.
Means in a column followed by the same letter are not significantly different at $p \leqslant 0.05$ by LSD.
[a] Percentages are based on air-dry weight.
[b] Percentages are based on oven-dry weight.

Table 3
Orthogonal design $L_9(3^4)$ of the experiment.

Treatment	EWCs (% in initial GW, dry weight)	CL (% in initial GW, dry weight)
T1	0	0
T2	0	15
T3	0	25
T4	0.30	0
T5	0.30	15
T6	0.30	25
T7	0.60	0
T8	0.60	15
T9	0.60	25

EWCs = earthworm casts; CL = clinoptilolite; GW = green waste.

adjusted to 25–30 by application of urea. The microbial inoculum was also added to the raw materials (5 ml/kg dry GW). Finally, different quantities of EWCs and CL were mixed together as indicated in Table 3.

A two-stage composting process was used in which the mixture was subjected to a PF and then to a SF. At the beginning of the PF, the mixtures were added on day 0 to each of 27 digester cells, which were non-covered cement containers (6 m long, 2 m wide, and 1.5 m high) with an automatic compost-turning and -watering system. The automatic compost-turning and -watering system is made by Beijing Jingpuyuan Biological Engineering Co., Ltd. (China) and is controlled by a computer. In addition, the turning system uses a "roller conveying" design and also can move up and down as needed for different depths of the composting materials (depth <1.7 m, width <5 m). Each treatment was represented by three replicate digester cells. The automatic system turned the mixture in each digester cell every day during the PF. When the temperature of the mixture increased to 60–70 °C, 2 ml of bamboo vinegar (diluted in 2 L of water) was added per 100 kg of GW (dry weight). The vinegar solution was sprinkled onto the mixtures as they were being turned. When the temperature dropped to 45–55 °C, the PF was considered complete. The temperatures in all treatments decreased to 45–55 °C by day 6. At this time, the mixtures were once again treated with the vinegar solution and also with EWCs and CL according to Table 3. In addition, the mixture was removed from each digester cell with an excavator and was placed in three windrows (three windrows per cell). The SF of all treatments began on day 6. Each windrow had a trapezoidal cross-section and was 2 m long, 1.5 m wide, and 1 m high. A mini-excavator was used to turn the windrows every 3 days in order to aerate and homogenize the mixtures and to stimulate microbial activities. EWCs, CL, and diluted bamboo vinegar were added every 6 days during the SF. When the temperature of a windrow decreased to the ambient temperature, the compost was considered mature.

2.3. Sampling and monitoring

Each replicate was sampled after 0, 1, 3, 6, 8, 15, 18, 21, 26, and 30 days of composting. Three subsamples (200 g per subsample) were collected from the top, middle, and bottom of each cell or windrow. The three subsamples from each cell or windrow were combined to form one composite sample per cell or windrow. The samples taken from the digester cells and windrows were divided into three portions. One portion was air-dried (3–5% moisture content) for determination of physical characteristics, pH, electrical conductivity (EC), total organic carbon (TOC), total Kjeldahl N (TKN), total phosphorus (TP), humic substances, and the contents of cellulose and hemicellulose. The second portion was oven-dried at 65 °C for determination of contents of total potassium (TK), mineral N (NH_4^+–N and NO_3^-–N), macro-nutrients (Ca, Mg, and S), and micro-nutrients (Fe, Cu, Mn, Zn, B, and Mo). The third portion, which was not dried, was used for quantification of microorganisms and enzyme activities; the third portion was also used for a seed germination test. When the same physical or chemical property was determined for the raw materials and the composting samples, the same procedure was used.

Temperature was measured daily at three points along the length of the middle of each digester cell and windrow using a self-made temperature sensor consisting of a temperature sensor attached to a 1-m-long rod. Ambient temperature was also recorded using the same temperature sensor. The three readings per cell or windrow per day were averaged.

2.4. Physico-chemical analyses

Bulk density (BD), water-holding capacity (WHC), total porosity (TPS), aeration porosity (AP), and water-holding porosity (WHP) of the raw materials and the final composts were determined by the ring knife method described by Zhang et al. (2013).

The pH and EC of a suspension of compost in water (1:10, v/v) were measured with an MP521 pH/EC meter (Shanghai, China). TOC was determined by oxidation with potassium bichromate. TKN was measured by the Kjeldahl method. TP was estimated by the Anti-Mo–Sb spectrophotometry method using a 721 spectrophotometer (Shanghai, China). TK was determined by flame photometry using a 425 flame photometer (Shanghai, China). Mineral N was extracted with 2 M KCl (1:100 ratio for 1 h) followed by colorimetric analysis of NH_4^+–N and NO_3^-–N, which are expressed per gram of dry weight of composting sample (Belyaeva and Haynes, 2009). Macro- and micro-nutrients were analyzed by inductively coupled plasma mass spectrometry (New Hampshire, America).

2.5. Humic substance extraction

Humic substances, including humic acids (HAs) and fulvic acids (FAs), were determined by the method of Francou et al. (2008). In the present study, HAs and FAs contents were expressed as a percentage of TOC.

2.6. Microbiological analyses

2.6.1. Estimation of microbial numbers

Throughout the composting process, the abundances of culturable bacteria, actinomycetes, and fungi in the composting sample were determined by serial dilution and plating as described by Sen and Chandra (2009), Shi et al. (2006).

2.6.2. Enzyme activities

CMCase and cellobiase activities were determined as recommended by Ghose (1987). Avicelase, xylanase, and dehydrogenase activities were determined according to the methods of Pardo (1996), Saha et al. (2005), Gabhane et al. (2012), respectively. The activities of these enzymes were measured in international units (IUs), where one unit (IU) of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glucose or xylose per minute under the given assay conditions (Liu et al., 2006). Activities are expressed per gram of dry weight of composting sample.

2.7. Fiber analyses

The proportions of cellulose and hemicellulose in the GW and in the final composts were determined by crude fiber analysis according to the method of Goering and Van Soest (1970). The degradation rates of cellulose and hemicellulose (organic matter loss) was calculated according to the initial and final ash levels. The GW and the final composts were ashed at 550 °C for 6 h in a muffle furnace.

Ash content was used to determine the indices for relative cellulose content (cellulose content/ash content) and relative hemicellulose content (hemicellulose content/ash content) because the ash content did not change during the composting (Liu et al., 2006). The index was then used to calculate cellulose and hemicellulose degradation rates for all treatments. Degradation rate (%) = (index of initial GW − index of the treatment) × 100%/index of initial GW.

2.8. Germination test

In a germination test, the seed germination rate and the germination index (48 h at 25 °C in the dark) were determined for 25 seeds of pakchoi (*Brassica rapa* L., Chinensis group) per replicate of the nine final compost products. The seeds were placed in a 90-mm Petri dish with 5 ml of compost extract (1:5, w/v) (Zhang et al., 2013). Controls were treated in the same way but with distilled water rather than compost extract as the culture solution. Each treatment was represented by three replicate dishes. According to Zhang et al. (2013), the seed germination rate (%) = mean number of germinated seeds per dish × 100%/number of seeds per dish, and the germination index (%) = (mean number of germinated seeds in the treatment × mean root length in the treatment × 100%)/(mean number of germinated seeds in the control × mean root length in the control).

2.9. Statistical analysis

One-way analyses of variance (ANOVAs) were used to determine whether the physical, chemical, and microbiological characteristics of the composting samples were affected by the treatments. When ANOVAs were significant, means were separated with an LSD test. As noted earlier, the samples collected at the same time from individual digester cells and from individual windrows were treated as replicates. All statistical analyses were performed with the SPSS16.0.

3. Results and discussion

3.1. Temperature

Temperature is an important parameter in the monitoring of the composting process and in the determination of compost quality (Liu and Price, 2011). Changes in the ambient temperature and the composting temperature in the current experiment are shown in Fig. 1. During the PF, temperatures in treatments T5, T6, T8, and T9 rapidly increased to the thermophilic phase (50–60 °C) on day 1, while temperatures in treatments T1, T2, T3, T4, and T7 gradually increased and reached the thermophilic phase on day 3. The maximum temperatures during the PF in treatments T1, T2, T3, T4, T5, T6, T7, T8, and T9 were 50.7, 51.5, 53.8, 51.0, 55.3, 58.3, 52.0, 56.1, and 57.2 °C, respectively. Subsequently, all the temperatures declined to 40–50 °C on day 6. Thereafter, the temperatures began to increase to a second thermophilic phase in the SF. The peak temperatures during the SF in treatments T1, T2, T3, T4, T5, T6, T7, T8, and T9 were 51.1, 52.8, 54.6, 52.6, 57.4, 60.1, 53.2, 58.0, and 58.6 °C, respectively. These temperatures were higher than those during the PF. During the maturation phase, all of the temperatures decreased and finally stabilized near the ambient temperature at 21 days for treatments T5, T6, T8, and T9; at 26 days for treatments T2, T3, T4, and T7; and at 30 days for treatment T1. During the

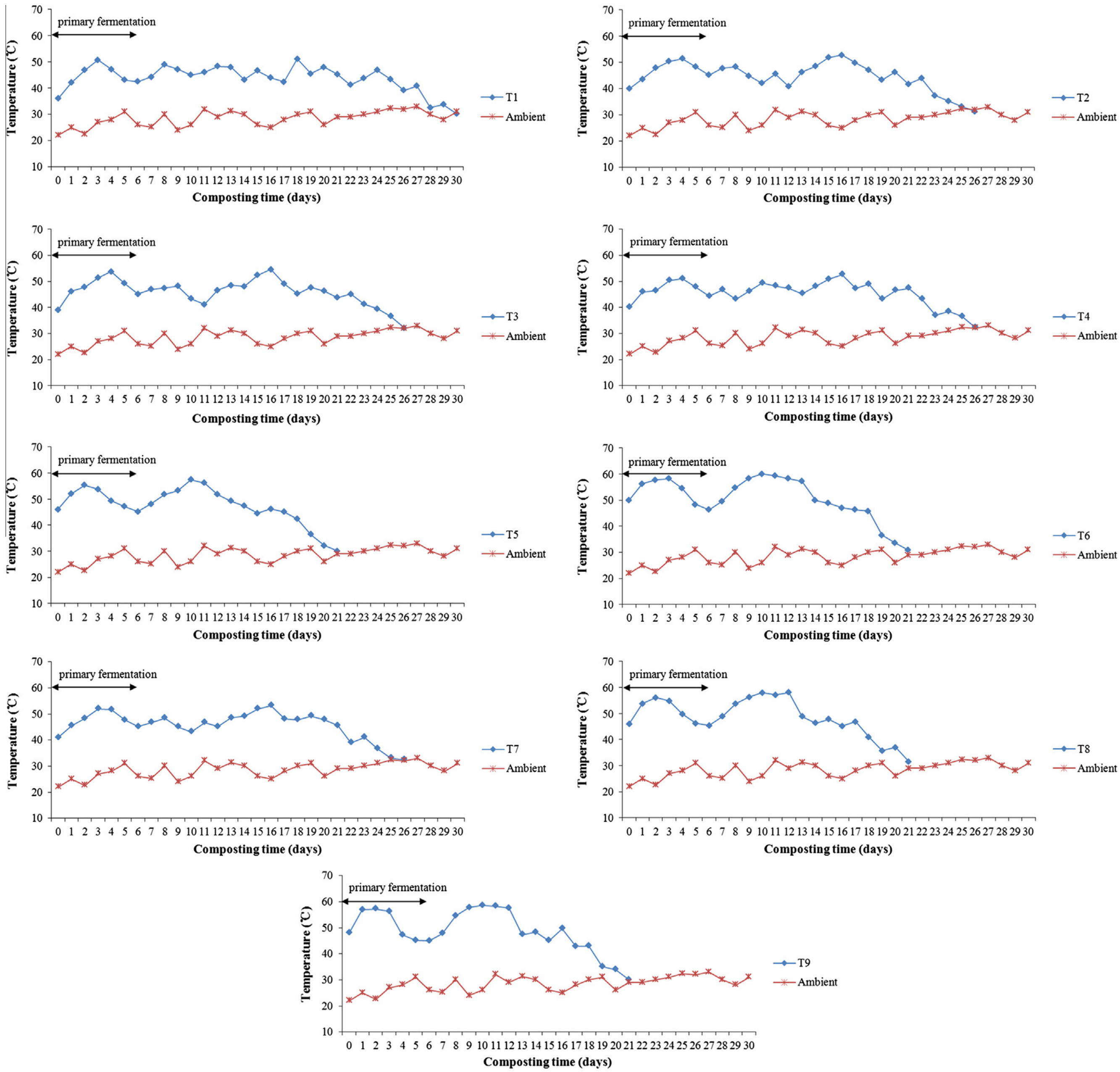

Fig. 1. Effects of earthworm casts (EWCs) and clinoptilolite (CL) on temperatures during the two-stage composting of green waste. Ambient air temperatures are also indicated and are the same for all treatments. EWC and CL levels in treatments T1–T9 are described in Table 3.

entire composting process, the temperature was highest in treatment T6 and lowest in treatment T1.

Many researchers believe that, during composting, the rate of oxidative degradation of organic waste is highest in the thermophilic phase (50–60 °C) (Belyaeva and Haynes, 2009). For all treatments except treatment T1, temperatures of 50–60 °C lasted longer than 3 days, which met the sanitation requirements according to Zhang et al. (2013). The duration of the thermophilic phase in treatments with the combined addition of EWCs and CL (treatments T5, T6, T8, and T9) ranged from 3 to 4 days and from 5 to 6 days in the PF and SF, respectively; without the combined addition of EWCs and CL, the thermophilic phase lasted for only 1–2 days in the PF and SF, respectively. The thermophilic phase was longest in treatment T6 (4 days during the PF and 6 days during the SF) and was shortest in treatment T1 (1 day during the PF and SF, respectively). The early start and extended duration of thermophilic phase in treatments T5, T8, T9, and especially in treatment T6 indicated that the combined addition of EWCs and CL may help to achieve and sustain the thermophilic temperature, which could enhance the degradation and stabilization of organic matter. This effect of the combined addition of EWCs and CL could be due to the instant supply of nutrients provided by these materials; the nutrients could enhance microbial metabolism and growth and thus enhance the generation of metabolic heat (Gabhane et al., 2012). According to Lalander et al. (2013), Venglovsky et al. (2005), the addition of EWCs and CL to GW could enhance aeration and moisture holding capacity, and thereby, increase decomposition efficiency.

Although traditional composting requires about 90–270 days to generate a stable product (Khalil et al., 2008), only 21 days were needed when two-stage composting was used with the combined addition of EWCs and CL in the current study. The best combination was 0.30% EWCs and 25% CL.

3.2. Physical properties of the final compost

The physical properties of the final composts are summarized in Table 1. BD values significantly differed ($p < 0.05$) among the treatments but were close to the standard of 0.40 g/cm^3 suggested by Zhang et al. (2013) in treatments T5, T6, T8, and T9. WHC was higher ($p < 0.05$) in treatments T5, T6, T8, and T9 than in the other treatments. WHC was highest in treatment T6 and lowest in treatment T1. The relationship for TPS among treatments was similar to that for WHC. TPS was highest in treatment T6, followed by treatments T9, T8, and T5, all of which were amended with both EWCs and CL; TPS was lowest in treatment T1.

The above results demonstrate that the combined addition of EWCs and CL, especially 0.30% EWCs and 25% CL, not only reduced the BD of the composting materials but also enhanced aeration and water retention in the compost product. These positive changes in physical characteristics probably contributed to an improvement in the formation of particle structures in the final composts amended with EWCs and CL, which agrees with Sen and Chandra (2009), Lu et al. (2014).

3.3. pH and electrical conductivity (EC)

During the PF, pH increased to a maximum on day 1 for treatments T5, T6, T8, and T9, and increased to a maximum on day 3 for treatments T1, T2, T3, T4, and T7 (Fig. 2). pH then decreased for all treatments until day 6. After day 6, the pH of all treatments increased and reached a second peak (on day 8 for treatments T5, T6, T8, and T9, and on day 15 for treatments T1, T2, T3, T4, and T7). The values of these second peaks were higher for treatments T1, T2, T3, T4, and T7 than for treatments T5, T6, T8, and T9. The final

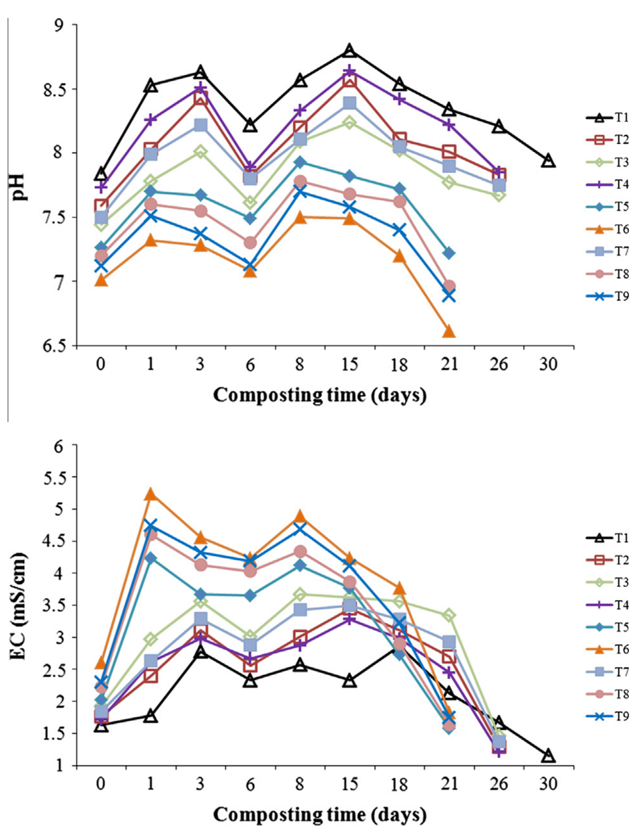

Fig. 2. Effects of earthworm casts (EWCs) and clinoptilolite (CL) on pH and electrical conductivity (EC) during the two-stage composting of green waste. EWC and CL levels in treatments T1–T9 are described in Table 3.

pH of treatments T5, T6, T8, and T9 were within the acceptable range (6.5–7.5) for mature compost as prescribed by Karak et al. (2013) (Table 1).

Jumnoodoo and Mohee (2011) suggested that the optimal pH during composting was between 7.0 and 8.0, because pH in that range benefitted microbial activities. During composting, the pH in treatments with the combined addition of EWCs and CL (treatments T5, T8, T9, and especially treatment T6) were more suitable for composting than in the other treatments, particularly between the thermophilic phases. The results indicate that the optimized pH resulting from the combined addition of EWCs and CL contributed to a greater rate of microbial degradation. Moreover, the pH between 7.0 and 8.0 may signify increased N retention (Raphael and Velmourougane, 2011). The too high pH of treatments without the combined addition of EWCs and CL probably resulted from an increase in ammonification and therefore an increase in ammonia release. EWCs are rich in organic acids that probably inhibit ammonia volatilization and regulate the pH during composting (Raphael and Velmourougane, 2011). In addition, because of its adsorptive and ion exchange properties, CL could also absorb ammonia and cations to decrease the pH during composting (Chiang et al., 2007).

The effects of treatments on EC were opposite to those on pH, i.e., treatments that caused the lowest pH caused the highest EC (Fig. 2). This is consistent with a previous report (Liu and Price, 2011). During composting, the EC was higher for treatments T5, T6, T8, and T9 than for treatments T1, T2, T3, T4, and T7. Although the EC of final compost was highest for treatment T6 and lowest for treatment T1, the values for all treatments were within the acceptable range ($\leqslant$4 mS/cm) for mature compost (Karak et al., 2013) (Table 1). In general, the EC is increased (as it was in treatments T5, T6, T8, and T9) because of the release of soluble salts like nitrate and phosphate during decomposition but is reduced (as it was in treatments T1, T2, T3, T4, and T7) because of the volatilization of ammonia and the leaching of mineral salts (Lazcano et al., 2008; Liu and Price, 2011). The results indicate that the treatments with the combined addition of EWCs and CL (especially treatment T6 with the combined addition of 0.30% EWCs and 25% CL) had greater nitrification and greater nutrient retention than the other treatments during the composting process, which also agrees with Sen and Chandra (2009), Venglovsky et al. (2005).

3.4. Humification of organic matter

As decomposition proceeds, HAs are generally rapidly produced while FAs may either slightly decrease or remain unchanged, and the mature compost product therefore contains high levels of HAs and low levels of FAs (Jouraiphy et al., 2005). The results of the current study were consistent with that generalization in that the HA content of all treatments increased while the FA content decreased only slightly (Table 4). The degree of humification and compost maturity are indicated by the humification ratio (HR), which is the ratio between HAs and FAs expressed as a percentage of TOC (Francou et al., 2008). In the present study, the HR of all treatments increased, and the final HR values were significantly higher ($p < 0.05$) for treatments with the combined addition of EWCs and CL (treatments T5, T6, T8, and T9) than for the other treatments. The HR was highest for treatment T6 and lowest for treatment T1.

Roletto et al. (1985) considered that an HR >1 indicates a mature compost. The HR was >1 for all treatments in the current study except treatment T1, suggesting that eight of the nine final composts were mature. The results indicate that treatments that combined EWCs and CL (especially at 0.30% EWCs and 25% CL) enhance the humification of organic matter and promote the formation of polycondensed humic structures (Jouraiphy et al.,

Table 4
Organic matter humification in the initial and final composts. Values are means (SD); $n = 3$. Treatments T1–T9 are described in Table 3.

Treatment	HAs (% of TOC)[a]		FAs (% of TOC)[a]		HAs/FAs ratio	
	Initial	Final	Initial	Final	Initial	Final
T1	2.64(0.03)g	10.23(0.10)e	13.90(0.10)a	10.29(0.09)a	0.19(0.02)f	1.00(0.02)h
T2	2.91(0.09)f	11.09(0.11)a	11.31(0.20)c	9.73(0.10)b	0.26(0.03)de	1.14(0.09)g
T3	3.37(0.06)e	10.42(0.17)d	10.20(0.17)e	7.90(0.08)c	0.33(0.04)c	1.34(0.03)e
T4	2.50(0.04)h	10.85(0.16)c	11.92(0.13)b	10.33(0.12)a	0.21(0.06)ef	1.05(0.01)h
T5	4.17(0.07)d	11.06(0.12)a	8.33(0.09)g	6.62(0.05)d	0.50(0.01)b	1.67(0.04)d
T6	4.85(0.02)a	8.38(0.09)g	7.83(0.14)i	3.48(0.07)g	0.62(0.05)a	2.41(0.07)a
T7	2.96(0.01)f	10.91(0.06)b	11.04(0.23)d	9.02(0.14)b	0.27(0.01)d	1.21(0.03)f
T8	4.73(0.10)b	10.84(0.13)c	9.19(0.11)f	5.93(0.03)e	0.51(0.07)b	1.83(0.06)c
T9	4.49(0.05)c	9.97(0.14)f	8.20(0.19)h	4.78(0.06)f	0.55(0.02)b	2.09(0.02)b

HAs = humic acids; FAs = fulvic acids; TOC = total organic carbon.
Means in a column followed by the same letter are not significantly different at $p \leqslant 0.05$ by LSD.
[a] Percentages are based on air-dry weight.

2005). Microorganisms are the main agents responsible for humus formation (Brown et al., 2013). As noted earlier, EWCs contain diverse enzymes and microorganisms, which can speed humification and induce the production of humic substances (Raphael and Velmourougane, 2011). In addition, humic substances generally can be absorbed by CL, which could inhibit the destruction of the molecular structure in HAs during decomposition and also accelerate the formation of humic-like substances (Lu et al., 2014).

3.5. NH_4^+–N, NO_3^-–N, and the NH_4^+–N/NO_3^-–N ratio

N transformations during composting are generally characterized by ammonification and nitrification. Addition of EWCs and CL significantly affected NH_4^+–N and NO_3^-–N contents and the NH_4^+–N/NO_3^-–N ratio (Fig. 3). The NH_4^+ contents of all treatments peaked during the PF and again during the SF. During composting, NH_4^+ contents were considerably higher in treatments T1, T2, T3, T4, and T7 than in treatments T5, T6, T8, and T9 and especially during the thermophilic stages of the PF and SF. The final NH_4^+ content was highest in treatment T1 and lowest in treatment T6 (0.30% EWCs and 25% CL) (Table 1). NH_4^+ content decreased during the last half of the composting process while NO_3^- generally increased throughout the process, probably because the bacteria responsible for nitrification were inhibited by the high temperatures (Fig. 3). Similar results were obtained by Ko et al. (2008). During the process, NO_3^- contents were higher in treatment T5, T6, T8, and T9, which were amended with both EWCs and CL, than in treatments T1, T2, T3, T4, and T7. The NO_3^- content in the final compost was highest in treatment T6 and lowest in treatment T1 (Table 1), indicating that addition of EWCs and CL favored the growth of the nitrobacteria that convert ammonia to nitrate.

Conservation of NH_3 during composting not only improves the nutritional value of final compost but also reduces environmental pollution (Venglovsky et al., 2005). Because of their buffering capability, EWCs have a great impact on N transformations in composting and help retain mineral N in the form of nitrate (Raphael and Velmourougane, 2011). CL is able to decrease the NH_3 through adsorption and ion exchange on its reactive surface, reducing ammonia release during the thermophilic phase (Venglovsky et al., 2005). Furthermore, if the compost product with the ammonium-saturated CL is used as a soilless medium or as a soil amendment, less N fertilizer is required (Zorpas and Loizidou, 2008).

Compost stability is closely related to the nitrification index (NH_4^+–N/NO_3^-–N ratio), and effective nitrification during the biostabilization process generally results in a final compost with an NH_4^+/NO_3^- ratio <1, indicating that the compost reached maturity (Jouraiphy et al., 2005). During composting, the NH_4^+/NO_3^- ratios were higher in treatments T1, T2, T3, T4, and T7 than in treatments T5, T6, T8, and T9, but the final ratios were <1 for all treatments

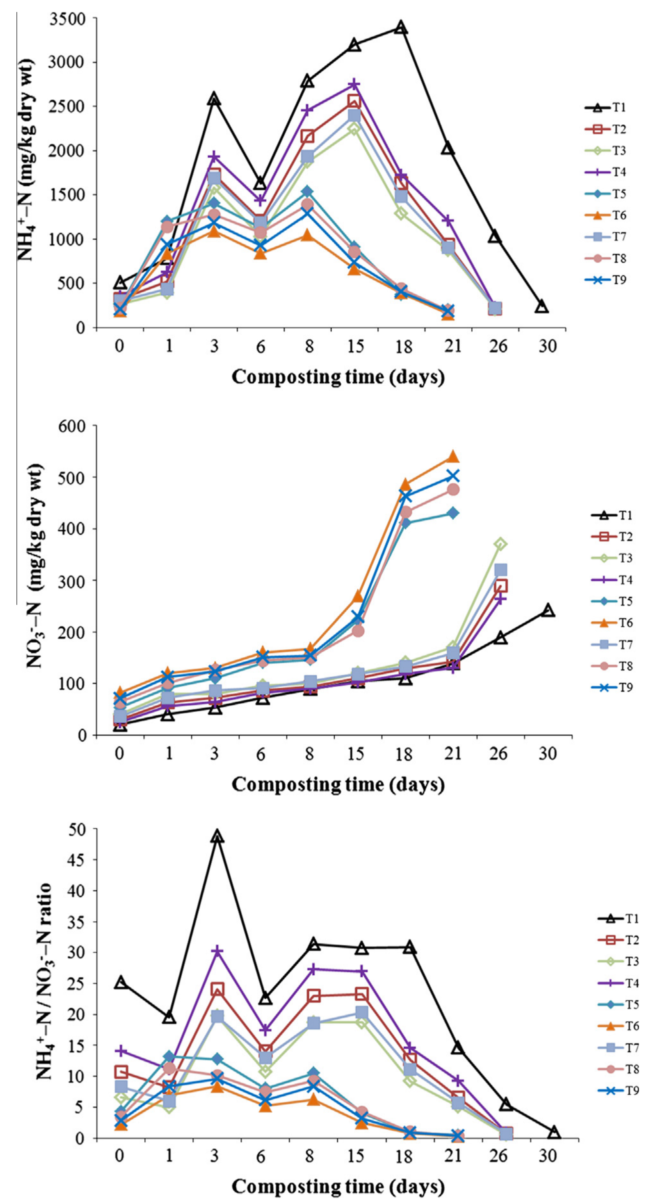

Fig. 3. Effects of earthworm casts (EWCs) and clinoptilolite (CL) on NH_4^+–N, NO_3^-–N, and the NH_4^+–N/NO_3^-–N ratio during the two-stage composting of green waste. EWC and CL levels in treatments T1–T9 are described in Table 3.

(Table 1 and Fig. 3). The NH_4^+/NO_3^- ratio in the final compost was highest in treatment T1 and lowest in treatment T6. These results indicate a rapid decrease in ammonium in treatments T5, T8, and T9 and especially in treatment T6, which was amended with 0.30% EWCs and 25% CL. The rapid decrease in ammonium in these treatments coincided with a rapid increase in nitrate concentration and an increased retention of N in the final compost.

3.6. Microbial numbers

Addition of EWCs and CL significantly affected the numbers of culturable bacteria, actinomycetes, and fungi during composting (Fig. 4). The numbers of culturable microorganisms increased sharply leading up to the thermophilic phase of the PF and then decreased slightly. During the SF, the numbers increased again and then dropped gradually until the end of the process. During the whole process, microbial numbers were higher in treatments T5, T6, T8, and T9 than in the other treatments. Numbers were highest in treatment T6 and lowest in treatment T1.

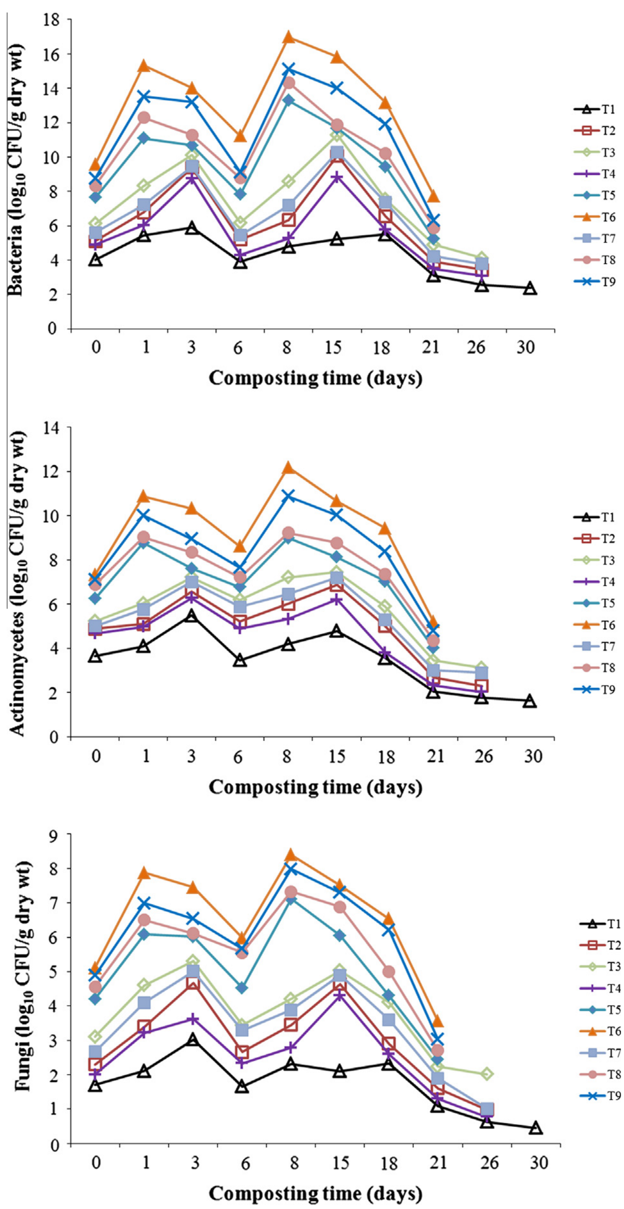

Fig. 4. Effects of earthworm casts (EWCs) and clinoptilolite (CL) on numbers of culturable bacteria, actinomycetes, and fungi during the two-stage composting of green waste. EWC and CL levels in treatments T1–T9 are described in Table 3.

The results, which agree with those of previous studies (Lalander et al., 2013; Zorpas et al., 2003), indicate that the combined addition of EWCs and CL, and especially the combined addition of 0.30% EWCs and 25% CL, stimulated microbial growth and activities. This is reasonable because EWCs and CL are enriched in nutrients and particularly in easily available carbon, N, phosphorus, and potassium. In addition, EWCs and CL could improve the moisture content and free air space of the composting materials, which can significantly enhance microbial activity (Venglovsky et al., 2005). EWCs also contain large numbers of fungi, bacteria, and actinomycetes, which can enhance microbial numbers during composting (Raphael and Velmourougane, 2011). Finally, because of its high porosity and large surface area, CL can retain nutrients, bind microorganisms, and serve as a stable substrate for microbial reproduction (Zorpas and Loizidou, 2008).

3.7. Enzyme activities

Enzyme activities have often been used as indicators of microbial metabolism during composting (Sen and Chandra, 2009). The current study determined the changes in the activities of CMCase, cellobiase, avicelase, xylanase, and dehydrogenase during composting (Fig. 5). The trends for enzyme activities were similar to those for microbial numbers. Enzyme activities were higher in all treatments that included the combined addition of EWCs and CL (treatments T5, T6, T8, and T9) than in the other treatments. Enzyme activities were highest in treatment T6 (0.30% EWCs and 25% CL) and lowest in treatment T1.

Singh et al. (2013) suggested that EWCs contain a large amount of enzymes that could contribute to the enhancement of enzyme activities and the GW degradation during composting. Clay minerals such as CL have been reported to enhance the production of enzymes (Zorpas et al., 2003). The uniform and porous structure of CL may provide a microenvironment that can stabilize enzymes so that they remain active during composting (Venglovsky et al., 2005).

According to Sen and Chandra (2009), dehydrogenase activity is a useful indicator of the overall oxidative activities of heterotrophic microorganisms. As noted earlier, dehydrogenase activity was increased by the combined addition of EWCs and CL, and the optimal combination was 0.30% EWCs and 25% CL. Additionally, a previous study also showed that the dehydrogenase activity in EWCs was enhanced when the casts were mixed with organic wastes in the composting process (Song et al., 2014).

3.8. Cellulose and hemicellulose degradation

Because the mass of the composting materials decreases during composting, the changes in percentages of cellulose and hemicellulose during composting do not correctly reflect their degradation. As described in Section 2.7, the degradation rates of cellulose and hemicellulose were calculated according to a relative index, which was based on ash content; a lower value for this index indicates a greater rate of degradation (Liu et al., 2006). As shown in Table 5, cellulose and hemicellulose degraded significantly faster ($p < 0.05$) in treatments T5, T6, T8, and T9 than in the other treatments. The degradation was highest in treatment T6 and lowest in treatment T1. These results indicate that the combined addition of EWCs and CL, and especially the combined addition of 0.30% EWCs and 25% CL in treatment T6, increased the decomposition of organic matter and reduced the duration of composting. The enhanced degradation of cellulose and hemicellulose in GW amended with both EWCs and CL was likely due to the effects of these amendments on the physical, chemical, and microbiological properties during composting, as previously discussed.

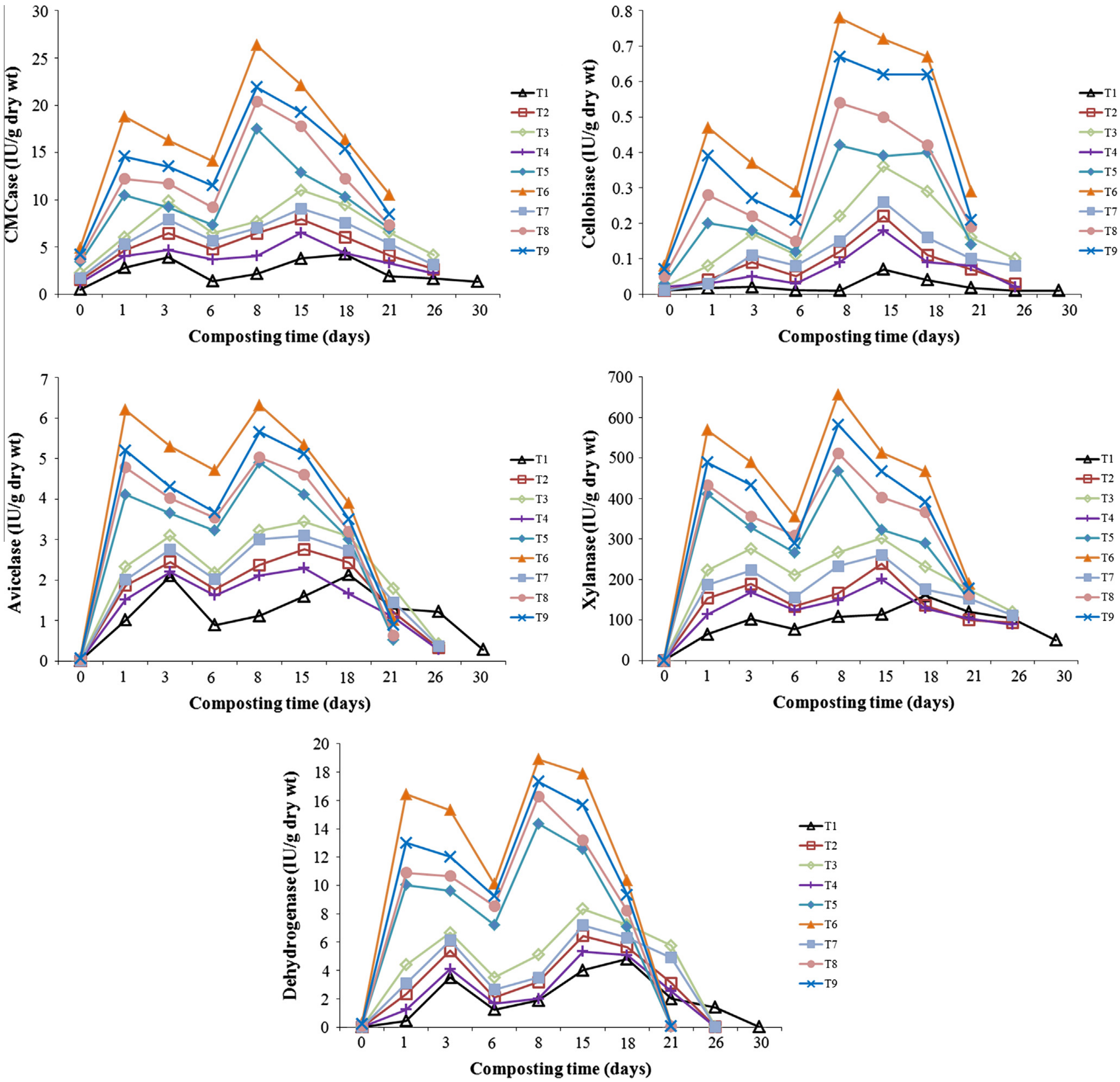

Fig. 5. Effects of earthworm casts (EWCs) and clinoptilolite (CL) on the activities of CMCase, cellobiase, avicelase, xylanase, and dehydrogenase during the two-stage composting of green waste. EWC and CL levels in treatments T1–T9 are described in Table 3.

3.9. Nutrient contents of the final compost

Compost quality depends in part on quantity of nutrients. Nutrient contents of the final composts were significantly higher ($p < 0.05$) in treatments T5, T6, T8, and T9 than in treatments T1, T2, T3, T4, and T7 (Tables 1 and 2). Nutrient contents were highest in treatment T6 and lowest in treatment T1.

These results show that the combined addition of EWCs and CL, and especially the combined addition of 0.30% EWCs and 25% CL, resulted in the final composts with high levels of nutrients. This is reasonable because EWCs and CL improved microbial activities and thereby enhanced the decomposition of GW and associated nutrient mineralization. The results are also consistent with those of a previous study (Hill et al., 2013). Song et al. (2014) pointed out that EWCs contain substantial quantities of nutrients (especially TN, TP, TK, Ca, and Mg), which are released into the compost and also contribute to the nutrient content of the final compost. In addition, Zorpas et al. (2003) found that addition of CL increased TN, TP, and TK during composting, suggesting that CL could also release some nutrients into composting materials when it was combined with water. CL can also enhance the nutrient content of the final compost because of its substantial absorptive and ion exchange capacities (Zorpas and Loizidou, 2008).

3.10. Seed experiment

The germination index, which is a measure of relative seed germination and relative seed root elongation, is used to evaluate compost maturity and its potential toxicity (Chiang et al., 2007). The germination index and seed germination rate were substantially higher ($p < 0.05$) in treatments that combined EWCs and CL (treatments T5, T6, T8, and T9) than in the other treatments (treatments T1, T2, T3, T4, and T7) (Table 6). The values were highest in

Table 5
Contents of cellulose, hemicellulose, and ash in the green waste (GW) and in the final composts and degradation rates of cellulose and hemicellulose. Values are means (SD); $n = 3$. Treatments T1–T9 are described in Table 3.

Material or treatment	Cellulose (%)[a]	Hemicellulose (%)[a]	Ash content (%)[a]	Cellulose		Hemicellulose	
				Index[b]	Degradation rate (%)	Index[b]	Degradation rate (%)
GW	29.59(0.15)	52.29(0.15)	10.23(0.11)	2.89(0.02)	0	5.11(0.08)	0
T1	16.78(0.11)g	24.12(0.06)a	7.53(0.10)h	2.23(0.05)a	22.96(0.11)i	3.20(0.08)a	37.34(0.15)i
T2	21.70(0.17)e	22.96(0.16)b	10.88(0.09)f	1.99(0.07)b	31.04(0.13)g	2.11(0.07)bc	58.71(0.11)g
T3	23.06(0.10)d	20.79(0.10)d	12.49(0.17)e	1.85(0.01)c	36.17(0.09)e	1.66(0.02)cde	67.44(0.18)e
T4	19.07(0.12)f	23.17(0.17)b	9.24(0.14)g	2.06(0.08)b	28.65(0.12)h	2.51(0.10)b	50.95(0.13)h
T5	24.37(0.06)c	20.49(0.12)d	14.26(0.13)d	1.71(0.12)d	40.92(0.08)d	1.44(0.03)def	71.89(0.16)d
T6	30.19(0.08)a	19.06(0.08)f	24.11(0.08)a	1.25(0.04)g	56.71(0.14)a	0.79(0.09)g	84.53(0.19)a
T7	22.75(0.14)d	22.38(0.14)c	12.02(0.15)e	1.89(0.06)c	34.56(0.10)f	1.86(0.06)cd	63.58(0.10)f
T8	26.02(0.13)b	19.68(0.09)e	16.27(0.12)c	1.60(0.09)e	44.70(0.16)c	1.21(0.01)efg	76.33(0.14)c
T9	26.50(0.09)b	19.41(0.11)ef	18.10(0.16)b	1.46(0.11)f	49.39(0.17)b	1.07(0.04)fg	79.02(0.12)b

Means in a column followed by the same letter are not significantly different at $p \leqslant 0.05$ by LSD.
[a] Percentages are based on air-dry weight.
[b] The relative index is defined as the ratio of (hemi) cellulose content/ash content (w/w).

Table 6
Seed germination rate and germination index of the final composts. Values are means (SD); $n = 3$. Treatments T1–T9 are described in Table 3.

Treatment	Seed germination rate (%)	Germination index (%)
T1	81(1)d	101(2)h
T2	90(2)c	124(2)fg
T3	93(1)bc	133(1)e
T4	84(1)d	120(3)g
T5	95(2)abc	142(2)d
T6	100(1)a	168(2)a
T7	93(1)bc	126(3)f
T8	95(3)abc	151(2)c
T9	98(2)ab	157(1)b

Means in a column followed by the same letter are not significantly different at $p \leqslant 0.05$ by LSD.

treatment T6 and lowest in treatment T1. The germination indices of all treatments were >80%, and thus, according to Zorpas and Loizidou (2008), all of the compost products were mature and non-phytotoxic. The above results indicate that combined addition of EWCs and CL, and especially the combined addition of 0.30% EWCs and 25% CL, greatly enhanced seed germination and root elongation and also efficiently decreased the toxicity of the compost product.

4. Conclusion

The present study indicates that EWCs and CL enhanced GW composting. The most efficient two-stage composting and the highest compost quality were obtained when GW was amended with the combination of 0.30% EWCs and 25% CL. This optimal combination extended the thermophilic phase, improved aeration and water permeability, moderated pH and EC, decreased ammonia volatilization, increased microbial numbers and enzyme activities, accelerated the humification and the degradation rate, and enhanced the nutrient content in the final composts. Two-stage composting of GW with 0.30% EWCs and 25% CL produced a high quality compost in only 21 days.

Some practical considerations about the application of this research in an industrial composting facility are presented below:

(1) The addition of additives: EWCs and CL should be added while the composting materials are being turned to evenly mix the materials and to increase the reaction areas between the additives and the GW.
(2) The control of composting moisture: During the whole two-stage composting process (the PF and the SF), the moisture content of composting materials should be checked every day and should be maintained at approximately 60%. If moisture content is too high or too low, it will reduce microbial activity.
(3) The monitoring of composting temperature: In the whole composting process, the period with temperatures between 50 and 60 °C should exceed 3 days because these temperatures will favor microbial activity and kill pathogens.
(4) The shift from composting in digester cells (PF) to composting in windrows (SF): When the composting temperature drops to 45–55 °C in the digester cells, the mixture should be placed in the windrows.
(5) Composting period: Difference in the length of the composting period is mainly due to differences in the original sources of composting wastes and additives, weather conditions, and practical operational methods. In two-stage composting with addition of EWCs and CL, the PF generally requires 5–7 days and the SF generally requires 15–23 days.
(6) Criteria for determining when the GW-derived compost is mature: Once the thermophilic temperature (50–60 °C) has lasted >3 days, the GW-derived compost can be considered mature if the product has the following characteristics: a pH of 6.5–7.5, an EC $\leqslant$ 4 mS/cm, a humification ratio >1, a NH_4^+–N/NO_3^-–N ratio <1, and a germination index >80%.

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