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Compost of Different Stability Affects the Molecular Composition and Mineralization of Soil Organic Matter

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ABSTRACT

This study investigated the C mineralization and chemical modification of a typical tropical soil amended with regional compost of different stability. Compost samples were produced from coffee pulp and fruit and vegetable waste in a method of small heap composting and the samples were collected in three different phases of composting. Both the fresh waste and compost samples were analyzed for their phytotoxicity. These samples were added to a tropical Nitisol at the rate of 48 t·ha^{-1} and a control was set up without amendment. The CO_2-C respired was determined during 98 days of incubation and the incubated samples were taken at the start and end of incubation for molecular-chemical analysis by Pyrolysis-Field Ionization Mass Spectrometry (Py-FIMS). The fresh waste yielded a germination index (GI) < 26% indicating phytotoxicity but this disappeared in all the composts (GI > 100%). The CO_2-C respired was best explained by a first order plus linear model. A soil amended with a compost taken at the thermophilic phase attained the lowest overall organic C loss. In general, the Py-FIMS revealed a significant enrichment of stable N-compounds during the incubation in all amended soils compared to the control. Furthermore, among the compost-soil mixtures Py-FIMS indicated significantly higher increases in the proportions of carbohydrates, peptides and phenols/lignin monomers at the expense of fatty acids and sterols in soil amended with composts from the thermophilic phase. Thermal volatilization curves of Py-FIMS indicated enrichments of stable N-compounds and peptides in compost amended soil. This was a result of enhanced decomposition and stabilization of decomposition products by physical protection through association with clay and soil aggregates. In summary, application of compost shortly after reaching the high temperature phase was shown to be more efficient in organic C sequestration in a clay-rich tropical agricultural soil than mature composts.

Keywords: Compost; Soil Organic Matter; Soil Respiration; Soil Carbon Sequestration; Pyrolysis-Field Ionization Mass Spectrometry; Molecular Composition

1. Introduction

Organic waste recycling in agriculture through composting is increasing as an environmentally sustainable waste management strategy [1]. It improves the soil fertility as compost increases the soil organic matter (SOM) content [2,3] and modifies the chemical, physical and biological properties of SOM [4-6]. Moreover, the added compost can lead to significant changes in the composition of SOM and minimize carbon (C) mineralization from the soil [3,7-9]. This will have a global implication as it is in line with the Kyoto protocol (1997) that aims at reducing greenhouse gas emissions through C sequestration in soils. Some authors suggested a significant impact of land application of organic residues to meet the emission reduction target agreed under the Kyoto protocol [10,11]. The strategy has more implications in tropical regions like Ethiopia where the degradation of SOM has been

accelerated by the high temperature and a wide set of soil fertility issues [12]. Moreover, tropical soils have lost a significant part of their original SOM pool, and therefore, have a capacity to sequester C by adopting recommended management practices. For these soils the estimated annual C sequestration potential is 0.34 to 0.52 Pg [13].

Coffee (*Coffea arabica* L.) is one of the abundant agricultural products generates expressive amounts of agricultural residues during processing [14]. Ethiopia is the third coffee producer country in the world with a production accounting for 61% of the annual commodity exports [15]. At present considerable amounts of coffee processing by-products like coffee pulp (40% of the wet weight) are generated and mainly damped to water bodies, unsanitary landfills and partly applied to agricultural fields without pre-treatment. This causes freshwater eutrophication, health problems, adverse effects on soil

properties such as induced anaerobic conditions and release of phytotoxic compounds impairing plant growth as well as offensive odour and greenhouse gases generation when collected, transported and disposed [15,16]. Moreover, a further increase of the amount of agro-industrial by-products can be assumed by the current development plan of Ethiopia. This triggers the need to find a sink for this growing amount of waste and at the same time opens up the chance for recycling the waste as compost for the improvement of soil fertility and the sequestration of C in soil. However, composting of organic waste has to be optimized to avoid adverse effects to plant growth and minimize overall C losses in the transformation process.

Aerobic incubation experiments were used to measure organic matter decomposition or soil C sequestration potential in compost-fertilized, inorganic fertilized or non-amended soils [7,17]. Moreover, better understanding of the decomposition dynamics need fitting the resulting carbon dioxide (CO_2) release data to different kinetic models used to describe the mineralization of organic waste. Incubation of different waste mixtures (prepared with sewage sludges, manures, city refuse, cotton waste, olive-mill wastewater, and sweet sorghum bagasse) after they had been composted for various periods with soil revealed decreases in CO_2 evolution with longer composting duration [17]. Moreover, references [5,7,8] evaluated the significance of adding compost of diverse origin in improving the soil C sequestration. All reported compost application increased the amount of stable compound retained in the soil and minimize the overall C loss. However, the majority of this information has been generated in temperate soils and little information is available on C mineralization of typical tropical soils amended with regional composts of different stability. The molecular-chemical composition of those compost-soil-mixtures has never been studied.

Pyrolysis-field ionization mass spectrometry (Py-FIMS) was used to characterize organic matter in sewage farm soils [18], in different stages of biowaste composting [19], and to study long-term compost effects on the molecular composition of SOM [3]. Recently Py-FIMS was used to characterize the organic matter composition of fresh agro-industrial wastes (including coffee pulp) and significant correlations between the organic composition and soil biochemical properties after application were reported [16]. However, neither Py-FIMS nor another highly sensitive speciation method has been applied so far to disclose effects of differently stabilized composts on C transformations in tropical soils.

Therefore, the objectives of the study were (1) to investigate how the CO_2-respiration from a tropical Nitisol was altered following the application of differently stabilized composts, and (2) to evaluate effects of the diffe-

rently stabilized composts on the molecular composition of SOM and its short-term modification during an incubation experiment.

The overall objective was to find out which compost is favorable to improve the soil fertility by sequestration of C in the soil based on a low CO_2-release to the atmosphere.

2. Materials and Methods

2.1 Soil and Composting Samples

The soil was sampled from a coffee plantation farm located in the southwestern highlands of Ethiopia and beloged to the major Soil Unit "Nitosol" (FAO, 1998). The sampling area is situated 36°36'E longitude and 7°56'N latitude (**Figure 1**). The elevation varies between 1533 - 1548 m a.s.l. Mean maximum and minimum temperature range from 26°C to 30°C and 11°C to 14°C, respectively, and the mean of precipitation ranges from 1131 to 1150 mm. Thus, the climate may be described as warm tropical. A total of 50 subsamples were taken from a total area of 5.91 h, traversed in a zig-zag pattern, from soil depth of 0 - 30 cm using a GPS and an Auger. The subsamples were well mixed in a plastic container and then a representative sample of 3 kg was placed in a labeled plastic bag. This soil sample was air-dried and milled to pass through a 2-mm-sieve. Its main characteristics were 84% clay, 14% silt and 1% sand, pH ($CaCl_2$) 5.2, electrical conductivity 2.39 mS $(cm)^{-1}$, 3.9% C_{org}, 0.3% N_t, C/N ratio of 12.0, and 0.1% S.

Composts were produced from 8 m^3 fruit and vegetable waste (dominated by fruit), 1600 kg wet coffee pulp waste and 300 kg garden trimmings (dry leaves, and young tree branches) in a method of small heap composting. Heaps were piled in a bamboo box of dimensions 1.5 m (l) × 1.0 m (w) × 0.8 m (h). The platform used has been considered as pilot compost production unit by the Addis Ababa city council environmental protection authority, Ethiopia.

The regional climate is tropical, with average temperatures between 15°C and 25°C. Three piles were constructed for experimental purposes with three replicates each and monitored as treatment COM1 (composed of coffee pulp waste), COM2 (fruit and vegetable waste), and COM4 (coffee pulp waste with fruit and vegetable waste in which the ratio was 50:50 by volume). The same quantity of soil was added as additional source of microbial colonization and garden trimmings to improve the structure. The experiment lasted from November 2010 to February 2011. The piles were manually turned each week during the active phase which lasts approximately 13 to 17 days, and then in a 15-day-interval during the maturation phase. Depending on the situation the piles
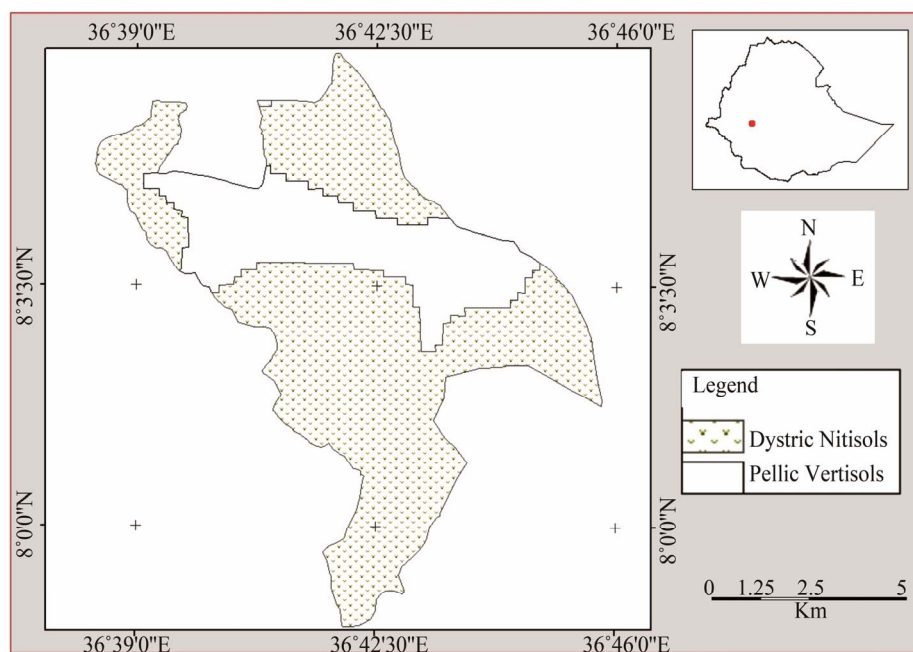
Figure 1. Map of Ethiopia showing area of study and soil type.

were watered so as to maintain the moisture above 40%. Temperatures were measured daily for the first two week and in a 3-day-interval during the next period always in the early morning using digital thermometers (0.5 and 1.0 m in length) at 2 different points of the heaps (25 and 60 cm depth). The composting was considered to be finished when the temperature of the mixture remained stable and near ambient (about 21˚C).

Representative compost samples (about 1 kg) were taken by mixing nine subsamples from different levels of the section in the pile along the whole profile at different phases of the composting, day 8 (thermophilic phase = T), day 24/36 (mesophilic phase = M, depending on the duration each treatment lasted in this phase), and 89/119 (final compost = F, depending on the duration each treatment lasted in this phase). In this way composts of different degree of stability were obtained from three different composting piles. The samples were air dried and ground to pass through a 0.5-mm-sieve. The main properties of the composts are shown in **Table 1**.

2.2 Phytotoxicity Test of Compost Samples

Raw coffee pulp waste (RCPW) and composts sampled at the end of the thermophilic phase (COM1-T, COM2-T, COM4-T), mesophilic phase (COM1-M, COM2-T, COM4-M) and final compost (COM1-F, COM2-F, COM4-F) were investigated for possible phytotoxic effects. A germination test was carried out using garden cress (*Lepidium sativum*) and radish (*Raphanus sativus*) seeds as suggested by [20]. In brief, Whatman filter papers were placed in a 145/20 mm disposable petri dish. The

filter paper was wetted with 5 ml of filtered compost-water-extract. The extract was prepared by putting 10 g of compost sample (dry weight) in a 100 ml flask filled with 100 ml of distilled water and shaking at 125 rpm for 16 hours at room temperature using an electric shaker. Then, 50 seeds of radish and cress were placed on the wetted paper. Distilled water was used as a control. The petri dish was covered and then kept in the oven at 25˚C. At day 3 and 6 germinated seeds were counted and roots length was measured for cress and radish, respectively. Germination percentages (G% = (No of seed germinated in each extract/No of seed germinated in control) × 100) and root growth percentage (RG% = (mean root length in each extract/mean root length in control) × 100) was determined. The germination index (GI%), which is sensitive for phytotoxicity, was then calculated as (GI% = (G% × RG%)/100) [20]. Each treatment was replicated four times.

2.3. Aerobic Incubation and Respiration Measurement

10 g dry weight of sieved soil samples (<2 mm) were thoroughly mixed with organic amendment at desired application rates (48 t·ha^{-1}) and placed in 100 ml incubation vessels in five replicates each. They were monitored as treatments S+RCPW, S+COM1-T, S+COM1-M, S+COM1-F, S+COM2-T, S+COM2-M, S+COM2-F, S+COM4-T, S+COM4-M and S+COM4-F. Soil controls were run without any amendment. Distilled water (1 to 5 ml) was added to the soil-compost-mixtures to keep the moisture at 60% of water-holding capacity. The in-

cubation vessels were placed in a glass container containing 25 ml of 0.05 M NaOH and made air tight with a film plaster. To maintain sufficient O_2 in the vessels they were opened several times in the first week and for two hours per day during the following weeks. Empty vessels were used as blanks. The CO_2 evolved was measured by titration of the NaOH solution with 0.05 M HCl after the carbonate was precipitated by adding excess 0.05 M $BaCl_2$ in 24-hour-intervals. The incubation was carried out in a temperature-controlled incubator at 25°C for 98 days. Subsamples (2 g) were taken destructively from each treatment at the start (day 1) and end (day 98) of incubation for chemical and mass spectrometric analyses. Percent increase and decrease in soil organic C as a result of added composts and subsequent mineralization were computed using a mathematical formula:% increase C_{org} $= (C_t/C_s) \times 100) - 100)$, % decrease $C_{org} = (100 - (C_a/C_b) \times 100)$ where C_t is total organic C of the compost-soil mixture, C_s is organic C of the control soil, C_b is organic C at the end of incubation, and C_a is organic C at the start of incubation.

2.4. Chemical Analyses: Basic Data and Molecular-Chemical Characterization

The soil samples were analyzed for pH, EC in 0.01 M $CaCl_2$ suspensions (1:2.5 w/v) and composting samples were analyzed for pH, EC in H_2O suspensions (1:10 w/v). Whereas the concentrations of total organic carbon (C_{org}), total nitrogen (N_t) and total sulfur (S_t) were determined for both soil and compost samples using a CNS analyzer (Vario EL III; Elementar Analysensysteme, Hanau, Germany).

For Py-FIMS analyses composting pile COM4 was selected because this treatment reached stability in a shorter time period than the other treatments and high analyses costs restricted the number of samples that could be measured. About 3 mg of the air dried, ground and homogenized soil samples amended with differently stabilized COM4 were thermally degraded in the ion source (emitter: 4.7 kV, counter electrode −5.5 kV) of a double-focusing Finnigan MAT 95 mass spectrometer. All samples were heated in a vacuum of 10^{-4} Pa from 50°C to 700°C in temperature steps of 10°C over a time period of 18 minutes. Between magnetic scans the emitter was flash-heated to avoid residues of pyrolysis products. About 65 magnetic scans were recorded for the mass range m/z 15 to 900.

Ion intensities were referred to 1 mg of the sample. For each of the single scans, the absolute and relative ion intensities of ten classes of compounds in the OM were calculated by summation of the ion intensities of indicator signals to obtain thermograms of their volatilization and averaged Py-FI mass spectra. This procedure was

done for each three replicate measurements per soil sample and the results were averaged for statistical analyses.

2.5. Statistical Analyses

The C-losses during the incubation experiment were fitted to mathematical models using the non-linear regression procedure. Means and standard errors were calculated for chemical and phytotoxicity parameters, CO_2-C volatilization data and ion intensities from Py-FIMS. Comparisons between means of ion intensities of compound classes of different treatments were done by applying One Way ANOVA test (LSD mean comparison method were used). All statistics were computed using data analysis and graphic software (Origin 8.1 G).

3. Results

3.1. Germination Index (GI)

In the experiment with garden cress the GI varied between 3% and 112% (**Table 1**). All the compost samples yielded GI greater than 100% whereas the RCPW yielded 3%. Moreover, a germination delay was observed in the treatments with fresh coffee pulp waste. In the test with radish the GI varied between 26% and 132%. Similar to the results with cress all the compost samples yielded GI greater than 100% where as the RCPW yielded 26%. The phytotoxicity of RCPW and the sanitation effect of composting were not reflected by the Germination percentages (GP) as all samples had GP above 80% (not shown).

3.2. Carbon Mineralization

In all treatments the cumulative respiration curves showed two distinct phases for CO_2-C evolution. An initial most intensive biological transformation phase was followed by a slower second phase (**Figure 2**). The maximum of CO_2-C release was achieved in the first three weeks of incubation in all treatments. The equation first order plus linear model $C_t = C_1(1 - e^{-k_1 * t}) + K_2 t$ gave the best fit for the cumulative CO_2-C respired. The different parameters in the equation have a biological meaning, *i.e.*, C_t is the amount of organic C mineralized, C_1 is the amount of the labile C (mg·g^{-1}), K_1 (day^{-1}) and K_2 (day^{-1}) are the rate constants for the mineralization of labile and recalcitrant C, respectively, and t is the incubation time in days. The CO_2-C release followed the order S+RCPW > S+COM-M > S+COM-T > S+COM-F > CONTROL in all treatments. However, the cumulative CO_2-C release curves of treatments S+COM4-T and S+COM4-F were not significantly different (**Figure 2**).

In the short time of three weeks between 35% and 69% of the total C had been evolved. The cumulative

Table 1. Germination index (GI) and chemical properties of row coffee pulp waste and composts of different degree of stability taken at different composting time (t). Different letters indicate that samples are significantly different (p < 0.05) according to Fisher LSD with in a column and values followed by the same letters with in a column were not significantly different at 5% probability level.

Compost samples	Composting time (days)	pH_{H_2O}	$C_{org.}$ $(g \cdot kg^{-1})$	N_{tot} $(g \cdot kg^{-1})$	S $(g \cdot kg^{-1})$	C/N	EC $(ms \cdot cm^{-1})$	GI (%)	
								Cress	Radish
COM1-T	8	8.81(0.03)ab	151.0(2.0)a	10.5(0.0)a	1.70(0.0)ad	14.37	1.99(1.3)a	106(8.9)a	121(8.5)a
COM1-M	36	9.29(0.03)a	149.0(4.0)a	12.7(0.5)b	2.22(0.0)b	11.72	2.13(0.0)a	109(4.6)a	120(8.4)a
COM1-F	119	9.07(0.02)ab	139.2(3.0)b	12.7(0.3)b	2.40(0.0)b	10.98	2.07(0.5)a	102(16.9a	125(4.8)a
COM2-T	8	8.70(0.2)ab	77.0(2.5)c	6.8(0.1)c	1.35(0.0)c	11.40	1.67(0.0)b	112(8.0)a	118(5.6)a
COM2-M	24	9.15(0.08)ab	73.2(2.0)c	7.0(0.1)c	1.80(0.0)d	10.48	1.09(0.0)c	113(9.0)a	120(7.5)a
COM2-F	89	8.96(0.27)ab	73.1(1.0)c	7.3(0.1)c	1.80(0.0)d	9.98	1.33(0.0)f	115(3.5)a	132(3.9)a
COM4-T	8	8.05(0.01)c	107.9(3.0)d	8.74(0.0)d	1.40(0.0)c	12.35	1.36(0.0)f	107(9.4)a	111(12.1)a
COM4-M	24	8.65(0.02)b	93.3(1.0)e	9.32(0.1)d	1.70(0.0)d	10.01	1.07(1.0)c	107(4.7)a	108(8.4)a
COM4-F	89	8.55(0.05)bc	91.49(3.0)e	8.99(0.0)d	1.88(0.0)d	10.18	1.11(0.0)c	108(8.8)a	115(9.3)a
RCPW	0	5.81(0.01)d	397.0(1.6)f	21.1(0.3)e	3.10(0.2)e	18.86	6.73(0.0)d	3(1.1)b	26(3.0)b

COM: compost; T: thermophilic phase; M: mesophilic phase; F: final stage; RCPW: row (fresh) coffee pulp waste; C_{org}: total organic carbon; N_{tot}: total nitrogen; C/N: carbon to nitrogen ratio; EC: electrical conductivity and standard errors in brackets.
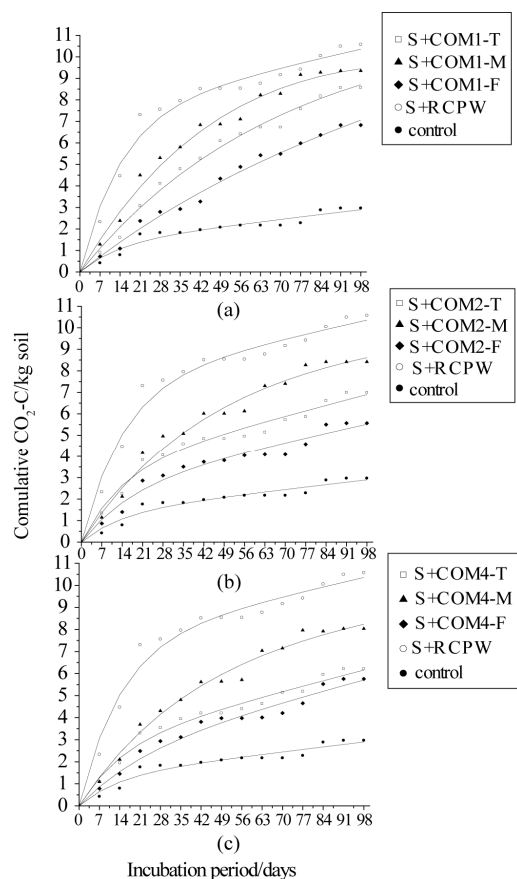

Figure 2. Comulative CO_2-C release from the control, soil amended with raw coffee pulp waste (S+RCPW), and composts of different degree of stability made up of mainly composting of: (a) coffee pulp waste (S+COM1-T, S+COM1-M, S+COM1-F); (b) fruit and vegetable waste (S+COM2-T, S+COM2-M, S+COM2-F); and (c) equal proportion of coffee pulp waste with fruit and vegetable waste (S+COM4-T, S+COM4-M, S+COM4-F).

CO_2-C respired from the amended soils were varied between 5.6 $g \cdot C \cdot (kg \cdot soil)^{-1}$ and 10.6 $g \cdot C \cdot (kg \cdot soil)^{-1}$ at the end of the incubation (**Table 2**). The rate constants K_1 were higher in treatment S+RCPW and lower in the control whereas the higher rate constants K_2 were observed in treatment S+COM-F. In all treatments the K_1 values exceeded the K_2 values (**Table 2**). During the whole incubation period the CO_2-C release from S+RCPW (10.6 $g \cdot C \cdot (kg \cdot soil)^{-1}$) was significantly higher than in soils amended with any of the compost materials.

The application of differently stabilized composts significantly affected the CO_2-C release from soil (**Table 2**). Addition of COM4-F to soil resulted in the lowest CO_2-C release (5.76 g C $(kg \cdot soil^{-1})$) followed by COM4-T (6.22 $g \cdot C \cdot (kg \cdot soil)^{-1}$) and COM4-M (8.03 $g \cdot C \cdot (kg \cdot soil)^{-1}$). Respiration of soils amended with differently stabilized COM1 and COM2 followed the same order. Considering the loss in percent of C_{org} derived from the C_{org} contents at the start and end of the incubation showed the smallest overall C loss (ranges from 1.42% to 2.43%) from the soil amended with COM-T during the incubation (**Table 2**).

3.3. Pyrolysis-Field Ionization Mass Spectrometry (Py-FIMS)

In the Py-FIMS analyses of the control and soils amended with differently stabilized COM4 the thermograms of total ion intensity (TII) showed a reduction in intensity and a shift towards higher pyrolysis temperature during incubation. For instance the thermal evolution of molecules reached maximum intensities in the temperature 460°C and 480°C at start of the incubation and shifted to 490°C and 510°C at the end of incubation (see upper right inserts in **Figure 3**).

Table 2. Carbon mineralized (g·C·kg^{-1} soil), total amount (g·kg^{-1}) of selected elements, C/N ratio, percent increase in soil organic carbon % inc. C$_{org}$, percent decrease of organic carbon (% dec. C$_{org}$), the labile pool rate constant K$_1$ (day^{-1}) and recalcitrant pool rate constant K$_2$ (day^{-1}) of the control and amended soil during the incubation. Values followed by the same letters with in a column were not significantly different at 5% probability level (S+COM: soil-compost mixture; T: thermophilic phase; M: mesophilic phase; F: final stage; S+RCPW: soil-row coffee pulp waste mixture). Numbers in brackets represent standard errors.

Treatments	K$_1$	K$_2$	CO$_2$-C	At day 1					At day 98				
				C$_{org}$	N$_{tot}$	S	C/N	% inc. C$_{org}$	C$_{org}$	N$_{tot}$	S	C/N	% dec. C$_{org}$
S+COM1-T	0.06	0.00	8.57(1.43)a	42.03	3.5	0.7	11.89	5.60	41.2	3.4	0.8	11.90	2.04
S+COM1-M	0.08	0.01	9.35(2.6)a	42.01	3.6	0.7	11.60	5.53	40.3	3.4	0.7	11.94	4.08
S+COM1-F	0.04	0.01	6.83(1.43)b	41.77	3.6	0.8	11.72	4.93	40.7	3.4	0.7	12.00	2.55
S+COM2-T	0.07	0.00	6.99(2.02)b	40.58	3.3	0.7	12.28	1.96	40.0	3.3	0.7	12.00	1.42
S+COM2-M	0.08	0.00	8.41(1.83)a	40.50	3.4	0.7	11.97	1.75	39.3	3.4	0.8	11.54	2.90
S+COM 2-F	0.06	0.01	5.57(1.67)c	40.46	3.4	1.3	11.78	1.66	39.2	3.3	0.7	11.91	3.05
S+COM4-T	0.07	0.00	6.22(1.2)c	41.20	3.4	0.8	12.17	3.51	39.9	3.3	0.7	12.03	3.26
S+COM4-M	0.07	0.00	8.03(2.08)a	40.93	3.4	0.7	11.96	2.82	39.3	3.4	0.8	11.63	4.09
S+COM4-F	0.05	0.00	5.76(0.85)c	40.77	3.5	0.7	11.74	2.44	39.8	3.4	0.7	11.71	2.50
S+RCPW	0.12	0.00	10.57(1.7)d	47.60	3.7	0.8	12.89	19.60	42.6	3.7	0.8	11.61	10.49
Control	0.04	0.00	2.97(1.41)e	39.80	3.3	0.7	12.15	0.00	38.3	3.2	0.7	11.82	3.66

In the Py-FI mass spectra, a particular enrichment of N-compounds during the incubation in compost treated soil was reflected by m/z signals 67, 110 and 117 being more intense in **Figures 3(b)**, **(d)** and **(f)** than in **Figures 3(a)**, **(c)** and **(e)**. This difference, however, was not shown in the control (**Figures 3(g)** and **(h)**). Comparison of mass spectra at the start and end incubation indicated increased proportions of carbohydrates (e.g. more intense m/z signals 84, 96, 110 in **Figures 3(b)**, **(d)**, **(f)** and **(h)** than in **Figures 3(a)**, **(c)**, **(e)** and **(g)**), lignin building blocks (e.g. more intense m/z signals 156, 168, 178, 192, 194, 202, 216 and 218 in **Figures 3(b)**, **(d)**, **(f)** and **(h)** than in **Figures 3(a)**, **(c)**, **(e)** and **(g)**). A decreased proportion of homologous series of free fatty acids and alkenes was indicated by m/z signals at 252, 266, 280, 294, 308 322, 336, and 350 (e.g. less intensive in **Figures 3(a)**, **(c)**, **(e)** and **(g)** than in **Figures 3(b)**, **(d)**, **(f)** and **(h)**) in all incubated treatments, but most pronounced in S+COM4-T.

The TII values were significantly larger in all amended soils than in the control at start of the incubation (**Table 3**). However, significantly higher TII's (p < 0.05) were recorded only in the treatment S+COM4-T than the control, both at the start and end of incubation. This well agreed with the C$_{org}$ concentrations at the start and end of the incubation experiment (**Table 2**).

The relative ion intensities (% TII) of compound classes indicated a significant (p < 0.05) enrichment of N-compounds during the incubation in all amended soils

(by 39.1% in S+COM4-T, by 28.6% in S+COM4-M and by 40.9% in S+COM4-F). However, in the control the proportions of N-compounds did not significantly change between the start and end of incubation (**Table 3**). Among compost amended treatments significantly higher increases in the proportions of carbohydrates (by 65.3%), phenols/lignin monomers (by 12.9%) and peptides (by 43.5%) at the expense of fatty acids and sterols were observed in the treatment S+COM4-T than in any other treatment. The compound classes phenols/lignin monomers and alkylaromatics are the major constituents of the SOM both at the start and end of incubation. The proportions of fatty acids and sterols were the smallest but an active component in the compost amended soils in which their proportion decreased largely during the incubation (**Table 3**). The proportions of alkylaromatics, lignin dimers and lipids did not differ among treatments, neither at the start nor at the end of incubation.

The temperature-resolved volatilization curves for N-compounds and peptides showed a clear difference in thermal stability between S+COM4-T and the control during the incubation (**Figure 4**). At start of the incubation the thermograms for these two compound classes indicated no change in the thermal stability between treatments S+COM4-T and the control. At the end of incubation, however, clear shifts of peaks to higher pyrolysis temperature were observed in S+COM4-T when compared with the control.
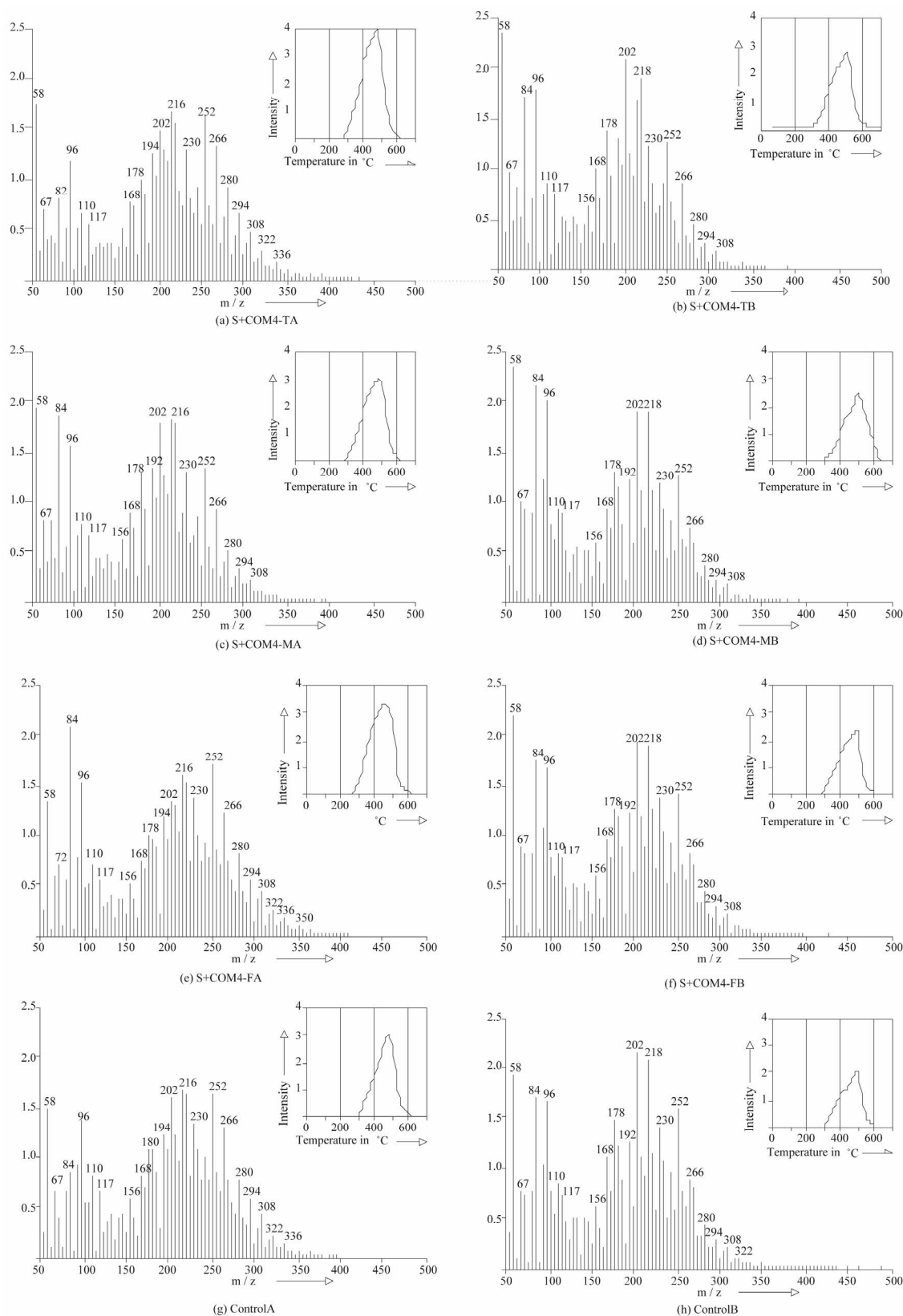
Figure 3. Summed and averaged pyrolysis-field ionization mass spectra and thermograms of total ion intensity (TII) (upper right inserts) of the non-treated control soil and soil amended with different degree of stability composts (COM: compost; T: thermophilic phase; M: mesophilic phase; F: final stage); at the start of incubation (---A) and end of incubation(---B).
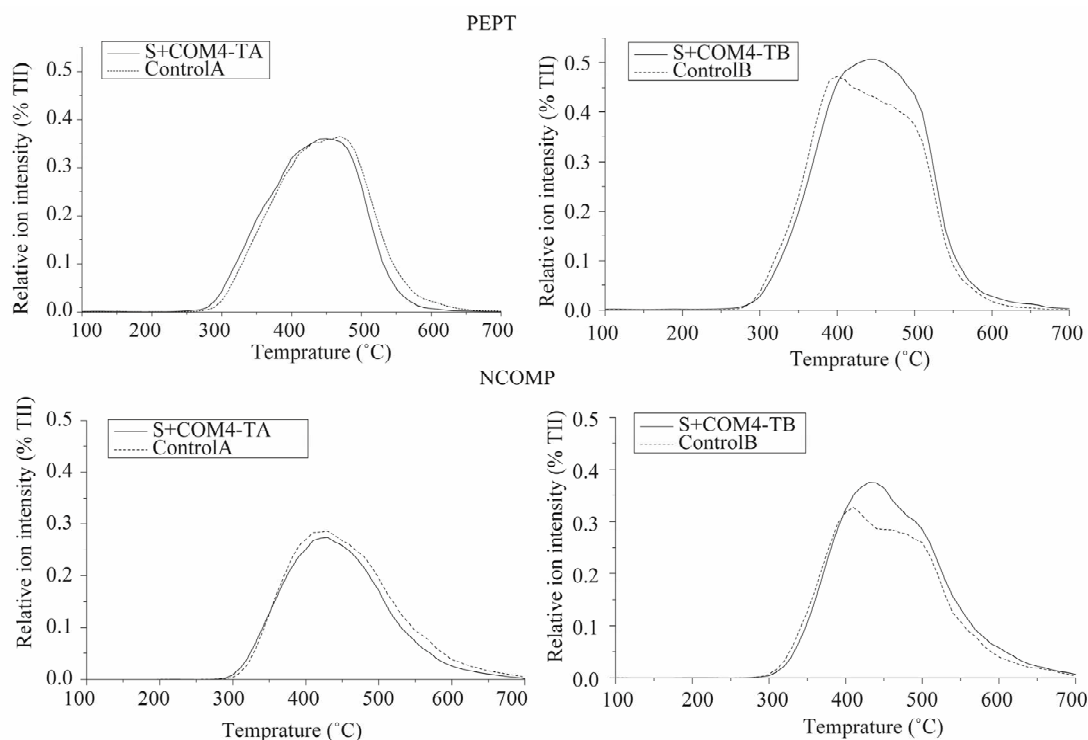
Figure 4. Thermograms for the evolution of peptides (PEPT) and mainly heterocyclic N-compounds (NCOMP) from non-treated control soil (Control) and soil amended with thermophilic compost (S+COMP4-T) at the start of incubation (Control A, S+COMP4-TA) and end of incubation (Control B, S+COMP4-TB).

Table 3. Total ion intensity (TII), volatile matters (VM %) and relative abundance (% TII) of principal classes of compounds from incubated samples at the start of incubation (---A) and end of incubation (----B). Values followed by the same letters with in a column and column without letters were not significantly different at 5% probability level.

Treatment	TII	CARB	PHLM	LDIM	LIPID	ALKY	NCOMP	STER	PEPT	SUBE	FATTY	VM (%)
S+COM4-T A	60.0(1.5)a	4.9(0.1)a	12.4(0.3)e	7.7(0.3)	3.1(0.3)	15.6(0.2)	4.6(0.1)a	0.3(0.1)a	6.2(0.1)a	0.0(0.0)	0.3(0.1)a	21.7(1.3)
S+COM4-T B	41.5(2.2)b	8.1(0.1)b	14.0(0.1)f	8.1(0.1)	2.8(0.1)	15.1(0.1)	6.4(0.1)b	0.1(0.0)b	8.9(0.1)bc	0.0(0.0)	0.0(0.0)b	23.7(0.5)
S+COM4-M A	49.7(4.3)b	7.8(0.1)b	13.5(0.3)f	8.0(0.1)	3.1(0.1)	15.5(0.3)	5.6(0.1)c	0.2(0.0)ab	8.3(0.1)d	0.0(0.0)	0.1(0.0)b	20.0(1.9)
S+COM4-M B	41.2(7.2)b	9.2(0.5)c	13.6(0.5)f	8.3(0.3)	2.8(0.4)	14.9(0.4)	7.2(0.2)d	0.1(0.0)b	9.4(0.4)c	0.0(0.0)	0.0(0.0)b	19.8(1.2)
S+COM4-F A	57.2(2.1)a	7.5(0.1)b	12.2(0.3)e	8.0(0.0)	3.5(0.1)	15.5(0.2)	4.4(0.1)a	0.3(0.0)a	7.4(0.0)e	0.0(0.0)	0.4(0.0)a	24.0(2.1)
S+COM4-F B	35.1(0.7)bc	7.8(0.2)b	13.5(0.2)f	8.3(0.0)	3.2(0.2)	15.1(0.3)	6.2(0.1)b	0.1(0.0)b	8.7(0.1)bd	0.0(0.0)	0.1(0.0)b	23.4(0.5)
Control A	42.9(0.5)b	5.4(0.1)d	13.1(0.1)f	8.3(0.0)	3.8(0.2)	15.6(0.1)	5.1(0.2)c	0.2(0.0)a	6.4(0.2)a	0.0(0.0)	0.1(0.0)b	18.8(0.1)
Control B	30.6(1.0)c	7.7(0.3)b	14.0(0.4)f	8.5(0.1)	3.4(0.1)	15.0(0.3)	5.6(0.1)c	0.2(0.1)ab	8.3(0.2)d	0.0(0.0)	0.1(0.0)b	23.3(0.7)

CARB: carbohydrates; PHLM: phenols/lignin monomers; LIPID: lipids; NCOMP: N-compounds; ALKY: alkylaromatics; LDIM: lignindimers; STER: sterols; PEPT: peptides; SUBE: suberin; FATTY: n-Fatty Acids (n-C_{16} to n-C_{34}) and standard errors in brackets; S: soil; COM: compost; T: thermophilic phase; M: mesophilic phase; F: final stage; RCPW: row (fresh) coffee pulp waste.

4. Discussion

4.1. Phytotoxicity of Starting Material and Composts

The low GI of 3% with cress and 26% with radish measured for the RCPW indicates its high phytotoxcity [20,21]. Moreover, the germination delay observed in this treatments can be attributed to the high salt content (electrical conductivity = 6.73 mS·cm^{-1}) of the sample [18]. A GI < 50% means high phytotoxicity, values between 50 and 80% a moderate phytotoxicity and values > 80% no phytotoxicity [20]. Therefore, the GI > 80% in all compost samples indicated that phytotoxicity of the raw material disappeared during the composting. The

observed values of GIs greater than 100% in all com-posting samples agreed with [21] who found a range of GIs between 25% and 151% in a study with 28 composts and cress seeds. This indicates the promotion of the ger-mination by compost samples [22]. A GI of >80% for radish in all samples including the RCPW indicates a lower sensitivity of this seedling against the RCPW.

4.2. Organic Matter Mineralization

The observed pattern in CO_2-C release agreed with the model used by [9]. They compared four different models to describe the mineralization dynamics from soil amended with alkaline treated municipal biosolids. The first rapid phase corresponded to the fast microbial de-composition of labile compounds [2,17]. The second slower phase corresponded to the slow decomposition of resistant organic compounds. The CO_2-C release from RCPW and compost-amended soils above that from the untreated control soil can be explained by the addition of labile C sources. This agrees with [7,23] who reported increased respiration of soils after amendment with or-ganic waste of diverse origins and stability from 7th to 30th day of incubation. Similarly, the observed difference in the CO_2-C release between the RCPW and compost amended soils resulted mostly from the first three weeks of decomposition. In the later incubation period a faster degradation of organic compounds was observed in soil amended with COMP-F ($K_2 = 0.008$) than in RCPW and soil amended with COM-T ($K_2 = 0.001$) (**Table 2**).

The variation in the CO_2-C release among the soils amended with differently stabilized composts despite of similar initial C_{org} contents and C/N ratios indicated a C immobilization. This immobilization was stronger in the soil amended with COM-T than in the soil amended with COM-M. This confirms reference [8] who observed that the degree of stabilization of added compost materials must not necessarily agree with the efficiency in C con-servation. Moreover, some authors found an inverse rela-tionship between the CO_2-C release and the degree of transformation/stabilization of compost materials added [7,17,23]. One explanation for the partial disagreement of our data for respiration and stabilization with the above three references might be the quantity of added labile components with COM-T. This quantity might have been to low to promote the microbial decomposi-tion significantly in the initial phase of incubation [19]. Another explanation is the stabilization of C by clay-organic matter associations [24]. Moreover, the lowest C_{org} loss (ranges from 1.4% and 2.4%) in the soil amended with COM-T indicated the suitability of com-posts from an early composting phase. This confirms that composts with a short composting period can be optimal for C sequestration in soil [7,9].

4.3. Changes in SOM Composition

The larger TII in the compost-amended treatments than in the control at the start of incubation coincided with the C_{org} concentrations in the treatments. It is explained by the additional organic matter from the composts [25,26]. However, the larger TII in S+COM4-T than in any other treatment at the end of incubation proved selective pres-ervation of compounds that were possibly mineralized in samples with longer composting time. The best explana-tion for such a selective protection is the binding of or-ganic matter at clay surfaces, and a consecutive aggrega-tion of clay-organic matter complexes [24,27,28].

The signal patterns of Py-FI mass spectra indicated the clearest impact of compost application by increasing proportions of N-compounds at the expense of fatty acids and sterols (**Figure 2**, **Table 3**). The stabilization of N-compounds in compost amended soils compared to the controls was also reflected by changes in the thermal volatilization of this compound class after 98 days of incubation (**Figure 3**). This suggests that the organic matter in compost-amended soils was relatively enriched in heterocyclic N-compounds which obviously resisted microbial decomposition. This indicates a residual rela-tive enrichment of rather stabile compounds during the decomposition [28-30]. This explanation agrees with reference [31], who described enrichments of heterocyc-lic N-compounds in SOM also beneath forest trees. In the same line, enrichments of N-compounds were detected at the end of composting by Py-FIMS and FT-IR spectros-copy [19]. However, these N-compounds were com-pletely decomposed in re-circulated leachates in larger scale lysimeters [32,33], likely caused by the lack of sorptive surfaces. This indirectly points to the importance of the sorptive surfaces of the clay minerals in the pre-sent experiment.

The temperature-resolved Py-FIMS showed that ther-mally stable carbohydrates, phenols/lignin monomers, and peptides were more enriched during the incubation of S+COM4-T than in any other compost amended treatment. This indicates that these compound classes were microbially synthesized during incubation or selec-tively preserved. The pronounced enrichment of phe-nols/lignin monomers agreed with [34] who stated that composition of SOM after compost amendment changed mainly by increases in the proportion of lignins. The stronger decrease in the lignin content in a soil amended with final compost than with early compost (treatment S+COM4-T) underlined the advantage of the early com-post. Moreover, significantly increased carbohydrate proportions in the variant S+COM4-T during the incuba-tion proved a selective carbohydrate preservation. This agrees with findings of [35,36] that progressive compost maturity reflected a decrease of the carbohydrate content.

In agreement with references [19,37], we found a progressive association of bio-labile peptides during composting.

4.4. Explanation of the Organic Matter Mineralization by Changes in the Molecular Composition of SOM

Computing the percentage decrease of ion intensities in the temperature range < 400°C (**Figure 3**) as indicator of thermally labile compounds during incubation revealed the largest decrease by 49% in S+COM4-M, followed by 35% in S+COM4-T, 28% in S+COM4-F whereas no decrease was observed in the control. This result is consistent with the order in CO_2-C release during the incubation experiment (**Figure 2**). Moreover, the changes in TII values ($l0^6$ counts·mg^{-1}) of the compound classes (not shown) confirmed the respired CO_2-C mainly resulted from reduced ion intensities of phenols/lignin monomers, lignin dimers, lipids, alkylaromatics, fatty acids and sterols during incubation for 98 days. On the other hand thermally stable compounds (> 400°C according to [38]) relatively increased by 12% in S+COM4-M, 16% in S+COM4-T and S+COM4-F but only by 1% in the control. Moreover, computing the relative increase in thermally stable carbohydrates (volatilized >400°C), N-compounds (volatilized > 380°C) and peptides (volatilized > 350°C - 400°C) confirmed that addition of composts particularly from the end of the thermophilic phase to soil significantly enriched the SOM during incubation by stabilized compounds of microbial and plant origin.

The decrease in TII mainly in the temperature range below 400°C (thermally labile compounds) in the compost amended soils only implied that a large portion of labile components of the compost material itself was transformed or degraded during the incubation process. This supports the idea that decomposition of the added composts in soil goes along with humification in the composts themselves [34]. Here we show that this decomposition process is accompanied by the pronounced development of thermally stable carbohydrates, phenols/lignin monomers, and peptides of plant and microbial origin if the composts from the early phase of composting were applied. This was really a positive contribution of adding compost to SOM composition in the clay dominated Nitisol because this was not observed following NPK fertilization of soil [36] and matured compost application in sandy soil [39].

5. Conclusions

1) The combination of laboratory incubation and Py-FIMS for the first time provided compelling evidence for effects of the compost stability on C mineralization and the molecular composition SOM when composts were applied to a typical clay-rich tropical soil.

2) The combined results revealed that it would be sufficient to end up the composting of coffee pulp and fruit waste immediately after a short thermophilc phase as its application better conserved the C (a) during composting and (b) during transformations in the soil.

3) Application of compost from the early phase of composting may result in a quicker mineralization of biologically labile organic matter from the compost and a better enrichment of SOM with stable compounds of plant and microbial origin such as carbohydrates, phenols/lignin monomers and peptides at the expense of free fatty acids and sterols compared to mature compost. This may have considerable economic implications as composting of coffee pulp in heaps may take 6 to 8 months to reach the mature stage.

4) Therefore, the application of compost from the early composting phase can be recommended not only as a soil amendment but also as a measure to mitigate CO_2-enrichment in the atmosphere. Forthcoming studies will be directed to disclose in more detail the stabilization mechanism of biologically labile, compost-derived organic molecules in tropical and other soils.

6. Acknowledgments

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