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Compost effects on soil organic matter: transformation, composition and stabilization

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Contents

Preface

This commulative dissertation consists of three manuscripts recently published or accepted in peer reviewed international scientific journals and other additional work (not yet published). The dissertation begins with an introduction that presents a general background and justification why studying soil organic matter (SOM) at the molecular level is currently an area of the research interest, fundamentals and impact of recycling organic waste as compost in agricultural soil on the composition, stabilization and transformation of SOM, and finally discusses the analytical methods used for investigating heterogeneous material like compost and SOM. The subsequent chapters were speparately published (chapters 2 to 4) or contain supplementary yet unpublished work (chapter 5) that are linked each other by presenting the effect of different composition and age of composts on the molecular composition of SOM and its implication in minimizing C mineralized from the soil (chapters 2 to 4). Furthermore, the suitability of the differently aged composts in relation to its use for plant growth was described in chapter 5. Chapters 6 and 7 summarize the findings of the study and gives directions for the future scientific research work. In general the research findings included in this thesis is an important additions to the existing knowledge in the area specifically SOM transformation, composition and stabilization in tropical Nitisols up on compost management.

1 Introduction

1.1 Background and rationale of the research

Soil organic matter (SOM) has been studied mainly for its central role in soil fertility and properties. In the past two decades additionally the role of SOM in moderating atmospheric CO_2 concentrations has emerged as a critical research area. For instance, Pacala and Socolow (2004) outlined the different options of stabilizing the atmospheric CO_2 concentration in which three of them were based on C sequestration in terrestrial ecosystems (comprises trees and soils). SOM is a major component of the world's terrestrial C reserves and soil management strategy that affect SOM can contribute to the mitigation potential of soils for elevated atmospheric CO_2 (Gruber *et al*., 2004; Lal, 2008).

The effect of SOM can be examined for (i) reducing C emissions from its mineralization, and (ii) sequestration of additional C into the SOM. These are achieved by minimizing soil disturbance, using integrated nutrient management strategies of applying manure/compost, using soil amendments such as biochar that improve soil humification efficiency and soil aggregation (Lal, 2008, 2011). However, SOM mineralization and stabilization are complex processes and involve a variety of physical, chemical, and biological factors (Janzen *et al*., 1998; Halvorson *et al*., 2002; Zimmermann *et al*., 2007; Lal and Follett, 2009). This influences SOM by shaping its molecular characteristics, the leading control of the SOM stability properties (Schulten *et al*., 1992). Therefore, fundamental knowledge on the effect of different management practices on the SOM composition, transformation and stabilization require molecular level investigations that take into account the different controlling factors.

Organic waste recycling in agriculture through composting has been considered as an environmentally favorable strategy to make use of the increasing amount of organic waste and foster SOM stabilization and C sequestration (Kutzner, 2000; Lal, 2004; Weber *et al*., 2007; Bustamante et al., 2010). Such a strategy based on the natural processes has numerous additional benefits, particularly in strongly weathered tropical soils such as to improve ecosystem services provided by soil, overcome the degradation of SOM and advance food security (Zech *et al*., 1997; Lal, 2002; Rivero *et al*., 2004; Scherr and Sthapit, 2009; Bruun *et al.*, 2010). The effects of compost on SOM composition and stabilization are depending on several parameters, e.g., the compost quality, the soil type, the application rate, trial duration, compost interactions with soil enzymes and mineral soil surfaces, baseline level of native SOM and other environmental conditions (Zech *et al*., 1997; González-Vila *et al.*, 1999; Grandy and Neff, 2008; Stewart *et al*., 2011). Compost quality itself depends on a number of factors, the main ones being the nature of the composted matter and the composting time (Bernal *et al*., 1998). Therefore, critical evaluation on how various initial quality composts taken from different phases of composting affect the molecular composition and stabilization of SOM is needed for efficient and sustainable compost use in agricultural soils.

Transformation and stabilization of SOM

Molecular compounds that constitute SOM are stabilized against biodegradation by selective preservation due to their association with the mineral particles, thereby lowering their potential to be degraded by microorganisms and their extracellular enzymes (Balesdent, 1996; Gregorich *et al*., 1996; von Lützow *et al*., 2006). For instance, accumulation of organic molecules such as lignin in the sand-sized fraction and of microbial products consisting primarily of polysaccharides, N-containing compounds, and hydrocarbons in the silt and clay-sized fractions were reported by Grandy and Neff (2008). According to Gregorich *et al*. (1996), Kögel-Knabner (2002) and Lorenz *et al*. (2007), the stabilization of SOM is an inherent property of the molecular structure (e.g., alkyl C chains in lipids, aromatic structures in aromatics and phenolics of the plant material). Baldock *et al*. (1992) found that alkyls were most dominant in tropical Oxisols and interpreted this as parts of the most stable soil C that resist temperature-enhanced microbial degradation in tropical soils. SOM stabilization can also be attained through the condensation and complexation of biomacromolecules which inhibits enzymatic degradation, and by non-enzymatic polymerization of low-molecular-weight compounds into macromolecules (Gleixner *et al*., 2001; de Leeuw *et al*., 2006). Coating of organic matter (OM) with hydrophobic compounds that contain aliphatic groups may also contribute to SOM stabilization (Piccolo *et al*., 1999; Hedges *et al*., 2000; Doerr *et al*., 2005). Furthermore, the location of organic substances within the soil structure which determine its accessibility to microbes and oxygen also affects SOM stability in soils (Six *et al.*, 2002).

Basics of Composting

Haug (1993) defines composting as “the biological decomposition and stabilization of organic substrates under conditions which allow development of thermophilic phases as a result of biologically produced heat, with a final product sufficiently stable for storage and application to land without adverse environmental effects.” Figure 1 breifly demonstrate processes takes place in the composting heap. Unlike the natural mineralization of organic compounds takes place in the upper layer of the soil, the temperature in composting heaps can increase by self-heating to the ranges of mesophilic (25–40 °C) and thermophilic microorganisms (50–70 °C), which is an important stage and needs to be investigated for a different compost application effects.

The time–temperature course of the composting process can be divided into 4 phases (Kutzner, 2000):

(1) During the first phase a diverse population of mesophilic bacteria and fungi proliferates, degrading primarily the readily available nutrients and, thereby, raising the temperature to about 45 °C. At this point their activities cease, the vegetative cells and hyphae die and eventually lyses and only heat resistant spores survive.

(2) After a short lag period (not always visible) there occurs a second more or less steep rise of temperature. This second phase is characterized by the development of a thermophilic microbial population comprising actinomycetes and fungi. The temperature optimum of these microorganisms is between 50 and 65 °C, their activities terminate at 70–80 °C.

(3) The third phase can be regarded as a stationary period without significant changes of temperature because microbial heat production and heat dissipation balance each other. The microbial population continues to consist of thermophilic bacteria, actinomycetes, and fungi.

(4) The fourth phase is characterized by a gradual temperature decline; it is best described as the maturation phase of the composting process. Mesophilic microorganisms having survived the high temperature phase or invading the cooling down material from the outside succeed the thermophilic ones and extend the degradation process as far as it is intended.

The terms stability and maturity are commonly used to define the degree of decomposition of organic matter during the composting process. Compost stability refers to the level of activity of the microbial biomass and can be determined by O_2 uptake rate, CO_2 production rate or by the heat released as a result of microbial activity (Iannotti *et al*., 1994; Conti *et al*., 1997). Compost maturity refers to the degree of decomposition of phytotoxic organic substances produced during the active composting stage (Wu *et al*., 2000). Seed germination indexes in compost or compost extracts are a common biological method to evaluate the degree of maturity of the material (Zucconi *et al*., 1985).

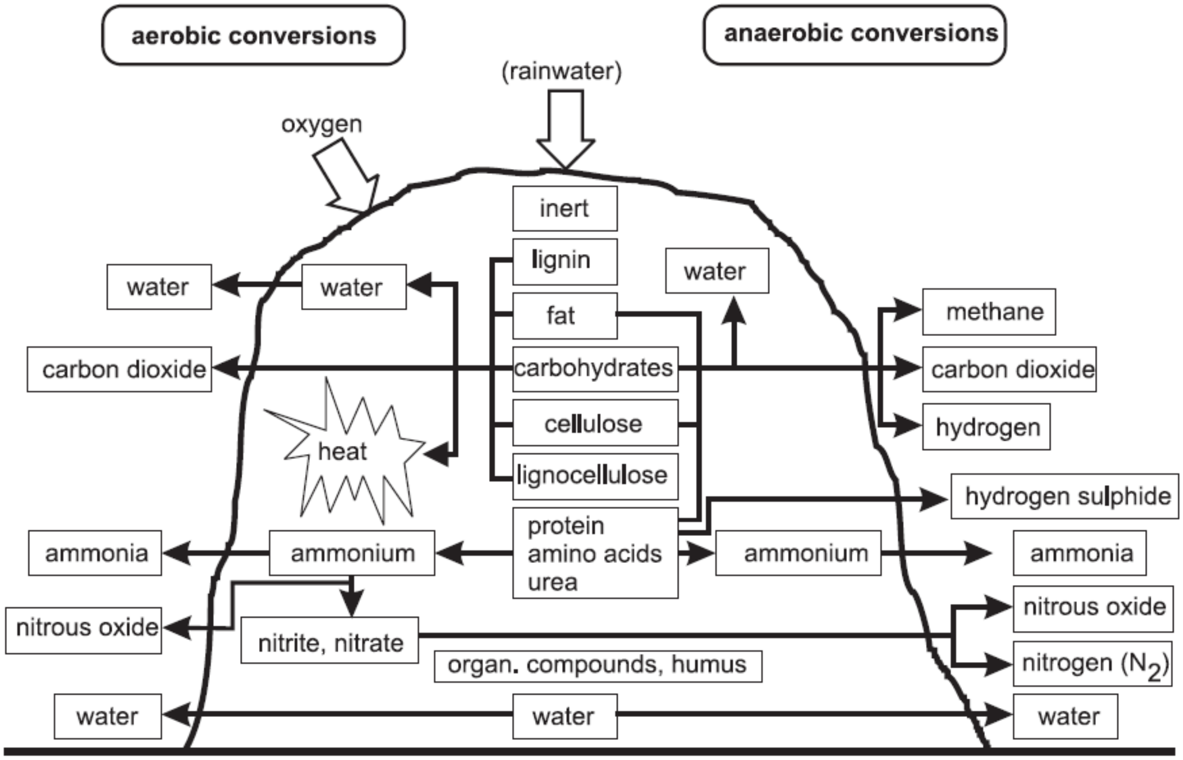

Figure 1 Substrates and products of microbial activity in a compost heap (adopted from Schuchardt, 2005).

The ingredients used for composting are organic by-products or waste materials of plant, animals and human origins usually blend together in suitable proportions for efficient composting processes. At present considerable amounts of municipal solid wastes, sewage sludge and agro-industrial wastes such as coffee processing by-products like coffee pulp (40% of the wet weight) are generated (Clapp *et al*., 2007). For instance, In Ethiopia these are mainly disposed to water bodies, unsanitary landfills and partly applied to agricultural fields without pre-treatment and causes freshwater eutrophication, health problems, adverse effects on soil properties such as induced anaerobic conditions and release of phytotoxic compounds impairing plant growth (Gezahegne *et al*., 2011; Negassa *et al*., 2011). They also compete with the scarce land resource for landfilling and generat offensive odour and greenhouse gases when collected, transported and disposed. All this triggers the need to find a sink for this growing amount of waste and, at the same time, opens up the chance for integrating agriculture and solid waste management by recycling the waste as compost for the improvement of soil fertility, food security and as a mitigation measure of climate change by the sequestration of C in soil. However, efficient composting of these organic wastes has to fulfill the agronomic requirements, economic effectiveness, and minimize overall C losses in the transformation process.

Previous studies reported that application of compost to agricultural soil improves soil physical, chemical and biological properties (Nardi *et al*., 2004; Adani *et al*., 2007; Lima *et al*., 2009). It enhanced the activity, size and composition of soil microbial communities (Garcia-Gil *et al*., 2000; Ros *et al*., 2006; Saison *et al*. 2006, Bustamante *et al*., 2010) and soil structural stability (Goulet *et al*., 2004). Moreover, it has been also evident that the incorporation of compost into soils results in an important supply of plant nutrient elements, such as nitrogen (N), phosphorus (P) and sulphur (S), exchangeable cations (calcium, potassium, magnesium and sodium) and micronutrients (Weber *et al*., 2007). Particularly in highly weathered soils composts can also decrease the rate of micronutrient leaching and level of P fixation in a soil (Obi and Ebo, 1995).

Likewise, composts can increase the pH in acidic soils and soil cation exchange capacity (CEC) which enhances the availability of nutrients. However, local limitations resulting from compost application to soil from, e.g., unnecessary nutrient enrichment and leaching, N immobilization or emission as potent GHG's such as N_2O need proper evaluation of change in plant available nutrient instead of total concentration during compost making and after application to different soils.

Furthermore, compost application effects on the SOM indicated a reduced mineralization of labile SOM compounds (Fortuna *et al*., 2003; Piccolo *et al*., 2004), and an increased humic acid (HA) over fulvic acid (FA) proportions (Rivero *et al*., 2004). The latter results were confirmed by Weber *et al*. (2007) who also reported a relative increase in HA relative to FA with time in soils amended with urban compost. Furthermore, the added compost can lead to significant changes in the molecular composition of SOM, thereby enhancing the role of SOM as a sink of organic C (Mondini *et al*., 2007; Fabrizio, 2009; Spaccini and Piccolo, 2009). Studies done by Leifeld et al. (2002) described improved SOM quantity and quality after compost application to soils. This will have a global implication as it minimizes greenhouse gas emissions through C sequestration in soils. The above results were based on chemolytic techniques using alkaline and acid solutions, or on the application of spectroscopic methods such as FTIR, ^{13}C-NMR and DRIFT. However, these methods do not yield sufficient chemical information for direct identification of individual/molecular subunits and they are consequently better suited to provide a chemical overview (Schulten and Hempfling, 1992).

On the bases of CO_2 respiration results during incubation of compost amended soils Mondini *et al*. (2007) and Sánchez-Monedero *et al*. (2010) reported an inverse relationship between the organic C mineralized and the degree of stability of the compost added. They concluded that addition of relatively stable compost always reduce the organic C respiration and, thus, stabilize the SOM. However, Sánchez-Monedero *et al*. (2008) observed that the efficiency of added olive mill composts for soil C conservation was independent on the degree of compost stability; rather prolonged composting might led to nitrogen and carbohydrate losses rather than contributing to the progressive accumulation of aromatized humic materials (González-Vila *et al.,* 1999). In summary these results are based on general chemical properties only and lack to identify the controls on suitability and stability of composts in soils. Furthermore, the fate of composts of different origin and stability in tropical soils is still completely unknown, because the citied studies all comes from temprate regions.

Relevant evidence on the impact of different age and composition of composts on the SOM contents, composition and stability requires the application of molecular-level sensitive analytical methods such as pyrolysis-mass spectrometry. The most important analytical pyrolysis methods widely applied to environmental samples are Curie point (flash) pyrolysis combined with electron impact (EI) ionization-gas chromatography mass spectrometry (Cp Py-GC/MS) and pyrolysis–field ionization mass spectrometry (Py-FIMS) (Leinweber *et al*., 2009). The Cp Py-GC/MS was used to characterize organic matter in compost (Dignac *et al*., 2004; González-Vila *et al*., 1999), organically fertilized agricultural soils (Nierop *et al*., 2001; Marinari *et al*., 2007), and plant materials (Stewart *et al*. 2011). Bochove *et al*. (1995) studied transformations of dairy manure and chopped straw by taking distinict samples at four different phases of composting (mesophilic, thermophilic, cooling and maturation) for investigation by Py-FIMS. The mass specra indicated the presence of carbohydrates, phenols/ lignin monomes, lignin dimers, n-alkyl (38-51) monoesters and n-alkyl (15-34) fatty acids, and N-containg compounds. The most important trends observed were: (i) increased proportions of carbohydrates and fatty acids during the thermophilic phase, (ii) further increased proportions of carbohydrates as well as degradation of lignin dimers to lignin monomes and of plant waxes to n-fatty acids during the cooling phase, and (iii) decreased contents of all identifiable moleucles in the maturation phase. Steroles showed intense signals in all samples. The observed changes of organic matter during composting wastes of plant and animal origin reflect the simplified processes of SOM formation and turnover under native or agricultural conditions. Moreover, Py-FIMS enabled to demonstrate management induced changes in SOM composition in whole soil samples (Schulten and Hempfling, 1992), to characterize SOM quality in sewage farm soils (Leinweber *et al*., 1996), to trace different phases of biowaste composting (Smidt *et al*., 2005) and to characterize the organic matter in fresh agro-industrial waste including coffee pulp (Negassa *et al*., 2011). Py-GC/MS and Py-FIMS have been also successfully applied in conjunction to evaluate forest soils (Hempfling and Schulten, 1990), amino acids in humic substances (Sorge *et al*. 1993), and changes of organic matter composition in re-circulated leachates in waste piles (Franke, 2006 & 2007). However, to the best of my knowledge neither of these complementary instruments nor another highly sensitive speciation method has been applied so far to evaluate effects of different composts on the molecular composition and stability of SOM in tropical soils.

1.2 Objectives of the research

Therefore, the specific objectives of the present study were: (1) to investigate how the CO_2-respiration from the topsoil of a tropical Nitisol was altered after the application of differently stabilized composts; (2) to evaluate the effect of the differently aged composts on the molecular composition of SOM and its short term modification in an incubation experiment; (3) to describe how compost amendment sustainably modifies and improves the SOM composition and stability in a long term pot experiment using novel pyrolysis based analytical methods; and (4) to determine and compare the available concentrations of macronutrients of distinct compost samples taken during composting of coffee pulp waste and after its application to a tropical topsoil from a Nitisol. The overall objective was to find out how applications of different composts affect the SOM stability and sequestration of C in the soil to lay a scientific base for its effective use as a soil amendement and approach for C sequestration in soil. Thus in summary the work aims at contributing to soil fertility and food security and mitigating climate change

References

Adani, F., Genevini, P., Ricca, G., Tambone, F. and Montoneri, E. 2007. Modification of soil humic matter after 4 years of compost application. *Waste Manage*., 27: 319–324.

Baldock, J. A., Oades, J. M., Waters, A. G., Peng, X., Vassallo, A. M., Wilson, M. A. 1992. Aspects of the chemical-structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. *Biogeochemistry,* 16: 1-42.

Balesdent, J. 1996. The significance of organic separates to carbon dynamics and its modeling in some cultivated soils. *Eur J Soil Sci.,* 47: 485–93.

Bernal, M. P., Sa´nchez-Monedero, M. A., Paredes, C., Roig, A. 1998. Carbon mineralization from organic wastes at different composting stages during incubation with soil. *Agric. Ecosyst. Environ.,* 69: 175–189.

Bochove van, E., Couillard, D., Schnitzer, M. and Schulten, H. -R. 1996. Pyrolysis-field ionization mass spectrometry of the four phases of cow manure composting. *Soil Sci. Soc. Am. J.,* 60: 1781–1786

Bruun, T. B., Elberling, B., Christensen, B. T. 2010. Lability of soil organic carbon in tropical soils with different clay minerals. *Soil Biol. Biochem*., 42: 888-895

Bustamante, M. A., Said-Pullicino, D., Paredes, C., Cecilia, J. A. and Moral, R. 2010. Influences of winery–distillery waste compost stability and soil type on soil carbon dynamics in amended soils. *Waste Manage*., 30(10): 1966-75.

Clapp, C. E., Hayes, M. H. B. and Ciavatta, C. 2007. Organic wastes in soils: Biogeochemical and environmental aspects. *Soil Biol. Biochem.*, 39: 1239–1243.

Conti, M., Arrigo, N. and Maralli, L. 1997. Relationships of soil carbon light fraction, microbial activity, humic acid production and nitrogen fertilization in the decaying process of corn stubble. *Biol. Fertil. Soils*, 25: 75-78.

de Leeuw, J. W., Versteegh, G. J. M. and van Bergen, P. F. 2006. Biomacromolecules of algae and plants and their fossil analogues. *Plant Ecol.,* 182: 20–233.

Dignac, M. F., Houot, S. and Derenne, S. 2004. How the polarity of the separation column may influence the characterization of compost organic matter by pyrolysis–GC/MS. *J. Anal. Appl. Pyrol*., 75: 128–139.

Doerr, S., et al. 2005. Extraction of compounds associated with water repellency in sandy soils of different origin. *Aust. J. Soil Res.,* 43: 225–237.

Fabrizio, A. 2009. Effect of compost application rate on carbon degradation and retention in soils. *Waste Manage*., 29: 174–179

Fortuna, A., Harwood, R. R., Kizilkaya, K. and Paul, E. A. 2003. Optimizing nutrient availability and potential carbon sequestration in an agro-ecosystem. *Soil Biol. Biochem*., 35: 1005–1013.

Franke, M., Jandl, G. and Leinweber, P. 2007. Analytical pyrolysis of re-circulated leachates: towards an improved municipal waste treatment. *J. Anal. Appl. Pyrol*., 79: 16–23.

Franke, M., Jandl, G. and Leinweber, P. 2006. Organic compounds in re-circulated leachates of aerobic biological treated municipal solid waste. *Biodegradation*, 17: 473–485.

Garcia-Gil, J. C.; Plaza, C., Soler-Rovira, P. and Polo, A. 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biol. Biochem*., 32: 1907-1913.

Gezahegne, B. Fikre, L. and Mulatu, W. 2011. Exploring the suitability of coffee pulp compost as growth media substitute in greenhouse production. *Int. J. Agr. Res.,* 6(3):255-267

Gleixner, G., Czimczik, C. J., Kramer, C., Luhker, B. and Schmidt, M. W. I. 2001. Plant compounds and their turnover and stabilization as soil organic matter. Global Biogeochemical Cycles in the Climate System. *Academic Press*, pp. 201–15.

González-Vila, F. J., Almendros, G. and Madrid, F. 1999. Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil. *Sci. Total Environ*., 236: 215–229.

Goulet, E., Dousset, S., Chaussod, R., Bartoli, F., Doledec, A. F. and Andreux, F. 2004. Water stable aggregates and organic matter pools in a calcareous vineyard soil under four soil-surface management systems. *Soil Use. Manage*., 20(3): 318–324.

Grandy, A. S. and Neff, J. C. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. *Sci. Total Environ*., 404: 297-307.

Gregorich, E. G., Monreal, C. M., Schnitzer, M. and Schulten, H.-R. 1996. Transformation of plant residues into soil organic matter: chemical characterization of plant tissue, isolated soil fractions, and whole soils. *Soil Sci*., 161: 680–693.

Gruber, N., et al. 2004.The vulnerability of the carbon cycle in the 21st century: An assessment of carbon-climate-human interactions, In: The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World, Eds: C. B. Field and M. R. Raupach, Island Press, Washington, D.C., pp. 45-76,.

Halvorson, A. D., Wienhold, B. J. and Black, A. L. 2002. Tillage, nitrogen and cropping system effects on soil carbon sequestration. *Soil Sci. Soc. Am. J.,* 66: 906–912.

Haug, R. T. 1993. The Practical Handbook of Composting. Boca Raton, FL: Lewis.

Hedges, J. I., et al. 2000. The molecularly-uncharacterized component of nonliving organic matter in natural environments. *Org. Geochem*., 31: 945–958.

Hempfling, R. and Schulten, H.-R. 1990. Chemical characterization of the organic matter in forest soils by Curie-point pyrolysis-gas chromatography/ mass spectrometry and pyrolysis-field ionization mass spectrometry. *Org. Geochem*., 15: 131-145.

Iannotti, D. A., Grebus, M. E., Toth, B. L., Madden, L. V. and Hoitink, H. A. J. 1994. Oxygen respirometry to assess stability and maturity of composted municipal solid waste. *J. Environ. Qual.,* 23:1177-1183

Janzen, H. H., et al. 1998. Management effects on soil C storage on the Canadian prairies. *Soil Till. Res.*, 47: 181–195.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem*., 34: 139–162.

Kutzner, H. J. 2000**.** Microbiology of composting. In: *Biotechnology* (Rehm HJ, Reed G. eds.) *Vol. 11c: Environmental Processes III*, Weinheim: Wiley-VCH pp. 35–100.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science*, 304:1623–1627.

Lal, R. 2011. Sequestering carbon in soils of agro-ecosystems. *Food Policy xxx : xxx–xxx*

Lal, R. 2008. Carbon sequestrian. *Phil. Trans. R. Soc. B.*, 363: 815-830.

Lal, R., 2002. The potential of soils of the tropics to sequester carbon and mitigate the greenhouse effect. *Adv. Agron.,* 74: 155– 192.

Lal, R. and Follett, R. F. 2009. Soil Carbon Sequestration and the Greenhouse Effect, 2nd ed. SSSA Special Publication 57. Madison, WI: Soil Science Society of America.

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. *Eur. J. Soil Sci*., 53: 299–309.

Leinweber, P., Blumenstein, O. and Schulten, H.-R. 1996. Organic matter composition in sewage farm soils: Investigations by ^{l3}C-NMR and pyrolysis-field ionization mass spectrometry. *Eur. J. Soil Sci*., 47: 71-80.

Leinweber, P., Schulten, H-R., Jandl, G., Eckhardt, K-U., Schlichting, A., and Hofmann, D. 2009. Analytical pyrolysis and soft-ionization mass spectrometry. In: Biophysico-chemical processes involving natural nonliving organic matter in environmental systems, pp. 539-588. Editors: N. Senesi, B. Xing and P.M. Huang. Wiley-IUPAC series, ISBN 978-0-470-41300-5.

Lima, G., et al. 2009. Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, 150: 38-45.

Lorenz, K., Lal, R., Preston, C. M. and Nierop, K.G.J., 2007. Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio (macro) molecules. *Geoderma*, 142: 1–10.

Marinari, S., Liburdi, K., Masciandaro, G., Ceccanti, B. and Grego, S. 2007. Humification-mineralization pyrolytic indices and carbon fractions of soil under organic and conventional management in central Italy. *Soil Till. Res*., 92: 10-17.

Mondini, C., Cayuela, M. L., Sinicco, T.; Cordaro, F., Roig, A. and Sánchez-Monedero, M. A. 2007. Greenhouse gas emissions and carbon sink capacity of amended soils evaluated under laboratory conditions. *Soil Biol. Biochem*, *39:* 1366-1374.

Nardi, S., Morari, F., Berti, A., Tosoni, M. and Giardini, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilizers. *Eur. J. Agron.,* 21: 357–367.

Negassa,W., Baum,C. and Leinweber, P. 2011. Soil amendement with agro-industrial products: Moluclar-chemical compostions and effects on soil biochemical activites and phosphrous fractions. *J. Plant Nutr. Soil Sci*., 174: 113-120.

Nierop, K. G. J., Pulleman, M. M. and Marinissen, J. C. Y. 2001. Management- induced OM differentiation in grassland arable soil: a study using pyrolysis techniques. *Soil Biol. Biochem.*, 33: 755-764.

Obi, M. E. and Ebo, P. O. 1995. The Effects of Organic and Inorganic Amendments on Soil Physical Properties and Maize Production in a Severely Degraded Sandy Soil on Southern Nigeria. *Bioresour. Technol.,* 51: 117-123.

Pacala, S. and Socolow, R. 2004. Stabilization wedges: solving the climate problem for the next 50 years with current technologies. *Science*, 305: 968–972.

Piccolo, A., Spaccini, R., Haberhauer, G. and Gerzabek, M. H. 1999. Increased sequestration of organic carbon in soil by hydrophobic protection. *Naturwissenschaften,* 86: 496–499.

Piccolo, A., Spaccini, R., Nieder, R. and Richter J. 2004. Sequestration of a biologically labile organic Carbon in soils by humified organic matter. *Clim. Change,* 67: 329-343.

Rivero, C., Chirenje, T., Ma, L. Q. and Martinez, G., 2004. Influence of compost on soil organic matter quality under tropical conditions. *Geoderma*, 123: 355–361.

Ros, M., Klammer, S., Knapp, B., Aichberger, K. and Insam, H. 2006. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. *Soil Use Manage*., 22: 209–218.

Saison, C., et al. 2006. Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. *Environ. Microbiol*., 8: 247–257.

Sánchez-Monedero, M. A., Cayuela, M. L., Mondini, C., Serramia, N. and Roig, A. 2008. Potential of olive mill wastes for soil carbon sequestration.*Waste manage.,* 28: 767-773.

Sánchez-Monedero, M. A., Serramia, N., Civantos, C.G.O., Fernandez-Hernandez, A. and Roig, A. 2010. Greenhouse gas emissions during composting of two-phase olive mill wastes with different agroindustrial by-products. *Chemosphere*, *81:* 18-25.

Scherr, S. J. and Sthapit, S. 2009. Farming and land use to cool the planet. In: Starke, L. (Ed.), State of the World 2009: Into a Warming World. Worldwatch Institute, WashinPgon, DC, pp. 30–49.

Schuchardt, F. 2005. Composting of organic waste. in: Jördening H.-J and Winter J. (eds), Environmental Biotechnology. Concepts and Applications. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. pp. 333-354.

Schulten, H.-R. and Hempfling, R. 1992. Influence of agricultural soil management on humus composition, quality and dynamics. *Plant Soil*, 142: 259-271.

Schulten, H.-R., Leinweber, P. and Reuter, G. 1992. Initial formation of soil organic matter from grass residues in a long-term experiment. *Biol Fertil Soils*, 14: 237-245.

Six, J., Conant, R. T., Paul, E. A. and Paustian, E. A. P. 2002. Stabilization mechanism of soil organic matter: implication for C-saturation of soil. *Plant Soil*, 241: 155–176.

Smidt, E., Eckhardt, K.-U., Lechner, P., Schulten, H.-R. and Leinweber, P. 2005. Characterization of different decomposition stages of biowaste using FT-IR spectroscopy and pyrolysis-fieldionization mass spectrometry. *Biodegradation*, 16: 67–79.

Sorge, C., Miiller, R., Leinweber, P. and Schulten, H.-R. 1993. Pyrolysis-mass spectrometry of whole soils, soil particle-size fractions, litter materials and humic substances: Statistical evaluation of sample weight, residue, volatile matter and total ion intensity. *Fresenius J. Anal. Chem.*, 346: 687-703.

Spaccini, R. and Piccolo, A. 2009. Molecular characteristics of humic acids extracted from compost at increasing maturity stages. *Soil Biol. Biochem.*, 41: 1164–1172

Stewart, C. E., Neff, J. C., Amatangelo, K. L. and Vitousek, P. M. 2011. Vegetation Effects on soil organic matter chemistry of Aggregate Fractions in a Hawaiian Forest. *Ecosystems*, 14: 382–397.

von Lützow, M., et al. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review. *Eur. J. Soil Sci.,* 57: 426-45.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Jamroz, E. and Kocowicz, A. 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biol. Biochem*., 39: 1294–1302.

Wu, L., Ma, L. Q. and Martinez, G. A. 2000. Comparation of methods for evaluating stability and maturity of biosolids compost. *J. Environ. Qual.,* 29: 424-429.

Zech, W., et al. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma*, 79: 117–161

Zimmermann, M., Leifeld, J., Schmidt, M. W. I., Smith, P., and Fuhrer, J. 2007. Measured soil organic matter fractions can be related to pools in the RothC model. *Eur. J. Soil Sci*., 58, 658–667.

Zucconi, F. A. Monaco, and Forte, M. 1985. Phytotoxins during the stabilization of organic matter. In: Gasser J. K. R. (edt.), Composting of Agricultural and other Wastes, Elsevier Applied Science Publication, *New York, NY, U.S.A. pp*. 73-86.

2 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.

2.1 Introduction

Organic waste recycling in agriculture through composting is increasing as an environmentally sustainable waste management strategy (Favoino and Hogg, 2008). It improves the soil fertility as compost increases the soil organic matter (SOM) content (Ceccanti *et al*., 2007; Eshetu *et al.,* 2007) and modifies the chemical, physical and biological properties of SOM (Nardi *et al*., 2004; Adani *et al*., 2007; Lima *et al*., 2009). Moreover, the added compost can lead to significant changes in the composition of SOM and minimize carbon (C) mineralization from the soil (Mondini *et al*., 2007; Sanchez-Monedero *et al*., 2008; Gillis and Price, 2011; Eshetu *et al.,* 2012). 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 (Lal, 1999; Smith *et al.,* 2001). 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 (Davidson *et al*., 2003). 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. (Lal, 2002).

Coffee (*Coffea arabica L*.) is one of the abundant agricultural products generates expressive amounts of agricultural residues during processing (Murthy *et al*., 2012). Ethiopia is the third coffee producer country in the world with a production accounting for 61% of the annual commodity exports (Gezahegne *et al*., 2011). 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

(Gezahegne *et al*., 2011; Negassa *et al*., 2011). 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 (Bernal *et al*., 1998; Mondini *et al*., 2007). 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 (Bernal *et al*. 1998). Moreover, Mondini *et al*. (2007), Sanchez-Monedero *et al*. (2008) and Adani *et al*. (2009) 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 (Leinweber *et al*. 1996), in different stages of biowaste composting (Smidt *et al*., 2005), and to study long-term compost effects on the molecular composition of SOM (Eshetu *et al.,* 2012). 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 (Negassa *et al*. 2011). 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 differently 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.

2.2 Materials and methods

2.2.1 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^{o}36’E longitude and 7^{o}56’N latitude (**Figure 2.1**). The elevation varies between 1533-1548 m a.s.l. Mean maximum and minimum temperature range from 26 to 30 ^{o}C and 11 to 14 ^{o}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.

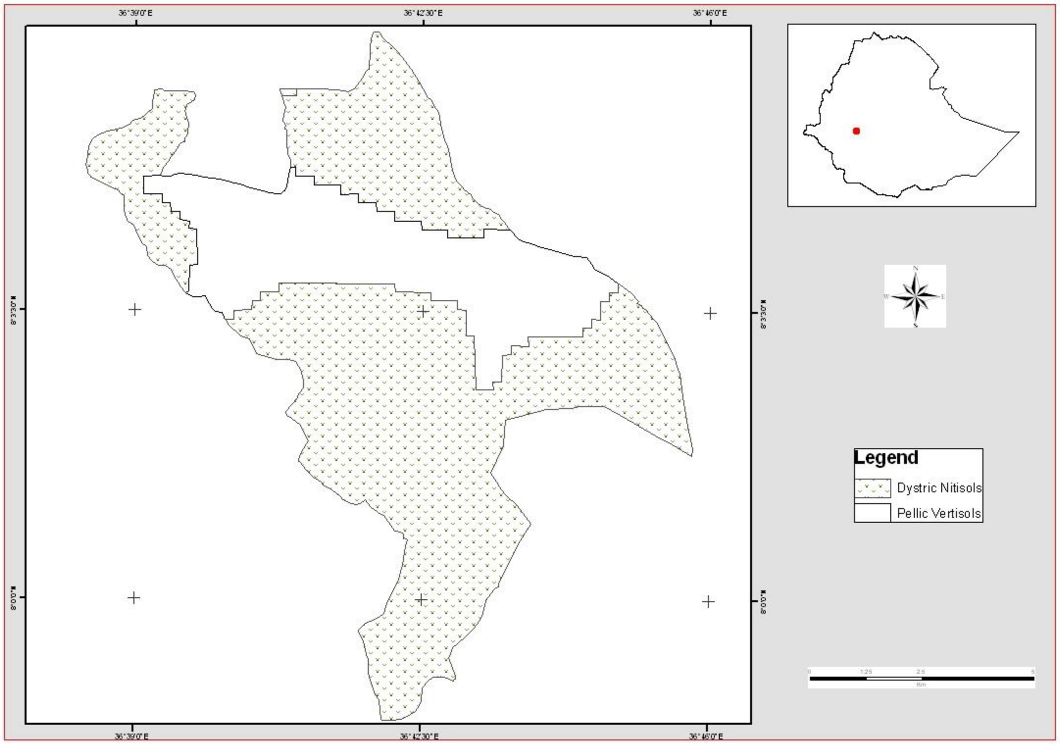

Figure 2.1. Map of Ethiopia showing area of study and soil type

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 30 days, and then in a 15-days-interval during the maturation phase. Depending on the situation the piles were watered so as to maintain the moisture above 40%. Temperatures were measured daily for the first two week and in a three-days-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.**

2.2.2 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 (Zucconi et al., 1985). 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 200 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) x 100) and root growth percentage (RG % = (mean root length in each extract/mean root length in control) x 100) was determined. The germination index (GI %), which is sensitive for phytotoxicity, was then calculated as (GI %=(G % x RG %) /100) (Zucconi et al., 1985). Each treatment was replicated four times.

2.2.3 Aerobic incubation and respiration measurement

10 g dry weight of sieved soil samples (<2 mm) were thoroughly mixed with 200 mg 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 incubation 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^{o}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) x 100) -100), % decrease C_{org} = (100-(C_a/C_b) x 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.

2.2.4 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

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.

2.2.5 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 intensi 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 8.1G).

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Table 2.1. 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_{H2O}	$C_{org.}$ ($g\ kg^{-1}$)	N_{tot} ($g\ kg^{-1}$)	S ($g\ kg^{-1}$)	C/N	EC ($ms\ cm^{-1}$)	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.

2.3 Results

2.3.1 Germination index (GI)

In the experiment with garden cress the GI varied between 3% and 115% (**Table 2.1**). 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)

2.3.2 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 2.2**). 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 $C_t = C_1\left(1 - e^{-k_1 * t}\right) + K_2 t$ 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 2.2**). In the short time of three weeks between 35 % and 69 % of the total C had been evolved. The cumulative CO_2-C respired from the amended soils were varied between 5.6 g C (kg soil)$^{-1}$ and 10.6 g C (kg soil)$^{-1}$ at the end of the incubation (**Table 2.2**). 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 2.2**). During the whole incubation period the CO_2-C release from S+RCPW (10.6 g C (kg soil)$^{-1}$) was significantly higher than in soils amended with any of the compost materials.

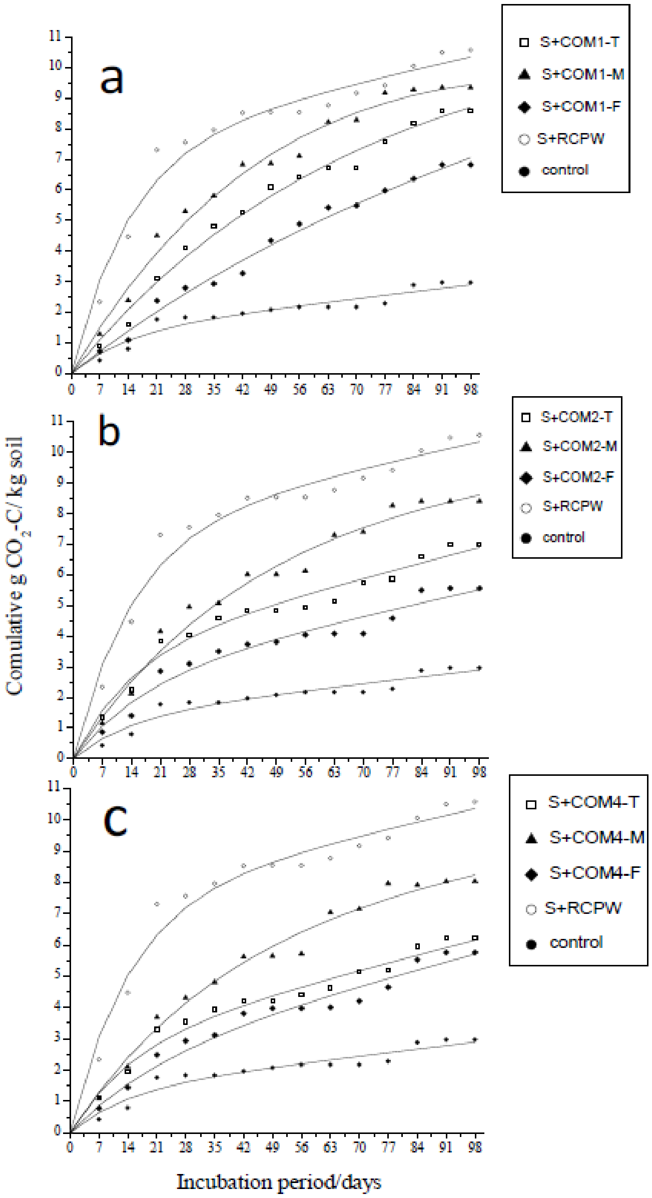

Figure 2.2. Cumulative CO_2-C release from the control , soil amended with raw coffee pulp waste (S+ RCPW), and composts of different degree of stability made up of mainly composting of: a) coffee pulp waste (S+COM1-T, S+COM1-M, S+COM1-F); b) fruit and vegetable waste (S+COM2-T, S+COM2-M, S+COM2-F); and c) equal proportion of coffee pulp waste with fruit and vegetable waste (S+COM4-T, S+COM4-M, S+COM4-F).

Table 2.2. 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	40.2	3.3	0.7	12.03	2.43
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

The application of differently stabilized composts significantly affected the CO_2-C release from soil (**Table 2.2**). Addition of COM4-F to soil resulted in the lowest CO_2-C release (5.76 g C (kg soil^{-1})) followed by COM4-T (6.22 g C (kg soil^{-1})) and COM4-M (8.03 g C (kg 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 2.2**).

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 and 480 °C at start of the incubation and shifted to 490 and 510 °C at the end of incubation (see upper right inserts in **Figure 2.3**).

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 Figure 3 b, d and f than in Figure 3 a, c and e. This difference, however, was not shown in the control (**Figure 2.3** 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 Figure 3 b, d, f and h than in **Figure 2.3** 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 **Figure 2.3** b, d, f and h than in **Figure 2.3** 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 **Figure 2.3** a, c, e and g than in Figure 3 b, d, f and h) in all incubated treatments, but most pronounced in S+COM4-T.

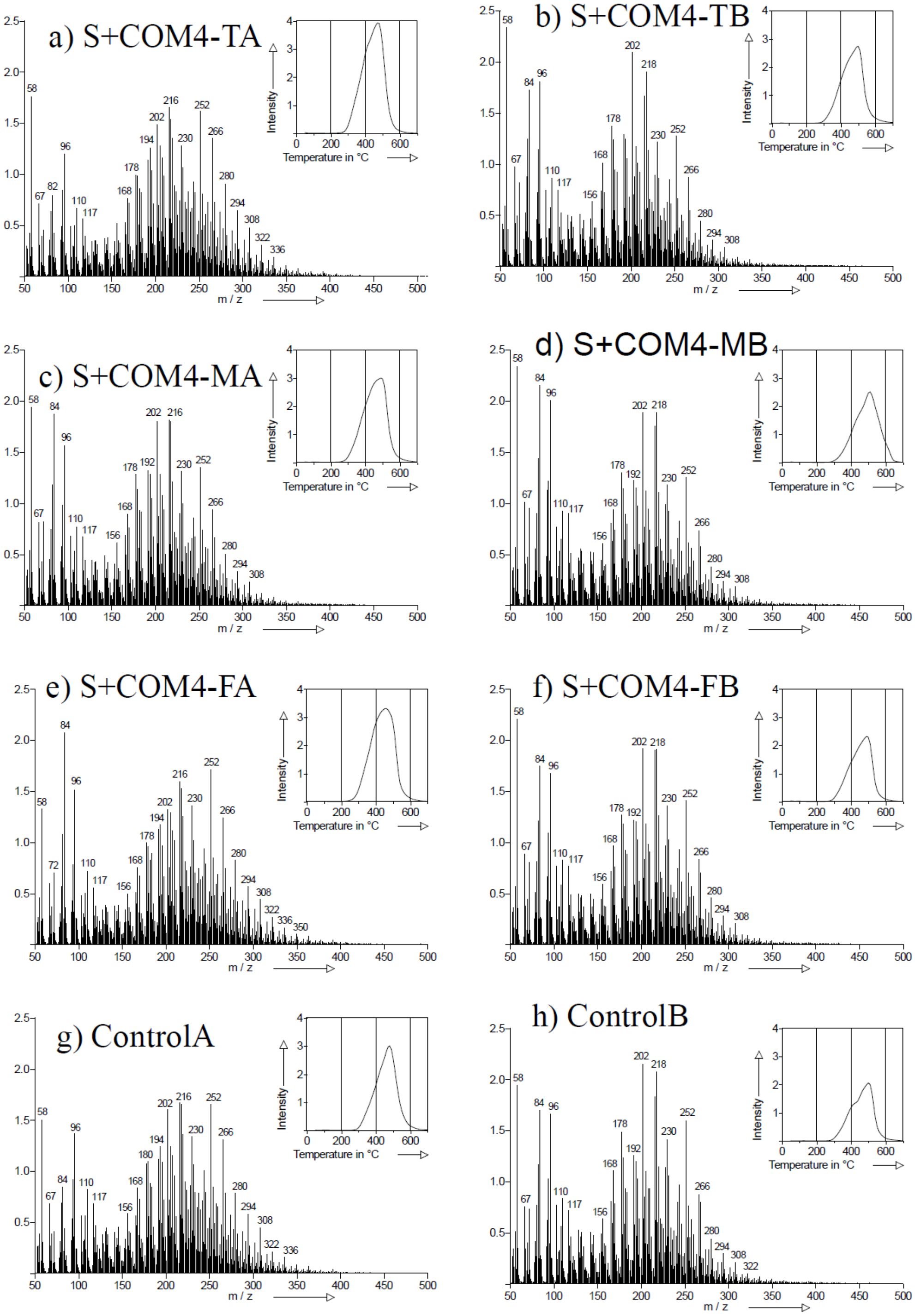

Figure 2.3 Summed and averaged pyrolysis-field ionization mass spectra and thermograms of total ion intensity (TII) (upper right inserts) of the non-treated control soil and soil amended with different degree of stability composts (COM – compost; T–thermophilic phase; M–mesophilic phase; F–final stage); at the start of incubation (-A) and end of incubation(-B)

The TII values were significantly larger in all amended soils than in the control at start of the incubation (**Table 2.3**). 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 2.2**).

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 2.3**). 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 2.3**). The proportions of alkylaromatics, lignin dimers and lipids did not differ among treatments, neither at the start nor at the end of incubation.

Table 2.3 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.

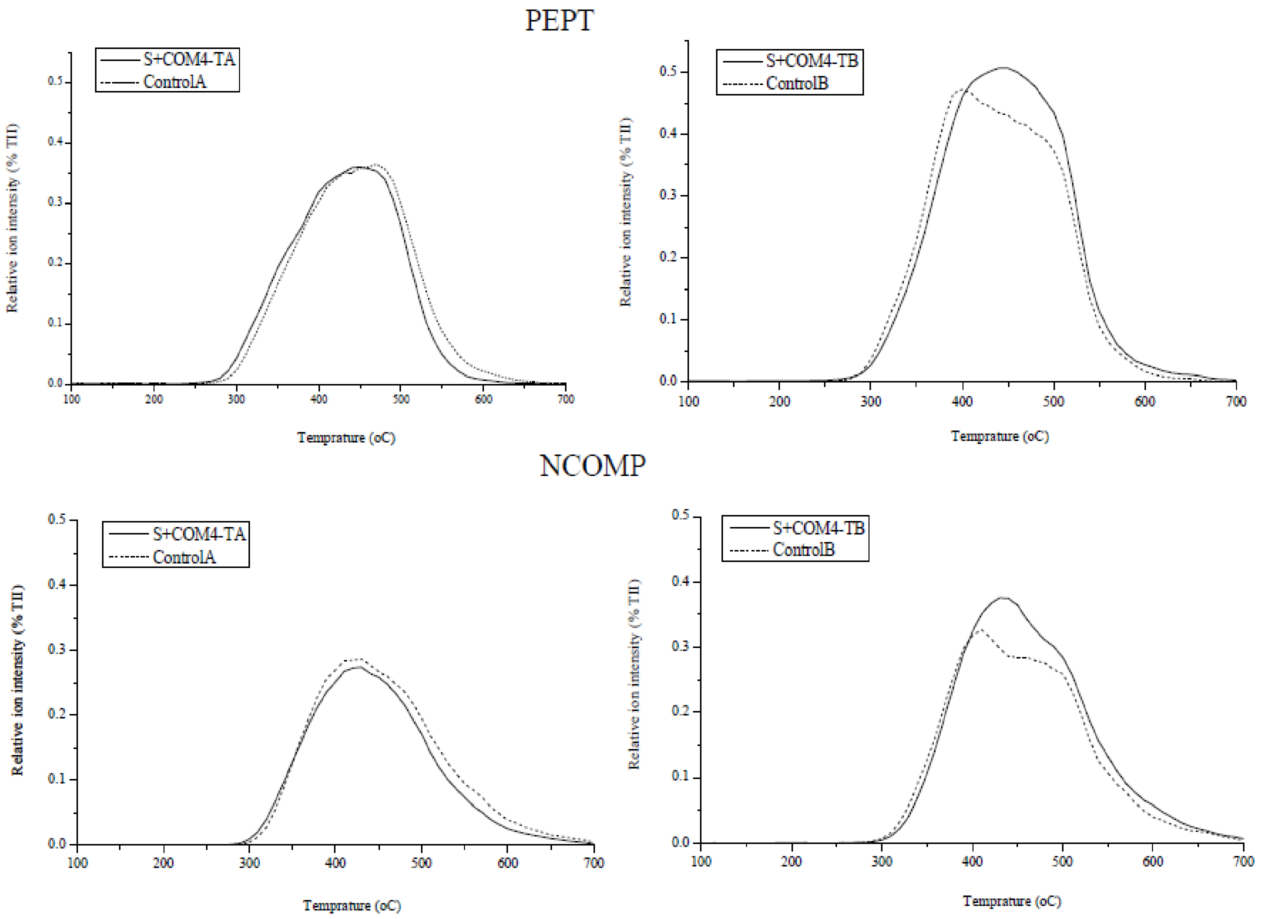

Figure 2.4 Thermograms for the evolution of peptides (PEPT) and mainly heterocyclic N-compounds (NCOMP) from non-treated control soil (Control) and soil amended with thermophilic compost (S+COMP4-T) at the start of incubation (Control A, S+COMP4-TA) and end of incubation (Control B, S+COMP4-TB).

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 2.4**). 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.

2.4 Discussion

2.4.1 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 (Zucconi *et al*., 1985; Lasaridi *et al*., 2006). 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 (Lasaridi *et al*., 2006). A GI < 50 % means high phytotoxicity, values between 50 and 80% a moderate phytotoxicity and values > 80% no phytotoxicity (Zucconi *et al*., 1985). 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 composting samples agreed with (Lasaridi *et al*., 2006) 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 germination by compost samples (Paradelo *et al*., 2008). A Gp of >80% for radish in all samples including the RCPW indicates a lower sensitivity of this seedling against the RCPW.

2.4.2 Organic matter mineralization

The observed pattern in CO_2-C release agreed with the model used by Gillis and Price (2011). 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 decomposition of labile compounds (Bernal *et al*., 1998; Ceccanti *et al*., 2007). 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 Pascual *et al*. (1998) and Mondini *et al.* (2007) who reported increased respiration of soils after amendment with organic 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 2.2**).

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 immobiliza was stronger in the soil amended with COM-T than in the soil amended with COM-M. This confirms Sanchez-Monedero *et al*. (2008) who observed that the degree of stabilization of added compost materials must not necessarily agree with the efficiency in C conservation. Moreover, some authors found an inverse relationship between the CO_2-C release and the degree of transformation/stabilization of compost materials added (Pascual *et al.,* 1998; Bernal *et al.,* 1998; Mondini *et al.,* 2007). 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 too low to promote the microbial decomposition significantly in the initial phase of incubation (Smidt *et al*., 2005). Another explanation is the stabilization of C by clay–organic matter associations (Bolan *et al*., 2012). Moreover, the lowest C_{org} loss (ranges from 1.4% and 2.4%) in the soil amended with COM-T indicated the suitability of composts from an early composting phase. This confirms that composts with a short composting period can be optimal for C sequestration in soil (Mondini *et al*., 2007; Gillis and Price, 2011).

2.4.3 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 (Leinweber and Schulten, 1993; Sorge *et al*., 1993). However, the larger TII in S+COM4-T than in any other treatment at the end of incubation proved selective preservation of compounds that were possibly mineralized in samples with longer composting time. The best explanation for such a selective protection is the binding of organic matter at clay surfaces, and a consecutive aggregation of clay-organic matter complexes (Schulten *et al*., 1993; Bustamante et al., 2010; Bolan *et al*., 2012).

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 2.2, Table 2.3**). 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 2.3**). 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 relative enrichment of rather stabile compounds during the decomposition (Janssen 1996; Schulten and Schnitzer, 1997; Bustamante *et al.*, 2010). This explanation agrees with Stewart *et al*. (2011), who described enrichments of heterocyclic 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 spectroscopy (Smidt *et al*. 2005). However, these N-compounds were completely decomposed in re-circulated leachates in larger scale lysimeters (Franke *et al*., 2007), likely caused by the lack of sorptive surfaces. This indirectly points to the importance of the sorptive surfaces of the clay minerals in the present experiment.

The temperature-resolved Py-FIMS showed that thermally 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 selectively preserved. The pronounced enrichment of phenols/lignin monomers agreed with Leifeld *et al*. (2002) 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 compost. Moreover, significantly increased carbohydrate proportions in the variant S+COM4-T during the incubation proved selective carbohydrate preservation. This agrees with findings of Chefetz *et al*. (1996) that progressive compost maturity reflected a decrease of the carbohydrate content. In agreement with Spaccini and Piccolo (2009) and Smidt *et al*. (2005), we found a progressive association of bio-labile peptides during composting.

2.4.4 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 2.3**) 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 2.2**). 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^{o}C according to Kalbitz *et al.*, 2003) 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^{o}C), N-compounds (volatilized >380 °C) and peptides (volatilized >350-400 °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 (Leifeld *et al*., 2002). 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 (Leinweber *et al*., 2008) and matured compost application in sandy soil (González-Vila et al., 1999).

2.5 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.

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References

Adani, F., Genevini, P., Ricca, G., Tambone, F. and Montoneri, E. 2007. Modification of soil humic matter after 4 years of compost application. *Waste Manage*., 27: 319–324.

Bernal, M. P., Sa´nchez-Monedero, M. A., Paredes, C. and Roig, A. 1998. Carbon mineralization from organic wastes at different composting stages during incubation with soil. *Agri. Ecosyst. Environ.,* 69: 175–189.

Bolan, N. S., Kunhikrishnan, A., Choppala, G. K., Thangarajan, R. and Chung, J. W. 2012. Stabilization of carbon in composts and biochars in relation to carbon sequestration and soil fertility. *Sci.total Environ*.,1(424): 264-270

Bustamante, M. A., Said-Pullicino, D., Paredes, C., Cecilia, J. A. and Moral, R. 2010. Influences of winery–distillery waste compost stability and soil type on soil carbon dynamics in amended soils. *Waste Manage*., 30(10): 1966-1975.

Ceccanti, B., Masciandaro, G. and Macci, C. 2007. Pyrolysis-gas chromatography to evaluate the organic matter quality of a mulched soil. *Soil Till. Res.,* 97: 71–78.

Chefetz, B., Hatcher, P.G., Hadar,Y. and Chen,Y. 1996 Chemical and biological characterization of organic matter during composting of municipal solid waste. J*. Environ. Qual.* 25: 776–785

Davidson, O., Halsnaes, K., Huq, S., Kok, M., Metz, B., Sokona, Y.and Verhagen, J. 2003. The development and climate nexus: the case of sub-Saharan *Africa. Climate Policy*, 31: 97-113.

Eshetu, B., Jandl G. and Leinweber, P. 2012. Compost Changed Soil Organic Matter Molecular Composition: A Study by Py-GC/MS and Py-FIMS. *Compost Sci. Util.,* 20: 230-238.

Favoino, E. and Hogg, D., 2008. The potential role of compost in reducing greenhouse gases. *Waste Manage. Res.,* 26: 61–69.

Franke, M., Jandl, G. and Leinweber, P. 2006. Organic compounds in re-circulated leachates of aerobic biological treated municipal solid waste. *Biodegradation*, 17: 473–485.

Franke, M., Jandl, G. and Leinweber, P. 2007. Analytical pyrolysis of re-circulated leachates: Towards an improved municipal waste treatment. *J. Anal. Appl. Pyrol*., 79: 16–23.

Gezahegne, B., Fikre, L. and Mulatu, W. 2011. Exploring the suitability of coffee pulp compost as growth media substitute in greenhouse production. *Int. J. Agri. Res*., 6(3): 255-267

Gillis, J. D. and Price, G. W. 2011. Comparison if novel model to three conventional models describing carbon mineralization from soil amended with organic residues.*Geoderma*, 160: 304-310.

González-Vila, F. J., Almendros, G. and Madrid, F. 1999. Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil. *Sci. Total Environ.*, 236: 215–229.

Janssen, B. H. 1996. Nitrogen mineralization in relation to C:N ratio and decomposability of organic materials. *Plant Soil*, 181: 39–45

Kalbitz, K., et al. 2003. Changes in properties of soil-derived dissolved organic matter induced by biodegradation. *Soil Biol. Biochem.,* 35: 1129–1142*.*

Lal, R. 1999. Soil management and restoration for C sequestration to mitigate the accelerated greenhouse effect. *Prog. Environ. Sci.,* 1, 307–326.

Lal, R., 2002. The potential of soils of the tropics to sequester carbon and mitigate the greenhouse effect. Adv. Agron., 74: 155– 192.

Lasaridi, K., Protopapa, I., Kotsou, M., Pilidis, G., Manios, T. and Kyriakou, A. 2006. Quality assessment of composts in the Greek market: The need for standards and quality assurance. *J. Environ. Manag***.**, 80: 58-65.

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. *Eur. J. Soil Sci*., 53: 299–309.

Leinweber, P., Blumenstein, O. and Schulten, H.-R. 1996. Organic matter composition in sewage farm soils: Investigations by ^{l3}C-NMR and pyrolysis-field ionization mass spectrometry. *Eur. J. Soil Sci*., 47: 71-80.

Leinweber, P., Jandl, G., Baum,C., Eckhardt, K.-U., Kandeler,E., 2008. Stability and composition of soil organic matter control respiration and soil enzyme activities. *Soil Biol. and Biochem.,* 40:1496-1505.

Leinweber, P. and Schulten, H.-R. 1993. Dynamics of soil organic matter studied by pyrolysis-field ionization mass Spectrometry. *J. Anal. Appl. Pyrol.*, 25: 123-136.

Lima,G., Diana L. D., Santos, M. S., Scherer, W. H., Schneider, J. R., Duarte, C. A., Santos, B. H. E. and Esteves, I. V. 2009. Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, 150: 38-45.

Mondini, C., Cayuela, M. L., Sinicco, T., Cordaro, F., Roig, A. and Sánchez-Monedero, M. A. 2007. Greenhouse gas emissions and carbon sink capacity of amended soils evaluated under laboratory conditions. *Soil Biol. Biochem*, *39:* 1366-1374.

Murthy, P. S. Manjunatha, M. R., Sulochannama G. and Naidu, M. M. 2012 Extraction, Characterization and Bioactivity of Coffee Anthocyanins. *Eur. J. Biol. Sci.,* 4: 13-19.

Nardi, S., Morari, F., Berti, A., Tosoni, M. and Giardini, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilizers. *Eur. J. Agron.,* 21: 357–367.

Negassa, W., Baum, C. and Leinweber, P. 2011. Soil amendement with agro-industrial products: Moluclar-chemical compostions and effects on soil biochemical activites and phosphrous fractions. *J. Plant Nutr. Soil Sci*., 174: 113-120.

Paradelo, R., Moldes, A. B., Rodríguez, M. and Barral, M. T. 2008. Relationship between heavy metals and phytotoxicity in composts. *Cienc. Tecnology Aliment.,* 6: 143-151.

Pascual, J. A., Hernandez, C., García C. and Ayuso, M. 1998. Carbon mineralization in arid soil amended with organic wastes of varying degrees of stability. *Communications in Soil Science and Plant Analysis,* 29: 835–846.

Sánchez-Monedero, M. A., Cayuela, M. L., Mondini, C., Serramia, N. and Roig, A. 2008. Potential of olive mill wastes for soil carbon sequestration.Waste manage. 28: 767-773.

Smidt, E., Eckhardt, K.-U., Lechner, P., Schulten, H.-R. and Leinweber, P. 2005. Characterization of different decomposition stages of biowaste using FT-IR spectroscopy and pyrolysis-fieldionization mass spectrometry. *Biodegradation*, 16: 67–79

Smith, P., Powlson, D.S., Smith, J. U., Falloon, P., Coleman, K., 2001. Meeting Europe's climate change commitments: quantitative estimates of the potential for carbon mitigation by agriculture. *Global Change Biol*., 6: 525–539.

Sorge, C., Miiller, R., Leinweber, P. and Schulten, H.-R. 1993. Pyrolysis-mass spectrometry of whole soils, soil particle-size fractions, litter materials and humic substances: Statistical evaluation of sample weight, residue, volatile matter and total ion intensity. *Fresenius J. Anal. Chem.*, 346: 687-703.

Schulten H.-R. and Schnitzer, M. 1997. The chemistry of soil organic nitrogen - a review". *Biol. Fertil Soils,* 26: 1–15.

Schulten, H.-R., Leinweber P. and Sorge, C. 1993. Composition of organic matter in particle size fractions of an agricultural soil. *J. Soil Sci.*, 44: 677–691.

Spaccini, R. and Piccolo, A. 2009. Molecular characteristics of humic acids extracted from compost at increasing maturity stages. *Soil Biol. Biochem*., 41: 1164–1172

Stewart, C. E., Neff, J. C., Amatangelo, K. L. and Vitousek, P. M. 2011. Vegetation Effects on soil organic matter chemistry of Aggregate Fractions in a Hawaiian Forest. *Ecosystems*, 14: 382–397.

Zucconi, F.; A. Monaco; and M. Forte (1985). Phytotoxins during the stabilization of organic matter. In: Gasser J. K. R. (Editor), Composting of Agricultural and other Wastes, Elsevier Applied Science Publication, New York, NY, U.S.A., pp. 73-86.

3 Soil organic matter alterations by short-term composted coffee pulp waste: evidence from pyrolysis-field ionisation mass spectrometry.

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Abstract

Understanding molecular changes in soil organic matter (SOM) from compost addition is important for efficient compost use as soil amendment and soil carbon (C) sequestration which is completely lacking for tropical soils. Therefore, this study investigates the impact of addition of compost from different phases of composting in molecular composition of SOM under controlled laboratory conditions. Compost samples produced from coffee pulp waste were collected after the thermophilic, the mesophilic and the final phase of composting, and an amount equivalent of 48 t ha^{-1} was added to a samples of a tropical Nitisol. The soil-compost-mixtures were incubated aerobically for 14 weeks, and samples from the end of incubation were analysed for elemental composition (C, N, S), amount of organic C mineralized and for molecular-chemical composition. The C_{org} concentrations and the total ion intensities of pyrolysis-field ionization mass spectrometry (Py-FIMS) were larger in compost amended treatments than in the control. Despite of similar initial organic C_{org} and N_t contents among compost amended treatments, less organic C was mineralized following the application of compost from the thermophilic than from later phases of composting. The SOM in the soil that received the “thermophilic” compost contained significantly higher proportions of carbohydrates, heterocyclic/nitrilic N-compounds, peptides, sterols and *n*-fatty acids than the other amended soils. Temperature-resolved volatilization curves for carbohydrates, N-compounds and peptides showed that the enriched compounds covered a wide range of thermal stability. It can be concluded that the application of compost from the thermophilic phase had the best effect in terms of biologically labile C sequestration and stabilization of native organic C in a clay-rich tropical Nitisol used in this study. If confirmed for other composts and soils, these findings may have also considerable economic implications because the time period required for composting can be shortened.

Keywords: compost; soil organic matter; coffee pulp waste; soil carbon sequestration; pyrolysis-field ionization mass spectrometry; molecular composition.

Zusammenfassung

Die Kenntnis von Veränderungen in der molekularen Zusammensetzung der organischen Bodensubstanzen (OBS) nach Kompostdüngung ist ebenso wichtig in abfallwirtschaftlichen Zusammenhängen wie zur effizienten Kompostnutzung als Bodenverbesserungsmittel und zur Kohlenstoffspeicherung im Boden. Allerdings sind entsprechende Daten für tropische Regionen nicht vorhanden. Es wurden Proben aus kompostierten Kaffeebohnenschalen untersucht, die nach der thermophilen, der mesophilen sowie der finalen Reifungsphase der Kompostierung entnommen wurden. Diese somit unterschiedlich stabilisierten Komposte wurden Bodenproben aus einem tropischen Nitisol in einer Aufwandmenge entsprechend 48 t ha^{-1} zugesetzt, und diese Gemische wurden 14 Wochen aerob im Dunklen inkubiert. Nach Abschluss der Inkubation wurden die Proben auf Gehalte an organisch gebundenem Kohlenstoff (C_{org}), Gesamt-Stickstoff N_t und Schwefel (S_t) sowie ihre molekular-chemische Zusammensetzung mit Pyrolyse-Feldionisation Massenspektrometrie (Py-FIMS) untersucht. Die C_{org}-Gehalte und die Gesamtionenintensitäten der Py-FIMS waren in den mit Kompost behandelten Proben größer als in der Kontrolle. Bei gleichen Anfangsgehalten an C_{org} and N_t innerhalb der verschiedenen Kompostvarianten zeigte die Variante mit Kompost aus der thermophilen Phase eine signifikant geringere C-Mineralisierung als die Varianten mit länger kompostierten Abfällen. Die OBS des mit Kompost aus der thermophilen Phase inkubierten Bodens enthielt signifikant größere Anteile an Kohlenhydraten, heterozyklischen/nitrilischen N-Verbindungen, Peptiden, Sterolen und *n*-Fettsäuren als die anderen Böden. Temperatur-aufgelöste Freisetzungskurven für Kohlenhydrate, N-Verbindungen und Peptide zeigten die Anreicherungen in einem weiten Bereich thermischer Stabilität. Aus den Daten folgt, dass der unmittelbar nach der thermophilen Phase entnommene Kompost die beste Wirkung hinsichtlich C-Speicherung in diesem tonreichen Nitisol hat. Bei weitere Bestätigung und breiter Anwendung dieser Erkenntnis könnten aus der Verkürzung der Kompostdauer beträchtliche ökonomische Vorteile erwachsen.

3.1 Introduction

The application of compost to soil has received much attention as an environmentally favorable strategy to use the increasing amount of organic waste and to improve the soil organic matter (SOM) status of agricultural land (Lal, 2004; Weber *et al*., 2007; Bustamante *et al*. 2010). Compost application to increase the quantity and improve the quality of SOM is particularly important in strongly weathered tropical soils to overcome the degradation of SOM and improve the soil carbon (C) sequestration (Zech *et al*., 1997; Lal, 2002, Rivero et al., 2004; Bruun et al*.*, 2010). Studies done by Leifeld *et al*. (2002), Spaccini *et al*. (2009) and Eshetu *et al*. (2012) clearly showed quantitative increases as well as improvements in the SOM quality following compost application to sandy or loamy soils. The effects of compost on composition and stability of SOM depend on various factors including the quality of compost, compost interactions with microbial enzyme systems and mineral soil surfaces (Zech *et al*., 1997; González-Vila *et al.*, 1999; Grandy and Neff, 2008; Stewart *et al*., 2011).

Previous research showed that compost application to soil modifies the chemical, physical and biological properties of SOM (Nardi *et al*., 2004; Adani *et al*., 2007; Lima *et al*., 2009). The addition of compost to soil reduced the mineralization of labile compounds of SOM (Fortuna *et al*. 2003; Piccolo *et al*. 2004) and increased the humic acid (HA) over the fulvic acid (FA) proportions (Rivero *et al*. 2004). The latter results were confirmed by Weber *et al*. (2007) who also reported a relative increase in HA relative to FA with time in soils amended with urban compost. Moreover, the added compost can lead to significant changes in the composition of SOM and minimize labile C mineralization from the soil thereby enhancing the role of SOM as a sink of organic C (Mondini *et al*., 2007; Spaccini *et al*., 2009). On the bases of CO_2 respiration results during incubation Mondini *et al*. (2007) and Sánchez-Monedero *et al*. (2010) reported an inverse relationship between the organic C mineralized and the degree of stability of the compost added. They concluded that addition of relatively stable compost always reduces the organic C respiration and, thus, stabilize SOM. However, Sánchez-Mondero *et al*. (2008) observed that the efficiency of added olive mills composts for soil C conservation was independent of the degree of compost stability. González-Vila *et al.* (1999) stated that extended composting did not contribute to the accumulation of progressively aromatized humic materials but it led to nitrogen and carbohydrate losses. However, most of these results on compost effects on SOM composition and stabilization are based on general chemical information rather than detailed molecular-level studies of SOM. Furthermore, the fate of composts of different origin and stability in tropical soils is completely unknown.

Effects of different management techniques such as compost application on the long term soil C sequestration are difficult to study because biologically induced fluxes however, take place in short time periods, e.g., hours and days to months whereas geochemical fluxes may take place over longer time periods such as hundreds to millions of years (Paul and Clark, 1989). Therefore, the popular laboratory incubation experiments which measure microbial activity, organic matter decomposition in amended soils (Bernal *et al*., 1998; Fernandez *et al*., 2007) and C sequestration potential of arable soils (Mondini *et al*., 2007; Sanchez-Monedero *et al*., 2008; Adani *et al*., 2009) have limitations in applying the results to field conditions and generalisation. For instance, under field conditions the decomposition and stability of organic C is a function of dynamic, heterogeneous and interactive variables such as the amount and quality of plant material entering the soil, the soil mineralogy and chemistry and other soil properties such as moisture, temperature, vegetation etc. The advantage of controlled laboratory incubation experiments is that these factors can be kept constant and restricted so that effects of one variable (e.g. compost quality) can be determined with more precision.

Coffee pulp waste may have significant adverse effects to the environment (Gezahegne *et al*., 2011; Negassa *et al*., 2011), but composted it may be a useful amendment of tropical soils. Therefore, it is important to disclose effects of coffee pulp waste composts to SOM contents, composition and stability. Relevant evidence requires the application of molecular-level sensitive analytical methods such as mass spectrometry. For instance, pyrolysis–field ionization mass spectrometry (Py-FIMS) enabled to describe management induced changes in SOM composition in whole soil samples (Schulten and Hempfling, 1992), to characterize SOM in sewage farm soils (Leinweber *et al*., 1996), to trace different phases of biowaste composting (Smidt *et al*., 2005) and to characterise the organic matter in fresh agro-industrial waste including coffee pulp (Negassa *et al*., 2011). Recently, Eshetu *et al*. (2012) used Py-FIMS in conjunction with pyrolysis gas chromatography/mass spectrometry to study long-term compost effects on the molecular composition of SOM developed in humus free Pleistocene loamy marl. Most recently we observed that composts taken after the thermophilic phase may have an overall better effect to soil C sequestration than mature composts (Eshetu et al., 2013). However, neither Py-FIMS nor another highly sensitive speciation method has been applied so far to disclose effects of differently aged or stabilised composts from coffee pulp waste on SOM in tropical soils.

Therefore, the objectives of the study were (1) to evaluate the effect of compost age on the molecular structure of SOM in a tropical Nitisol amended with compost derived from different phases of composting of coffee pulp waste, and (2) to investigate which constituents of organic matter were decomposed or relatively enriched, when differently stabilized composts were incubated in the Nitisol sample to simulate the microbial decomposition.

3.2 Materials and methods

3.2.1 Soil and composting samples

The soil was sampled at a coffee plantation farm located in the South-western highlands of Ethiopia and classified as Nitosol (FAO, 1998). The sampling area is situated 36^{o}36'E longitude and 7^{o}56'N latitude. The elevation varies between 1533-1548 m a.s.l. Mean minimum and maximum temperatures range from 11 to 14 °C and 26 to 30 °C, respectively, and the mean of precipitation ranges from 1131 to 1150 mm. Thus, the climate may be indicated as warm tropical. A total of 50 subsamples were taken from 5.91 ha land, 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. The soil sample was air-dried and milled to pass a 2 mm sieve. Main characteristics are compiled in **Table 3.1**.

The compost was produced from 800 kg raw coffee pulp waste (RCPW) 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. Piles were constructed for experimental purposes with duplicates. About 5 % (w/w) soil and 10 % (w/w) of garden trimmings (dry leaves, and young tree branches) were added as additional source of microbial colonization and to improve the pile structure. The experiment lasted from November 2010 to February 2011. The moisture content of the compost was manually estimated on site by hand squeezing the compost tightly. Moisture contents above 40 % were indicated by a formation of a ball up on squeezing of the compost. Depending on the situation the piles were watered so as to always maintain > 40 % (w/w) moisture. Samples were taken once a week for pH moisture content measurements in the laboratory. Temperatures were measured daily for the first two weeks and in a three-days-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 piles were manually turned each week during the thermophilic phase (45°C - 69 °C) which lasts 30 days, and then in a 15-days-interval during the maturation phase (< 43 °C). Temperature was considered as a major indicator how well the composting process was progressing. The composting was considered to be finished when the temperature of the mixture remained stable and near ambient (about 21 °C) after 16 weeks.

Representative samples (about 1 kg) were taken by mixing nine subsamples from different levels of the section in the pile at different phases of the composting, day 8 (thermophlic phase = COM-T), day 36 (mesophilic phase = COM-M) and day 119 (final compost = COM-F). In this way composts of different stability were obtained. The samples were air dried and ground to pass a 0.5 mm sieve. Main properties are shown in **Table 3.1**.

Aerobic incubation of the soil-compost-mixtures was done by mixing 10 g dry weight of sieved soil samples (<2 mm) with fresh and composted coffee pulp waste at desired application rates (48 t ha^{-1}) and placed in 100 ml incubation vessels in five replicates each. They were monitored as treatment S+RCPW, S+COM1-T, S+COM1-M and S+COM1-F. Soil controls were run without any amendment. Distilled water (1 to 5 ml) was added to the mixtures to keep the moisture at 60 % of water-holding capacity. 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 end of incubation for elemental and Py-FIMS analyses.

3.2.2 Chemical analyses: basic data and molecular-chemical characterization

The soil samples were analyzed for pH and EC in 0.01 *M* $CaCl_2$ suspensions (1:2.5 w/v), and fresh and composed waste samples were analyzed for pH and EC in H_2O suspensions (1:10 w/v). The concentrations of total organic carbon (C_{org}), total nitrogen (N_t) and total sulfur (S_t) were determined for soil, compost and amended soil samples using a CNS analyzer (Vario EL III; Elementar Analysensysteme, Hanau, Germany). RCPW and Compost phytotoxicity was determined according to the modified method of Zucconi *et al*. (1985) and described and discussed in Eshetu *et al*. (2013).

For Py-FIMS about 3 mg of the air dried, ground and homogenized samples 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 a sample. For each of the single scans, the absolute and relative ion intensities of ten classes of compounds in the organic matter 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 analysis. Information on the molecular size distribution of pyrolysis products was obtained by calculating the molecular weight averages M_w (weight-based) and M_n (number-based), and the polydispersity ($I = M_w / M_n$) (Lattimer and Schulten, 1983). Detailed descriptions of the Py-FIMS methodology are published in Leinweber *et al*. (2009).

3.2.3 Statistical analyses

Means were calculated for physicochemical properties of soil, composts, amended soils and ion intensities from Py-FIMS. Comparisons between means of ion intensities of compound classes and polydispersity in different treatment were made by a One Way ANOVA test. Multivariate statistical evaluation of Py-FI mass spectra was done by principal component analysis to test which m/z signals contributed to differences among samples. All statistics were computed using data analysis and graphic software (Origin 8.1G).

Table 3.1: Main characteristics of soil, fresh waste and differently stabilized compost samples (RCPW: fresh coffee pulp waste, COM: compost, T: thermophilic phase, M: mesophilic phase, F: final stage, C_{org}: total organic carbon, N_{tot}: total nitrogen, S_t: total sulfur, C/N: carbon to nitrogen ratio, EC: electrical conductivity).

Parameter	Soil	RCPW	COM1-T	COM1-M	COM1-F
pH	5.2	5.8	8.8	9.3	9.1
$C_{org.}$(g kg^{-1})	38.6	397.0	151.0	149.0	139.2
N_{tot} (g kg^{-1})	3.2	21.1	10.5	12.7	12.7
S_t (g kg^{-1})	0.8	3.1	1.7	2.2	2.4
C/N	11.9	18.9	14.4	11.7	11.0
EC (mS cm^{-1})	2.4	6.7	2.0	2.1	2.1
Soil texture					
% Sand	1%		ND	ND	ND
% Silt	14%		ND	ND	ND
% Clay	84%		ND	ND	ND
Phytotoxicity (Germination index in %)					
With cress		3	106	109	102
With Radish		26	121	120	125

3.3 Results and Discussion

3.3.1 Elemental composition

Organic amendments increased the C_{org} concentrations by 7.8 g kg^{-1} (RCPW) and about 2.0 g kg^{-1} (with the addition of composts) at start of the incubation (Table 2). The increases in N_{tot} concentrations were proportionally. These differences in C_{org} and N_{tot} concentrations originate from the different amounts of organic matter applied with RCPW and the composts (Lima *et al*., 2009). At the end of incubation all samples had lower C_{org} and N_{tot} (except the S + RCPW) concentrations. The C_{org} losses were larger in the soil amended with RCPW (10.5%) than in the samples amended with composts (2% to 4.1%). This difference in C_{org} losses by respiration are explained by the decomposition of easily degradable organic compounds from the RCPW which in the composts had been either mineralized (Bernal *et al*., 1998) or otherwise stabilized. Furthermore, the observed smallest C_{org} losses by only 2.0% in the samples amended with compost from the thermophilic phase disagrees with the findings of (Leifeld *et al*., 2002; Mondini *et al*., 2007). These authors found an inverse relationship between the organic C mineralized and the degree of stability of the compost added in a laboratory incubation experiment and concluded that composts require a maturation phase for humification and organic matter stabilization.

Table 3.2: Elemental composition (g kg^{-1}) of control soil sample, and soil samples amended with row coffee pulp waste (S+RCPW) and composts from coffee pulp waste taken at the thermophilic (S+COM1-T), mesophilic (S+COM1-M) and final maturation (S+COM1-F) phase of composting at start (Day 1) and end of an aerobic incubation (Day 98). The right column indicates the relative decrease in the C_{org} concentrations during the incubation.

Treatments	Day 1				Day 98				
	C_{org}	N_{tot}	S_{tot}	C/N	C_{org}	N_{tot}	S_{tot}	C/N	C_{org} loss (%)
Control	39.8	3.3	0.7	12.2	38.3	3.2	0.7	11.8	3.7
S+RCPW	47.6	3.7	0.8	12.9	42.6	3.7	0.8	11.6	10.5
S+COM1-T	42.0	3.5	0.7	11.9	41.2	3.4	0.8	11.9	2.0
S+COM 1-M	42.0	3.6	0.7	11.6	40.3	3.4	0.7	11.9	4.1
S+COM 1-F	41.8	3.6	0.8	11.7	40.7	3.4	0.7	12.0	2.6

Explanation of this disagreement requires a detailed molecular-chemical investigation of the different composts and soil-compost-mixtures as described below.

3.3.2 Pyrolysis – field ionization mass spectrometry (Py-FIMS)

In the Py-FIMS the thermograms of total ion intensity (TII) shows single peaks for all the control soil and the soil amended with RCPW (**Figure 3.1**), and similar for the three samples with compost amendment **(Figure 3.2)**. Addition of the organic materials slightly changed the peak temperatures of maximum volatilization of organic molecules from about 500 °C in the control to 470 to 500 °C in the samples with organic amendments (see **Figure 3.1** and compare with **Figure 3.2**). Furthermore, the pronounced shoulder at 420 °C seen at the control soil sample (**Figure 3.1**) disappeared in the samples with organic amendments (**Figure 3.2**), indicating some stabilization of labile organic matter by the organic amendments.

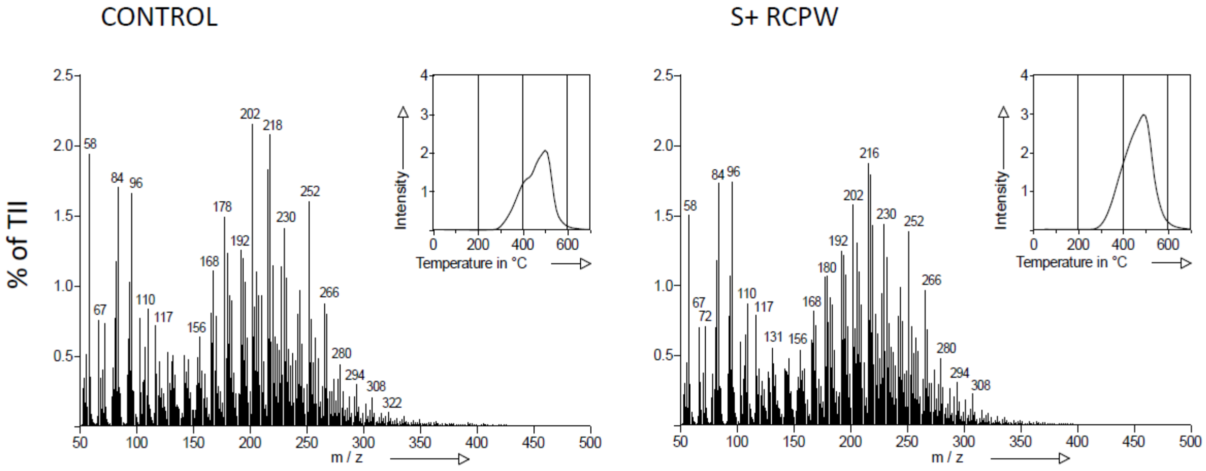

Figure 3.1 Summed and averaged pyrolysis-field ionization mass spectra and thermograms of total ion intensity (TII, upper right inserts) of a Nitisol soil sample amended with fresh coffee pulp waste (S+RCPW) and the non-amended control sample of the Nitisol

The signal patterns of the Py-FI mass spectra revealed differences among compost samples originating from the different duration of the composting. For instance the prominent signals at *m/z* 58, 72, 96, and 110 indicate a particularly high relative intensity of carbohydrates in sample S+COM1-T (**Figure 3.2**). The very intense *m/z* 84 in this treatment can be explained by pyrolysis products of carbohydrates and peptides. Furthermore, *m/z* 67 (pyrrole) and *m/z* 117 (indole) seem to be more abundant in this than in the other composts. Moreover, the signals with highest molecular masses originating from sterols and long-chained *n*-fatty acids (e.g., *m/z* 390, 424) were detected in this treatment only (**Figure 3.2**). On the other hand, intense signals of phenols/lignin monomers *(m/z* 168, 180, 194), lipids *(m/z* 202, 216, 230), homologous series of alkanes (*m/z* 252, 266, 280, 294, 308) and alkylaromatics *(m/z* 218) were found in all spectra.

A more detailed spectra evaluation by PCA indicated that 92.4 % of the total variance in the whole data set from the three soil-compost-mixtures could be explained by the first two principal components (not shown). For 34 *m/z* signals the univatiate wilks's lambda was significant at a level of $p<0.01$. The signals with the highest discriminating powers among treatments S+COM1-F, S+COM1-M and S+COM1-T are *m/z* 114, 71, 176, 434, 399, 113, 85, 177, 189, 468, 157, 84, 219, 83, 69, 253, 228, 143, 44, 46, 60, 221, 252, 202, 141, 72, 53, 269, 107, 82, 200, 88, 152, 233, 443 and 167 (arranged with decreasing importance for discrimination). For instance the pentose marker *m/z* 114 from plant material was significantly higher in treatment S+COM1-T but showed no significant difference between composts from the mesophilic and final phase. On the other side, the relative proportions of the lignin marker *m/z* 178 (coniferyl aldehyde) (Leinweber et al., 2009) was significantly higher in S+COM1-M and S+COM1-F than in S+COM1-T. This provides unequivocal evidence that duration of the composting has an effect on the molecular composition not only of the compost (Smidt et al., 2005) but also of a soil amended with this compost.

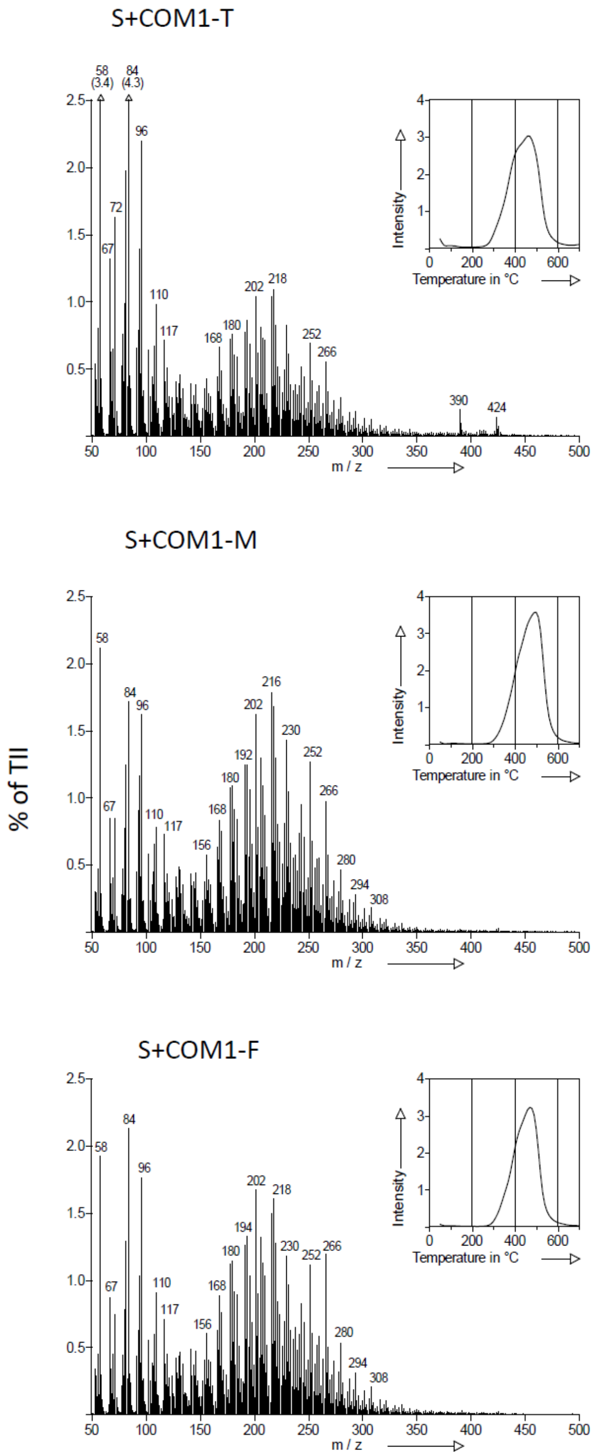

Figure 3.2 Summed and averaged pyrolysis-field ionization mass spectra and thermograms of total ion intensity (TII, upper right inserts) of Nitisol soil samples amended with composts taken in different phases of composting (COM – compost; T – thermophilic phase; M – mesophilic phase; F – final stage)

The data derived from the Py-FI mass spectra pointed to similarities but also significant differences among samples at the end of the incubation (**Table 3.3**). The proportions of volatile matter, ranging from 20 to 25%, differed insignificantly, indicating that organic matter and adsorbed water were not greatly affected by the soil amendments. The TII values were significantly larger in all amended soils than in the control without significant differences ($p<0.05$) among the amended soil samples. Assignment of markers signals to important compound classes of SOM revealed significantly larger intensities of carbohydrates, peptides and sterols in S+COM1-T than in the other treatments. Difference were largest between S+COM1-T and the control accounting for 4.4×10^6 counts mg^{-1} for carbohydrates, 3.9×10^6 counts mg^{-1} for peptides and (0.2×10^6 counts mg^{-1} for sterols (**Table 3.3**). The ion intensities of phenols/lignin monomers, alkylaromatics, and N-compounds were significantly lower in the control than in the amended soils but did not significantly differ among the amendments.

The relative ion intensities of compound classes indicated that carbohydrates (12.8%) and peptides (12.2%) were the major SOM constituents in S+COM1-T (**Table 3.3**, see values in parentheses). Furthermore, this sample contained significantly more N-compounds (7.1%) and sterols (0.6%) than any other sample. Phenols/lignin monomers (13.1-14.0%) and alkylaromatics (14.9-15.4%) were more abundant in the other amended soil samples and the control. Suberin was the compound class with smallest proportions in all samples.

Table 3.3: Results of pyrolysis-field ionization mass spectrometry: volatile matter (VM, %), total ion intensity (TII, 10^6 counts mg^{-1}), ion intensities for 10 important compound classes of organic matter (CARB: carbohydrates, PHLM: phenols/lignin monomers, LIPID: lipids, NCOMP: N-compounds, ALKY: alkylaromatics, LDIM: lignindimers, STER: sterols, PEPT: peptides, SUBE: suberin, FATTY: n-C$_{16}$ to n-C$_{34}$ fatty acids in 10^6 counts mg^{-1}), relative ion intensity (values in parenthesis, % TII) and molecular weight averages (g mol^{-1}).

Treatment	VM	TII	CARB	PHLM	LDIM	LIPID	ALKY	NCOMP	STER	PEPT	SUBE	FATTY	M_n	Mw	Mw/Mn
CONTROL	23.3a	30.6b	2.4	4.3	2.7	1.1	4.6	1.8	0.1	2.6	0.0	0.0	175.7a	206.5a	1.2a
			(7.7)a	(14.0)a	(8.8)ab	(3.4)a	(15.0)a	(5.8)a	(0.2)a	(8.3) b	(0.0)	(0.1)a			
S+RCPW	21.1a	46.4a	3.6	6.1	3.9	1.5	7.1	2.6	0.1	3.6	0.0	0.0	178.5a	207.3a	1.2a
			(7.7)a	(13.1)b	(8.3)ab	(3.3)ab	(15.2)b	(5.7)b	(0.1)a	(7.8)ab	(0.0)	(0.0)a			
S+COM1-T	20.1a	53.3a	6.8	5.8	2.6	1.0	5.9	3.8	0.3	6.5	0.0	0.1	141.5b	194.2b	1.4b
			(12.8)b	(11.0)ab	(5.0)a	(1.9)a	(11.1)b	(7.1)c	(0.6)b	(12.2)c	(0.0)	(0.1)b			
S+COM1-M	22.1a	54.3a	4.2	7.2	4.4	1.6	8.1	3.2	0.1	4.5	0.0	0.0	171.6a	204.2a	1.2a
			(7.7)a	(13.2)b	(8.1)b	(3.0)b	(14.9)b	(6.0)bc	(0.2)a	(8.2)a	(0.0)	(0.1)ab			
S+COM1-F	25.5a	46.6a	3.9	6.4	3.4	1.3	7.2	2.7	0.1	4.0	0.0	0.0	174.4a	205.7a	1.2a
			(8.5)a	(13.8)b	(7.4)ab	(2.8)ab	(15.4)b	(5.8)ab	(0.2) a	(8.5)a	(0.0)	(0.1)ab			

For explanation of treatments see **Table 3.1**). Values followed by the same letters with in a column and column without letters were not significantly different at the 5% probability level.

The molecular weight averages M_n (142 g mol^{-1}) and M_w (194 g mol^{-1}) of S+COM1-T were significantly lower than those of the other treatments (M_n: 171-175 g mol^{-1}; M_w: 205-207 g mol^{-1}). This was due to the dominance of low molecular-weight organic compounds such as carbohydrates and N-compounds. However, a significantly higher M_w/M_n ratio (1.4) in treatment S+COM1-T indicated a higher polydispersity of the SOM in this treatment compared to the other organic amendments (1.2). This was due to the fact that the SOM in treatment S+COM1-T are complex mixtures of low molecular mass compounds (such as carbohydrates) to high molecular masses compounds (such as *n*-fatty acids and sterols) (**Figure 3.1, Table 3.3**).

The TII larger in the compost fertilized than in other treatments (**Table 3.3**) agreed with the C_{org} concentrations (**Table 3.2**). This confirms previous studies in which the TII from Py-FIMS also corresponded to the C_{org} concentrations of different agricultural soils (Schulten and Hempfling, 1992), organic waste materials (Franke *et al*., 2006) and compost-amended soils (Eshetu *et al*., 2012). The results provide evidence for an exogenous C input to these treatments from compost and microbial biomass (Leinweber and Reuter, 1992; Spaccini *et al*., 2009; Eshetu *et al*., 2012).

The signal patterns (**Figure 3.2**) and summed intensities of compound classes (**Table 3.3**) indicated the clearest impact of compost from the thermophilic phase on the SOM composition. The particularly high ion intensities of carbohydrates, peptides and heterocyclic N compounds are explained by their enrichment in the thermophilic phase of composting. Obviously, these compounds were not mineralized when the “thermophilic compost” is incubated with the Nitisol sample. This resistance of otherwise labile compounds (carbohydrates and peptides) can be explained by stabilization at reactive surfaces (Gregorich *et al*., 1996; Zech *et al*., 1997; Grandy and Neff, 2008; Bruun *et al*., 2010; Bustamante *et al*., 2010) of the clay-rich (84 % < 2 µm) Nitisol. The abundance of reactive clay surfaces is crucial, because both carbohydrates and N-compounds were completely decomposed in re-circulated leachates from organic waste in large-scale lysimeters that offered no contact with reactive surfaces (Franke *et al*., 2007). Unlike the high content of *n*-fatty acids and sterols in the raw coffee pulp waste (Negassa *et al*., 2011) signals of these compounds were detected only in treatment S+COM1-T. This can be explained by the decomposition of these compounds in the later stages of compositing. Smidt *et al*. (2005) also reported that fatty acids and sterols changed most during composting of yard and kitchen wastes studied by Py-FIMS and FT-IR spectroscopy.

The similar intensities of phenols/lignin monomers, lignin dimers, lipids and alkylaromatics among the compost-amended soils revealed that these compound classes were little affected by the degree of stabilization of composts and subsequent short-term incubation. However, their higher contents in amended soils compared to the control indicate enrichments of non-decomposable stable compounds by compost addition, irrespective of the compost maturation (Buurman *et al*., 2007; Stewart *et al*., 2011). For instance similarly large proportions of phenols/lignin monomers, lipids and alkylaromatics were also observed in both fresh and decomposed plant materials (Leinweber and Schulten, 1999), in a fresh coffee pulp waste (Negassa *et al*., 2011), and in long-term compost-fertilized soils (Eshetu *et al*., 2012). The small suberin proportions in all samples (**Table 3.3**) are explained by the origin of suberin from bark and outer covering of twigs (Hempfling *et al*., 1991) which were absent in these samples. The average molecular weights of the compost-amended soils agree with the results recorded for SOM accumulated after 19 and 34 years of a humus-formation experiment (Schulten *et al*., 1992), greater than values observed in soils under potato cultivation and crop rotation (Schulten and Hempfling, 1992) and below values from pig slurry fractions (Aust et al., 2009).

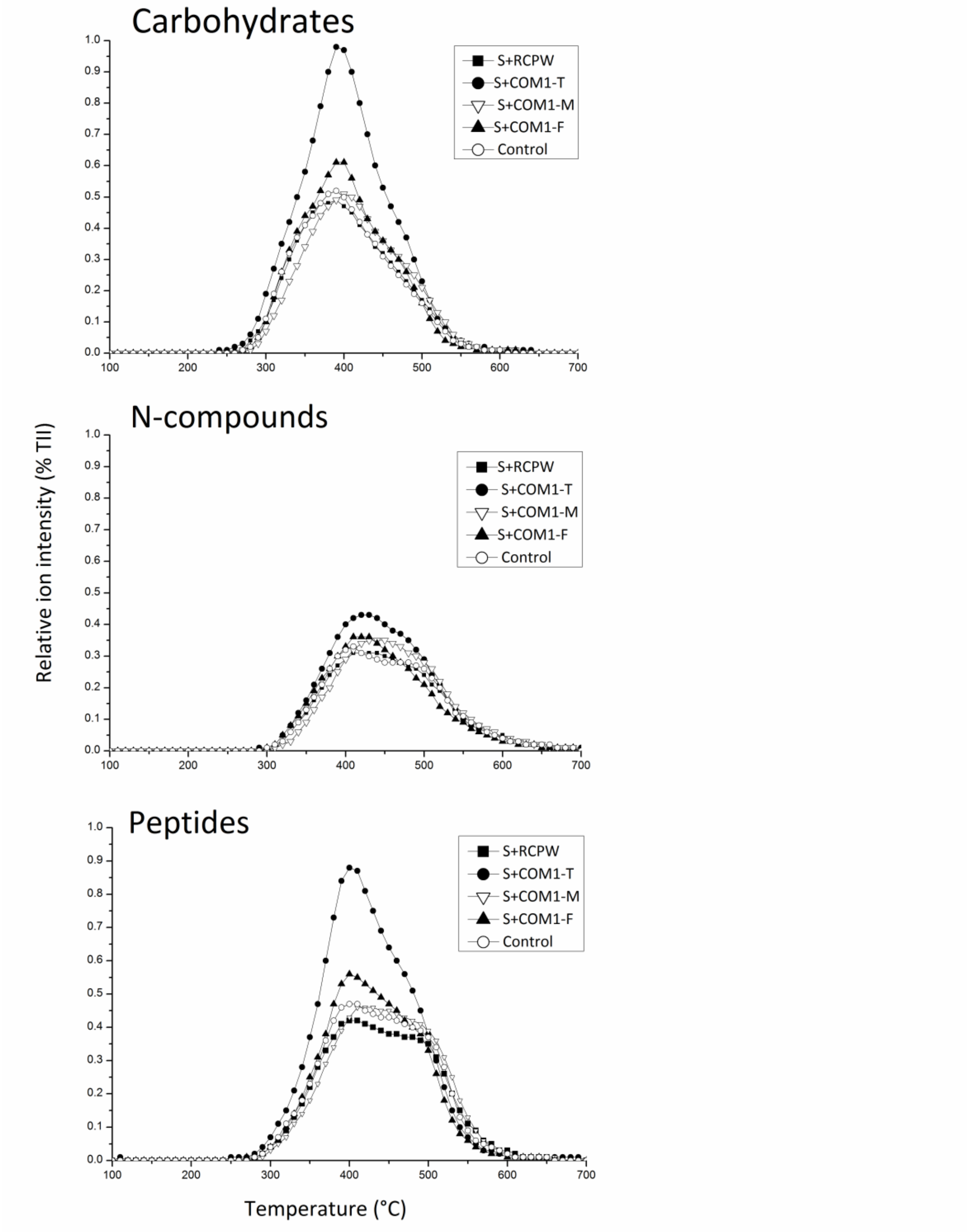

Figure 3.3 Thermograms for the evolution of carbohydrates, N-compounds, and peptides derived from the pyrolysis-field ionization mass spectra.

Figure 3.3 shows the temperature-resolved volatilization curves of those compound classes that were particularly enriched in the soil incubated with compost from the thermophilic phase. The maximum volatilization occurred at

390°C (carbohydrates), 420°C (N-compounds) and 410 °C (peptides). The thermograms indicate that compounds enriched in treatment S+COM1-T were volatilized over the whole temperature range. In other words, there is no enrichment in a particularly low or high temperature range. This was also true for the comparison of the other organic amendments and the control (**Figure 3.3**)

The thermograms for N-compounds and peptides in Figure 3.3 show some differences to the corresponding thermograms obtained for similarly treated compost from coffee pulp waste and fruit waste (Eshetu *et al.*, 2013). In the latter more complex compost the volatilization maxima were at 10 to 50 °C higher pyrolysis temperature, indicating that the organic parent materials affect the stability of the compost in soil even when it was removed from the composting in an early phase. The broad temperature range in which the enriched compound classes were thermally volatilized point to the presence of both, labile and rather stabile humified, organics which originate from microbes and the compost (Schulten and Hempfling, 1992; Stuczynski *et al*., 1997; Nierop *et al*., 2001; Kögel-Knabner, 2002). The lowest proportion of these fractions in S+RCPW provides evidence that non-altered plant materials scarcely contributed to the observed enrichment when the compost COM1-T was incubated with soil. More likely is the origin of these carbohydrates and protein from the microbial biomass such as fungi and bacteria (Zech *et al*., 1997) which possibly reach a maximum abundance in the thermophilic phase of composting. Furthermore, the described enrichment in carbohydrates and protein-derived compounds well agrees with findings from Lima *et al*. (2009) who reported larger proportions of these compound classes in compost-amended soils than in soil that received fresh manure. Their soil was dominated by the silt fraction (76 %) and compost effects were determined by ^{13}C NMR spectroscopy. Thus, the abundance of reactive surfaces from silt (Lima *et al.*, 2009) or clay (Nitisol of the present study) supports the stabilization of these labile organic compounds of microbial origin, making them non-decomposable in short-term incubation under optimized aerobic and moisture conditions. The observed lower proportions of carbohydrates, peptides and N-compounds in the soil amended with the raw coffee pulp waste or composts taken at later stages of composting can be explained by the decrease in microbial activity and microbial residues after the rapid consumption of easily degradable organic matter and high temperatures during the thermophilic phase (Dignac *et al*., 2005) and a progressive decrease of carbohydrate content during composting (Chefetz *et al*., 1996; Spaccini and Piccolo, 2007).

3.4 Conclusions

The Py-FIMS for the first time provided compelling evidence that the phase after which compost is applied to soil finally determines how stable it is in the soil. Furthermore, the results from this study suggests that it may be better to end up the composting of coffee pulp waste immediately after a short thermophilc phase and apply this "fresh" compost to soil rather than processing the compost through a mesophilic (cooling) and maturation phase of composting. Since the Nitisol used in this study was particularly rich in clay, binding of intermediate decomposition products and microbial metabolites to reactive surfaces or in clay aggregates is the best explanation for the enrichment of carbohydrates and various non-cyclic and cyclic N-containing compounds. However, our results based on small scale laboratory incubation experiment are restricted to a single soil type and under exclusion of factors such as temperature/moisture fluctuations, meso- and macrofauna or plants and other field heterogeneity in general, so that further research in the field or using field replicates as well as investigating different soil types is recommended.

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References

Adani, F., Genevini, P., Ricca, G., Tambone, F. and Montoneri, E. 2007. Modification of soil humic matter after 4 years of compost application. *Waste Manage.,* 27: 319–324.

Aust, M. –O., Thiele-Bruhn, S., Eckhardt, K –U. and Leinweber, P. 2009. Composition of organic matter in particle size fractionated pig slurry. *Bioresour Technol.,* 100: 5736-5743

Bernal, M. P., Sánchez-Monedero, M. A., Paredes, C. and Roig, A. 1998. Carbon mineralization from organic wastes at different composting stages during incubation with soil. *Agric. Ecosyst. Environ.,* 69: 175–189

Bolan, N. S., Kunhikrishnan, A., Choppala, G. K., Thangarajan, R. and Chung, J. W. 2012. Stabilization of carbon in composts and biochars in relation to carbon sequestration and soil fertility. *Sci. Total Environ.,* 1(424): 264-270

Bruun, T. B., Elberling, B. and Christensen, B. T. 2010. Lability of soil organic carbon in tropical soils with different clay minerals. *Soil Biol. Biochem*. 42: 888-895

Buurman, P., Peterse, F. and Martin, G. A. 2007. Soil organic matter chemistry in allophanic soils: a pyrolysis-GC/MS study of a Costa Rican Andosol catena. *Eur. J. Soil Sci.,* 58: 1330–1347

Bustamante, M. A., Said-Pullicino, D., Paredes, C., Cecilia, J. A. and Moral, R. 2010. Influences of winery–distillery waste compost stability and soil type on soil carbon dynamics in amended soils. *Waste Manage.*, 30(10): 1966-1975

Chefetz, B., Hatcher, P. G., Hadar, Y. and Chen, Y. 1996. Chemical and biological characterization of organic matter during composting of municipal solid waste. *J. Environ. Qual*., 25: 776–785

Dignac, M. F., Houot, S., Derenne, S., Francou, C. and Derenne, S. 2005. Pyrolytic study of compost and waste organic matter. *Org. Geochem,* 36: 1054–1071

Eshetu, B., Jandl, G. and Leinweber, P. 2012 Compost changed soil organic matter molecular composition: a study by Py-GC/MS and Py-FIMS. *Compost Sci. Util.*, 20: 230-238.

Eshetu, B., Baum, C. and Leinweber, P. 2013. Compost of different stability affects the molecular composition and mineralization of soil organic matter. Open Journal of Soil Science, 3(1): xxx-xxx.

Fernández, J. M., Hernández, D., Plaza, C. and Polo, A. 2007. Organic matter in degraded agricultural soil amended with composted and thermally-dried sewage sludge. *Sci. Total Environ.,* 378: 75–80.

Franke, M., Jandl, G. and Leinweber, P. 2006. Organic compounds in re-circulated leachates of aerobic biological treated municipal solid waste. *Biodegradation*, 17: 473–485.

Franke, M., Jandl, G. and Leinweber, P. 2007. Analytical pyrolysis of re-circulated leachates: Towards an improved municipal waste treatment. J. Anal. Appl. Pyrol., 79: 16–23.

Fortuna, A., Harwood, R. R., Kizilkaya, K. and Paul, E. A. 2003. Optimizing nutrient availability and potential carbon sequestration in an agro-ecosystem. *Soil Biol. Biochem.*, 35: 1005–1013.

Gezahegne, B., Fikre, L. and Mulatu, W. 2011. Exploring the suitability of coffee pulp compost as growth media substitute in greenhouse production. Int. J. Agr. Res., 6(3): 255-267

Gleixner, G., Poirier, N., Bol, R. and Balesdent, J. 2002. Molecular dynamics of organic matter in a cultivated soil. *Org. Geochem*., 33: 357–366

González-Vila, F. J., Almendros, G. and Madrid, F. 1999. Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil. *Sci. Total Environ*., 236: 215–229

Grandy, A. S. and Neff, J. C. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. *Sci. Total Environ.,* 404: 297-307

Gregorich, E. G., Monreal, C. M., Schnitzer, M. and Schulten, H. –R. 1996. Transformation of plant residues into SOM: chemical characterization of plant tissue, isolated soil fractions, and whole soils. *Soil Sci*., 161: 680–693.

Hempfling, R., Simmleit, N. and Schulten, H.–R. 1991. Characterization and chemodynamics of plant constituents during maturation, senescence and humus genesis in spruce ecosystems. *Biogeochemistry*, 13:27–60

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.,* 34: 139–162

Lattimer, R. P. and Schulten, H. –R. 1983. Field desorption of hydrocarbon polymers. *Int. J. Mass Spectrom. Ion Phys.*, 52: 105-116

Lal, R. 2002. The potential of soils of the tropics to sequester carbon and mitigate the greenhouse effect. *Adv. Agron.,* 74: 155– 192

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science*, 304: 1623–1627

Leinweber, P., Blumenstein, O., Schulten H.–R. 1996. Organic matter composition in sewage farm soils: Investigations by ^{l3}C-NMR and pyrolysis-field ionization mass spectrometry. *Eur. J. Soil Sci.,* 47: 71-80

Leinweber, P., Jandl, G., Eckhardt, K.-U, Schlichting, A., Hofmann, D. and Schulten, H.–R. 2009. Analytical pyrolysis and soft ionization mass spectrometry, in Senesi N, Xing B, Huang PM (eds.): Biophysico-chemical processes involving natural non-living organic matter in environmental systems. John Wiley & Sons, Inc New York, pp. 533–582

Leinweber, P. and Schulten, H –R. 1993. Dynamics of soil organic matter studied by pyrolysis-field ionization mass spectrometry. *J. Anal. Appl. Pyrol*., 25: 123-136

Leinweber, P. and Reuter, G. 1992. The influence of different organic fertilization practices on concentrations of organic carbon and total nitrogen in particle-size fractions during 34 years of a soil formation experiment in loamy marl. *Biol. Fertil. Soils,* 13: 119–124

Leinweber, P. and Schulten, H.–R. 1999. Advances in analytical pyrolysis of soil organic matter. *J. Anal. Appl. Pyrol*., 49: 359-383

Lima, G., Diana, L. D., Santos, M. S., Scherer, W. H., Schneider, J. R., Duarte, C. A., Santos, B. H. and Esteves, I. V. 2009. Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, 150: 38-45

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. *Eur. J. Soil Sci*., 53: 299–309

Mondini, C., Cayuela, M. L., Sinicco, T., Cordaro, F., Roig, A. and Sánchez-Monedero, M. A. 2007. Greenhouse gas emissions and carbon sink capacity of amended soils evaluated under laboratory conditions. *Soil Biol. Biochem*., 39: 1366-1374

Nardi, S., Morari, F., Berti, A., Tosoni, M. and Giardini, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilizers. *Eur. J. Agron*. 21: 357–367

Negassa, W., Baum, C. and Leinweber, P. 2011. Soil amendment with agro-industrial products: Molecular-chemical compositions and effects on soil biochemical activities and phosphrous fractions. *J. Plant Nutr. Soil Sci*. 174: 113-120

Nierop, K. G. J., Pulleman, M. M. and Marinissen, J. C. Y. 2001. Management-induced OM differentiation in grassland arable soil: a study using pyrolysis techniques. *Soil Biol. Biochem*., 33: 755–764

Paul, E. A. and Clark, F. E. 1989. Soil microbiology and biochemistry.Academic press, San Diego, California, USA.

Piccolo, A., Spaccini, R., Nieder, R. and Richter, J. 2004. Sequestration of a biologically labile organic Carbon in soils by humified organic matter. Clim. Change, 67: 329-343

Rivero, C., Chirenje, T., Ma, L. Q. and Martinez, G. 2004. Influence of compost on soil organic matter quality under tropical conditions. *Geoderma*, 23: 355–361

Sánchez-Monedero, M. A, Cayuela, M. L, Mondini, C., Serramia, N. and Roig, A. 2008 Potential of olive mill wastes for soil carbon sequestration. *Waste Manage*., 28: 767-773

Sánchez-Monedero, M. A, Serramia, N., Civantos, C. G. O., Fernandez-Hernandez, A. and Roig A. 2010. Greenhouse gas emissions during composting of two-phase olive mill wastes with different agro-industrial by-products. *Chemosphere*, 81: 18-25

Schulten, H.–R. and Hempfling, R. 1992. Influence of agricultural soil management on humus composition and dynamics: Classical and modern analytical techniques. *Plant Soil*, 142: 259-271

Schulten, H.-R, Leinweber, P. and Reuter, G. 1992. Initial formation of soil organic matter from grass residues in a long-term experiment. *Biol. Fertil. Soils*, 14: 237-245

Smidt, E., Eckhardt, K.–U., Lechner, P., Schulten H–R. and Leinweber, P. 2005. Characterization of different decomposition stages of biowaste using FT-IR spectroscopy and pyrolysis-fieldionization mass spectrometry. *Biodegradation,* 16: 67–79

Spaccini, R., Sannino, D., Piccolo, A. and Fagnano M. 2009. Molecular changes in organic matter of a compost amended soil. *Eur. J. Soil Sci*., 60: 287–296

Spaccini, R. and Piccolo, A. 2007. Molecular characterisation of compost at increasing stages of maturity. II: Thermochemolysis-GC-MS and 13C-CPMAS-NMR spectroscopy. *J. Agr. Food Chem*., 55: 2303–2311.

Stewart, C. E, Neff, J. C., Amatangelo, K. L. and Vitousek, P. M. 2011. Vegetation effects on soil organic matter chemistry of aggregate fractions in a Hawaiian Forest. *Ecosystems*, 14: 382–397

Stuczynski, T. I., McCarty, G. W., Reeves, J. B. and Wright, R. J. 1997. Use of pyrolysis GC/MS for assessing changes in soil organic matter quality. *Soil Sci*., 162: 97-105

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Licznar, S., Jamroz, E. and Kocowicz, A. 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil. Biol. Biochem*., 39: 1294–1302

Zech, W., Senesi, N., Guggenberger, G., Kaiser, K., Lehmann, J., Miano, T. M., Miltner, A. and Schroth, G. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma*, 79: 117–161.

Zucconi, F., Monaco, A. and Forte, M. 1985 Phytotoxins during the stabilization of organic matter. In: Gasser JKR. (Editor), Composting of Agricultural and other Wastes, Elsevier Applied Science Publication, New York NY USA, pp. 73-86.

4 Compost Changed Soil Organic Matter Molecular Composition: A Study by Py-GC/MS and Py-FIMS

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Abstract

Changes in the molecular composition of soil organic matter (SOM) resulting from compost application are not sufficiently known at the molecular scale even though this is major issue for soil fertility and soil carbon sequestration. Therefore, the present study investigated effects of long-term compost application in comparison to mineral fertilizer on the molecular composition of SOM in a 34-year-old experiment. Soil samples were taken after 19 and 34 years of constant management and analyzed by Curie point Pyrolysis-Gas Chromatography/Mass Spectrometry (Cp Py-GC/MS) and Pyrolysis-Field Ionization Mass Spectrometry (Py-FIMS). In general, compost application increased the organic carbon (C) content. The Cp Py-GC/MS revealed larger relative intensities of alkylphenols/lignin monomers at the expense of carbohydrates in the compost treatments. Py-FIMS indicated higher proportions of labile *n*-fatty acids, lipids and sterols in the compost than in the mineral fertilizer treatment. Permanent cropping of grass between years 19 and 34 revealed similar signal patterns, which is also maintained after conversion of soil from permanent grass to arable use. Thermograms of volatilization indicated enrichments of stable (compounds volatilized in between 370 and 570°C) phenols/lignin monomers, lipids and alkylaromatics between years 19 and 34 in compost fertilized soils. This was a result of enhanced losses of compounds that are considered easily metabolized by microorganisms (e.g. carbohydrates) after compost addition as derived from Py-GC/MS and Py-FIMS. In summary, long-term application of mature compost was shown to have a positive, long lasting effect on the organic carbon sequestration in agricultural soils.

4.1 Introduction

Soil organic matter (SOM) in agricultural soil was formed over time periods from different vegetation covers and the corresponding decomposer populations. Moreover it is also a result of anthropogenic inputs such as manure (Schulten and Leinweber 1991), sewage sludge (Leinweber *et al*. 1996), and compost (Leinweber and Reuter 1992; Lal 2004). The addition of compost to soils built-up SOM over time (Leinweber and Reuter 1992; Gregorich *et al*. 1996; Ceccanti *et al*. 2007), and modified the chemical, physical and biological properties of SOM (Quedraogo *et al*. 2001; Nardi *et al*. 2004; Adani *et al*. 2007; Lima *et al*. 2009). Recently, changes in SOM quality resulting from compost addition have gained importance as a major issue for soil quality and soil carbon (C) sequestration (Bustamante *et al*. 2010). However, long term effects of compost application on the molecular composition and soil C sequestration, that is stability of SOM, are not sufficiently known.

The addition of compost to soil reduced the mineralization of labile compounds of SOM (Fortuna *et al*. 2003; Piccolo *et al*. 2004), enhanced the activity, size and composition of soil microbial communities (Saison *et al*. 2006), and increased the humic acid (HA) over the fulvic acid (FA) proportions (Rivero *et al*. 2004). The latter results were confirmed by Weber *et al*. (2007) who also reported a relative increase in HA relative to FA with time in soils amended with urban compost. However, most of these results provided an overall chemical characterization of SOM rather than detailed information at the molecular level.

The molecular-chemical characterization of heterogeneous mixtures can be done by analytical pyrolysis and/or chemolytic degradation of the macromolecule into small fragments (Leinweber and Schulten 1999). Various analytical pyrolysis techniques such as Curie Point pyrolysis combined with electron impact (EI) ionization-gas chromatography mass spectrometry (Cp Py-GC/MS) were used to characterize organic matter (OM) in compost (Dignac *et al*.

2004; González-Vila *et al*. 1999), organically fertilized agricultural soils (Nierop *et al*. 2001; Marinari *et al*. 2007), and plant materials (Stewart *et al*. 2011). Pyrolysis–field ionization mass spectrometry (Py-FIMS) was used for the investigation of OM composition in sewage farm soils (Leinweber *et al*. 1996), and in agro-industrial byproducts (Negassa *et al*. 2011). Py-GC/MS and Py-FIMS have been also successfully applied in conjunction to evaluate forest soils (Hempfling and Schulten 1990), amino acids in humic substances (Sorge *et al*. 1993), and changes of OM composition in re-circulated leachates in waste piles (Franke *et al*. 2006 & 2007). Nevertheless, to the best of our knowledge up to now these complementary mass spectrometric methods were not applied in conjunction to investigate compost effects in the molecular composition and stability of SOM.

Therefore, the objectives of the study were:

(i) to characterize the molecular composition of the SOM in a well-defined experimental soil fertilized with compost and mineral fertilizer using two independent mass-spectrometric methods,

(ii) to investigate how sustainable the compost fertilization is in the C sequestration if land use is converted from continuous grass grown more intensively aerated arable.

4.2 Materials and Methods

Soil samples from the Rostock long term (34 years) pot-experiment on soil formation in practically humus free Pleistocene loamy marl (about 20% clay, 42% silt, 3% $CaCO_3$; 0.22% OM) were used. Experimental conditions, principal results concerning the development of SOM and other physico-chemical soil properties (Reuter and Kahle 1992), organic-mineral size-fractions (Leinweber and Reuter 1992) and of SOM molecular subunits (Schulten *et al*. 1992) have been reported.

In the present study we investigated soil samples from the 19th and 34th years under permanent grass grown, and converted to arable, and fertilized with mineral fertilizer and compost. The compost was prepared according to the Indore composting method at the experimental station of the Agricultural Faculty, Rostock University, during the month June to September. Feedstock materials were sewage sludge (27 % w/w), foliage (11 % w/w), green plant material (19 % w/w), garden trimmings (e.g. *Ligustrum* spp.) (25 % w/w), soil (13 % w/w), and municipal waste water (5 % w/w) (all dry on weight basis). Foliage, green plant material and garden trimmings made the basis, which subsequently was moistened by sprinkling waste water, followed by a sewage sludge layer. This layering was repeated until the heap was about 1.5 m high, and finally covered by soil. The pile was turned after 4 and 8 weeks. Temperature was considered as a major indicator how well the composting process was progressing. The temperature maximum during the thermophilic phase was 63^{o}C (which lasts for about a six weeks) followed by a cooling phase (<40 ^{o}C) for about seven weeks. The composting was considered to be finished when the temperature remained stable and near ambient (17^{o}C) after about 12 weeks. The final compost had lost 24% (w/w) of the sum of the feedstock materials (42 % w/w on organic matter basis), and the dry matter consisted of 61 % ash and 39 % organic matter with a C/N-ratio 19, and 47% of total C in bound in humic substances. The compost and mineral fertilizer were applied in the experimental period year 1 to 19 in two-year-intervals. More details on the compost and mineral fertilization, as well as sample codes are compiled in Table 1. The six samples were air-dried, gently milled (< 0.2 mm) and sieved to be used for analytical pyrolysis. Total organic carbon (C_{org}) content was determined by dry combustion (Wösthoff, Bochum, Germany and subtraction of $CaCO_3$-C, total nitrogen (N_t) content was determined using CNS analyzer (Vario EL III; Elementar Analysensysteme, Hanau, Germany) (**Table 4.1**).

For Cp Py-GC/MS approximately 15 mg of the soil sample were pyrolyzed at 500°C in a Curie-Point Pyrolyzer Fischer1040 PSC. Pyrolysis products were separated using a gas chromatograph Varian 3800 (Varian USA)- equipped with a 25m capillary column BPX 5 (SGE, Australia) that was coated with 0.25µm film thickness and had an inner diameter of 0.32 mm. The injector temperature was adjusted at 300°C. The carrier gas helium 5.0 was set up with a constant flow of 1 ml min^{-1}. Following split injection up to 45 s (splitless), the split ratio was

1:100 from 45 up to 90 s and 1:5 from 90 s on. The temperature program of the GC oven was set with the starting temperature for the gas chromatographic program at 28°C (5 min), and heated at a rate of 5°C min^{-1} to 280°C (40 min). The GC was connected to a double- focusing MAT 95 mass spectrometer (MS) (Finnigan, Bremen, Germany). Conditions for mass spectrometric detection in the electron impact mode were 4.7 kV acceleration voltage, 70 eV electron energy, 1.2 kV multiplier voltage, and *m/z* 48 to 600 mass range, 0.3 s (mass decade) scan rate and 0.6 s interscan time. Peaks were assigned by comparing of their mass spectra with Wiley mass spectral library, software edition 6.0. The relative composition of the OM is based on the proportions of the peak areas of the compound classes to the total peak area of the chromatograms.

Table 4.1 Sample code, input and management practices and organic carbon (C_{org}), total nitrogen (N_t) content and C/N ratio of whole soil samples taken at 19th and 34th year of the experiment.

Sample code	Input and management	C_{org} (g kg^{-1})	N_t (g kg^{-1})	C/N
MIN19-grass	Continuous mineral fertilization using nutrient solution (KNO_3 +KH_2PO_4) (years 1-19) and continuously grown with the grass species *Phleum pratense* (years 1 - 11) and *Lolium multiflorum* lain. vat. westerwoldicum (years 12 - 19).	10	1	10.0
COM19-grass	Compost input of total amount 403 g organic dry matter (ODM) per pot (yearly dose of 6.75 t ha^{-1}.) (Years 1 - 19) and continuously grown with the grass species *Phleum pratense* (years 1-11) and *Lolium multiflorum* lain. vat. westerwoldicum (years 12 - 19).	27	2	13.5
MIN34-grass	Continuous mineral fertilization using nutrient solution (KNO_3 +KH_2PO_4) (years 1 - 34) and continuously grown with the grass species *Phleum pratense* (years 1 - 11) and *Lolium multiflorum* lain. vat. westerwoldicum (years 12 - 34).	9	1	9.0
COM34-grass	Compost of total amount 403 g ODM per pot (years 1-19), No more compost input (years 20-34 year) and continuously grown with the grass species *Phleum pratense* (years 1 - 11) and *Lolium multiflorum lain. vat. westerwoldicum* (years 12 - 34)	25	2	12.5
MIN34-arable	Mineral fertilization (KNO_3 +KH_2PO_4) and continuously grown with the grass species(years 1 - 19) and two-year rotation: potato – barely (years20 - 34)	16	1	16.0

COM34-arable	Compost of total amount 403 g ODM per pot (years 1 - 19) and continuously grown with the grass species (1-19) and two-year rotation: potato–barely (20 - 34)	12	2	6.0

For Py-FIMS, about 3 mg of the air dry, ground and homogenized whole soil samples 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 analysis.

Statistical analyses such as means and standard deviations were calculated for the results of Py-FIMS and pair means were compared among treatments by applying the two-way t-test. Differences between the means were considered to be statistically significant at $p \leq 0.05$. All statistics were computed using data analysis and graphic software (Origin 8.1G).

4.3 Results and Discussion

Evaluation of the Cp Py-GC/MS data in **Table 4.2** revealed that the proportion of total chromatogram area assigned to five compound classes reflected the C_{org} content (**Table 4.1**). For instance, the lowest percentage in MIN34-grass (53%) was obtained in the treatment containing least C_{org} (9 g kg^{-1}) and the highest percentage in COM19-grass (62%) agreed with the highest C_{org} content (27 g kg^{-1}).

After the experimental period with organic amendments COM19-grass had more phenols/lignin monomers (26 %) but less carbohydrates (17 %) than MIN19-grass (**Table 4.2**).The proportions of lipids, alkylaromatics and N-compounds were the same in both treatments. The high proportions of phenols/ lignin monomers 26% in COM19-grass decreased to 21% in COM34-grass along with a slight increase in proportions of N-containing compounds, whereas the other compound classes remained unchanged. The same was true for the comparison MIN34-grass vs. MIN34-arable and COM34-grass vs. COM34-arable (alkylaromatics, and lipids). The sample COM34-arable had slightly more carbohydrates and phenols/lignin monomers at the expense of N-compounds than COM34-grass.

Table 4.2 Relative abundance (%) of compound classes in whole soil samples of different experimental years (19 and 34 years) and under different management practices (fertilized with compost and mineral fertilizer) as assigned by Curie point pyrolysis-gas chromatography/mass spectrometry.

Sample	CARB	PHLM	LIPID	NCOMP	ALKY	Sum of assigned compounds	Not assigned
MIN19-grass	21	13	5	8	9	56	44
COM19-grass	17	26	4	8	7	62	39
MIN34-grass	24	12	3	8	6	53	47
COM34-grass	17	21	4	11	5	58	42
MIN34-arable	26	16	3	9	6	60	40
COM34-arable	20	23	3	8	6	60	40

CARB: carbohydrates, PHLM: phenols/Lignin monomers, LIPID: lipids, NCOMP: N-compounds, and ALKY: alkylaromatics**.**

The compound classes assigned, in general, were similar with compounds obtained from compost (Marinari *et al*. 2007), plant litter in forest ecosystems (Stewart et al., 2011), manure (Leinweber and Reuter 1992), waste pile leachates (Frank *et al*. 2006), aquatic humic substances (Gonzalez *et al*. 2001) and differently managed soils (Nierop *et al*. 2001; Lorenz *et al*. 2006) as determined by FT-IR, semi-direct FT-IR, ^{13}C-NMR, Cp Py-GC/MS and Py-FIMS. The high proportions of phenols/lignin monomers at the expense of carbohydrates in all the treatments with compost confirmed studies that showed more phenols and lignin building blocks than carbohydrates in compost (Ceccanti *et al*. 2007) and slowly decomposing matter (Stewart *et al*. 2011). This indicates that these two compound classes rather than others can be used as early indicators of compost effects on SOM quality. The similarity of COM19-grass and COM34-grass in the proportions of alkylaromatics and lipids points to a long-term effect of compost, lasting at least for 15 years after the application was stopped. Furthermore, the constant proportions of these compound classes even in a more oxidative environment such as COM34-arable suggest that these compound classes were stable against microbial decomposition.

The decrease in the proportions of phenols/lignin monomers and subsequent increase of N-containing compounds from COM19-grass to COM34-grass agreed with the same trend over time observed for lignin derived phenols and N-containing compounds in field soils under organic-arable management and permanent grass (Nierop *et al*. 2001) and those from forest ecosystems (Stewart *et al*. 2011). This suggests that upon lignin degradation N-containing compounds are formed, most likely by enhanced microbial communities as reported as well for compost applications by (Chefetz *et al*. 2002 ; Bustamante *et al*. 2010).

In the Py-FIMS the total ion intensity (TII)-thermograms show two peaks for all six samples (**Figure 4.1**). The thermal evolution of molecules reached maximal intensities in the lower temperature range at 380 to 390 ^{o}C and the higher temperature range at 460 to 470 ^{o}C. The integrals of TII-thermograms and the amounts of TII (**Table 4.3**) confirm larger ion intensities in the treatments with compost than those receiving mineral fertilizer following the order COM19-grass (44.2) > MIN19-grass (10.2), and COM34-grass (60.4) > COM34-arable (54.1) > MIN34-arable (22.6) > MIN34-grass (18.0). This is well agreed with the decrease in concentrations of C_{org} (**Table 4.1**).

The comparison of TII-thermograms in the treatments COM19-grass and COM34-grass showed a buildup of thermally stable (volatilized >400 ^{o}C pyrolysis temperature) at the expense of labile compounds (volatilized <400 ^{o}C pyrolysis temperature). This is indicated by a transformation of major peak appeared at 380 ^{o}C in COM19-grass to 460 ^{o}C in COM34-grass (**Figures. 4.1b & 1d**).

However, this change in the TII-thermograms was not observed in the mineral fertilized treatments (**Figures. 4.1a & 1c**).

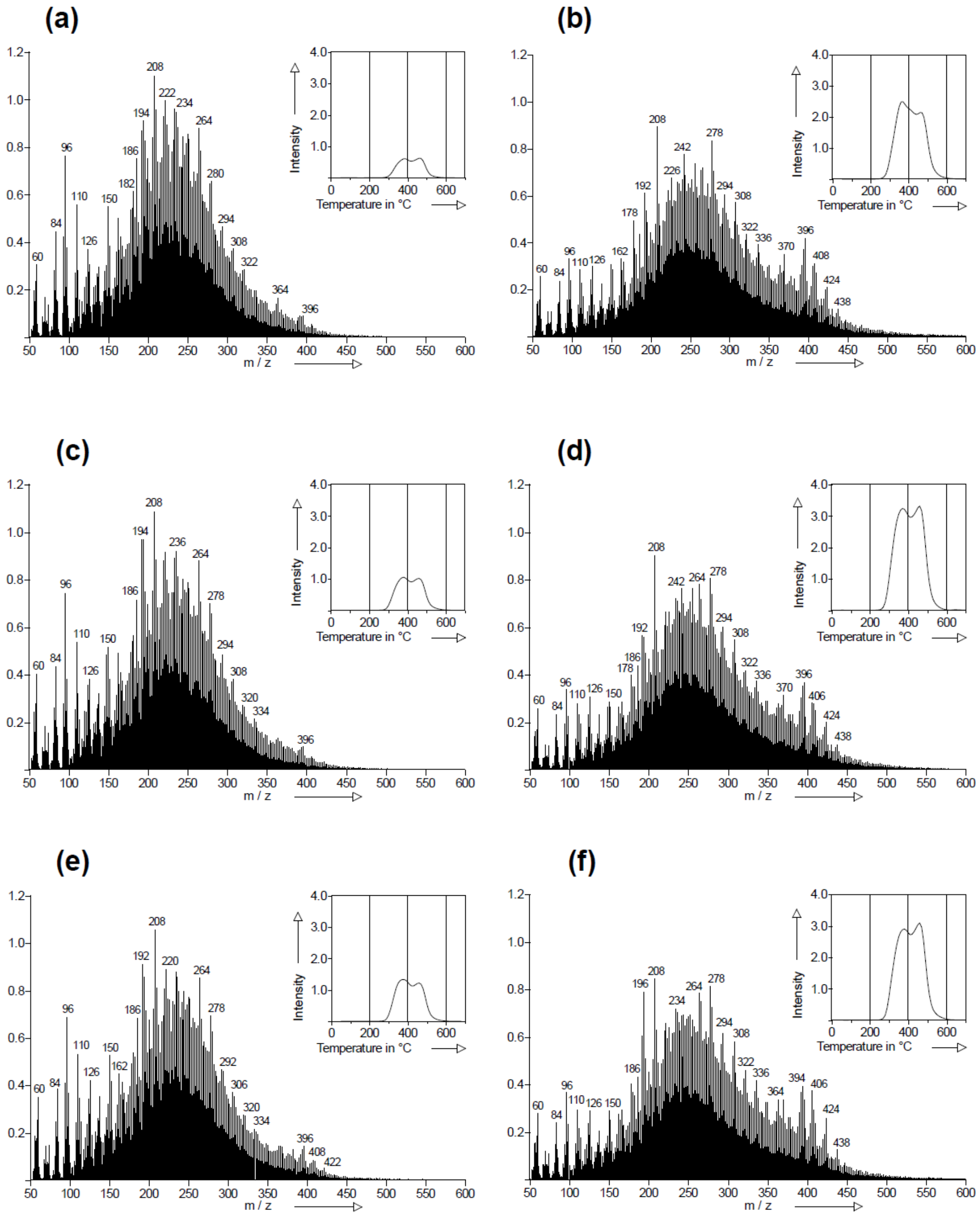

Figure 4.1 Summed and averaged pyrolysis-field ionization mass spectra and thermograms of total ion intensity (TII) (upper right inserts) of whole soil samples taken in the 19th experimental year under continuous grass, fertilized with a) mineral fertilizer, b) compost, and taken in the 34th experimental year under continuous grass fertilized with c) mineral fertilizer and d) compost, and under arable use fertilized with e) mineral fertilizer and f) compost.

In the Py-FI mass spectra, signals of phenols/lignin monomers appearing at *m/z* 178 (coniferyl aldehyde) and *m/z* 196 (syringyl acetaldehyde) were detected only in COM34-grass and COM34-arable. Signals of lipids (*m/z* 394, 396), *n*-fatty acids (*m/z* 396, 424,438) and sterols (*m/z* 396, 408) were also dominated in the compost treatments. However, intense signals at *m/z* 60, 84, 96, 110, 126 indicating carbohydrates and signals indicating phenols/lignin monomers (*m/z* 150,182, 208), and alkylaromatics (*m/z* 192) were more intense in the mineral than the compost fertilized treatments. Signals indicative of lignin dimers (*m/z* 270, 272, 284, and 298) were equal in all treatments.

Comparison of ion intensities of compound classes among treatments COM19-grass and MIN19-grass in **Table 4.3** shows larger intensities of all compound classes in compost than in mineral fertilized soils with the largest differences for lipids (4.4×10^6 counts mg^{-1}), alkylaromatics (3.2×10^6 counts mg^{-1}) and phenols/lignin monomers (2.0×10^6 counts mg^{-1}). The higher ion intensity of all compound classes in the treatments fertilized with compost agreed with the corresponding C_{org} concentrations (**Table 4.1**).

The TII-proportions of compound classes (values in parenthesis of Table 3) shows significantly larger proportions of *n*-fatty acids (*n*-C_{16} to C_{34}), lipids, sterols and suberin in the compost than in the mineral fertilized treatment ($P \leq 0.05$). In the Py-FI mass spectra, these were clearly reflected by patterns of signal intensities in the higher mass range (*m/z* 350-500).

Comparison of the Py-FI mass spectra of COM19-grass (**Figure 4.1b**) and COM34-grass (**Figure 4.1d**) shows similar signal patterns as also confirmed by the relative signal intensities of all compound classes. Moreover, transformation from continuous grass grown to arable use in the previous compost treatments did not change the SOM composition after 15 years. This is obvious when comparing signal intensities (**Figure 4.1**) and relative ion intensities (%) for the compound classes (**Table 4.3**) between COM34-arable with COM 34-grass.

Table 4.3 Total ion intensity (TII), ion intensity (I0^6 counts mg^{-1}) and relative abundance (% TII) of principal classes of compounds of whole soil samples taken at experimental years 19 and 34 under different management practices as assigned by pyrolysis-field ionization mass spectrometry.

Sample	TII	CARB	PHLM	LIPID	NCOMP	ALKY	LDIM	STER	PEPT	SUBE	FATTY
MIN19-grass	10.2	0.5 (4.4)	1.1 (10.8)	0.9 (9.0)	0.7 (6.4)	1.4 (13.4)	0.6 (5.7)	0.1 (0.9)	0.2 (1.9)	0.0 (0.0)	0.1 (0.6)
COM19-grass	44.2	1.2 (2.8)	3.1 (7.0)	5.3 (12.1)	2.3 (5.3)	4.6 (10.3)	2.4 (5.4)	1.7 (3.8)	0.6 (1.3)	0.1 (0.3)	1.3 (2.9)
MIN34-grass	18.0	0.8 (4.6)	1.9 (10.3)	1.6 (9.0)	1.1 (6.3)	2.3 (13.1)	1.0 (5.4)	0.2 (1.0)	0.4 (2.0)	0.0 (0.0)	0.2 (0.8)
COM34-grass	60.4	1.7 (2.8)	4.2 (6.9)	7.0 (11.6)	3.1 (5.2)	6.4 (10.6)	3.1 (5.2)	2.1 (3.5)	0.8 (1.3)	0.2 (0.3)	1.8 (3.0)
MIN34-arable	22.6	1.0 (4.5)	2.3 (10.2)	2.1 (9.2)	1.5 (6.4)	2.8 (12.5)	1.1 (5.0)	0.3 (1.2)	0.4 (1.8)	0.0 (0.1)	0.3 (1.2)
COM34-arable	54.1	1.5 (2.8)	3.9 (7.1)	6.2 (11.5)	2.8 (5.2)	5.8 (10.7)	3.0 (5.6)	2.0 (3.7)	0.6 (1.3)	0.1 (0.3)	1.3 (2.5)

CARB: carbohydrates, PHLM: phenols/lignin monomers, LIPID: lipids, NCOMP: N-compounds, ALKY: alkylaromatics, LDIM**:** lignin dimers, STER**:** sterols, PEPT**:** peptides, SUBE**:** suberin, and FATTY**:** *n*-fatty acids (*n*-C_{16} to *n*-C_{34})

The larger TII in the compost fertilized treatments might be due to an input from compost, re-synthesized microbial derived stable compounds, increased stabilization through mineral-OM association due to high amount of OM in the compost fertilized treatment, and microbial residues by degrading the labile OMs (Leinweber and Schulten 1993; Six *et al*. 2002; Dignac *et al*. 2004).

Some compound classes were more abundant in the mineral than in the compost treatments (carbohydrates, phenols/lignin monomers, and alkylaromatics) (**Table 4.3**). One explanation could be a larger input of these compounds from grass. Nierop *et al.* (2001) also reported that mineral fertilizer application yielded more grass dry matter than different organic amendments. Another explanation may be an accelerated decomposition of the most labile proportions of these compounds upon addition of compost (Schulten and Hempfling 1992; Gleixner *et al*. 2002; Bustamante *et al*. 2010).

Higher proportions of lipids, sterols, suberin and *n*-fatty acids in compost fertilized soils (**Table 4.3**) agree with the same changes found in soils fertilized with farmyard manure plus mineral fertilizer (Leinweber and Schulten 1993), extracts of municipal waste composts (Keeling *et al*. 1994,), time series of forest soils (Hempfling and Schulten 1988), re-circulated waste leachates (Franke *et al*. 2006) and in soils amended with agro-industrial wastes (Negassa *et al*. 2011). These relative increases can be explained by a reduced decomposition, additional input from compost, and re-synthesis by enhanced activity of microorganisms (Schulten. 1992; Stewart *et al*. 2011).

The similar signal patterns in the Py-FI mass spectra (**Figure 4.1**) as also confirmed by insignificant differences in the relative intensity of compound classes (**Table 4.3**) between COM19-grass and COM34-grass indicated that stabilizing effect of compost on SOM has been continued long after its final application year. This stabilizing effect was also observed after permanent grass cropping was converted to arable use. This suggested compost application enhances long-term SOM stability even in a more degradative environment such as disturbance of soil aggregation and aeration.

For a more detailed analysis of compost effects on the stabilization of compounds, thermal volatilization curves were computed for individual compound classes (**Figure 4.2**). The curves representing lipids, alkylaromatics, and phenols/ lignin monomers show two maxima at about 340-360 ^{o}C and 460-470^{o}C (**Figure 4.2**). Lignin dimers and fatty acids have one single maximum of ion intensity. The volatilization curve for sterols is broad with a maximum and a lower intensity shoulder.

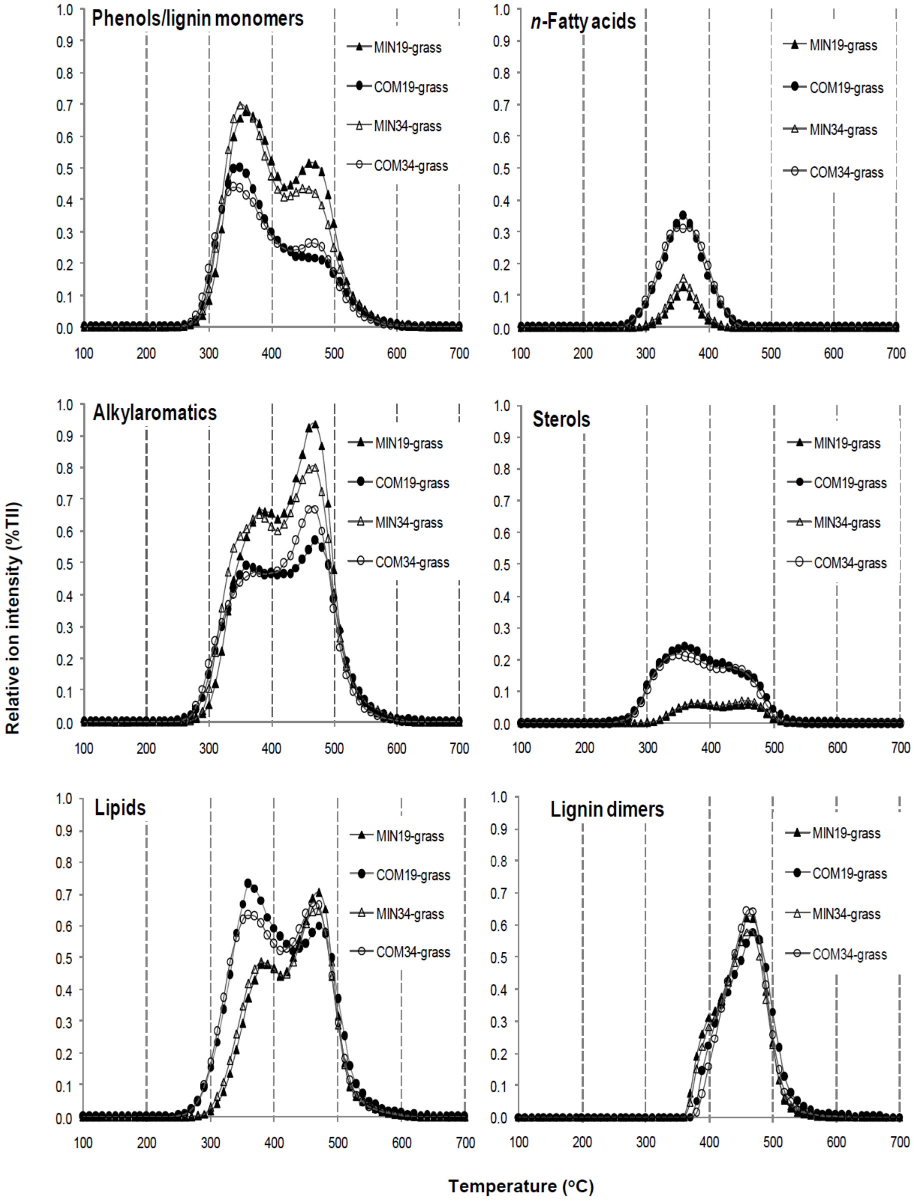

Figure 4.2 Thermograms for the evolution of important soil organic matter compound classes from compost and mineral fertilized samples collected in the 19th and 34th year of the experiment.

The thermal volatilization curves for sterols, lipids and *n*-fatty acids are larger in the compost fertilized treatments than mineral fertilized treatments in agreement with their relative abundances (%) in **Table 4.3**. Moreover, slight intensity increases for lipids, phenols/lignin monomers, and alkylaromatics at 420 to 480 °C indicate enrichments of a relative stable proportion of these compound classes in compost fertilized soil from 19th to the 34th experimental year. Clear increases for lipids volatilized between 270 and 420 °C indicate that compost enriched a relatively labile fraction of this compound class (**Figure 4.2**).

The two maxima for the volatilization of lipids, alkylaromatics, N-containing compounds, and phenols/lignin monomers indicate that these compound classes consisted of compounds with different stability (Leinweber and Schulten 1993). The broader curve shape for sterols might be due to heterogeneity of chemical linkages and physical interaction (Hempfling and Schulten 1988). The similar intensity and thermal volatilization at high temperature of lignin dimers irrespective of fertilization treatments indicates these compounds were stabilized already in an early period of the experiment.

The enrichments of phenols/lignin monomers, alkylaromatics and lipids with thermally stable compounds from compost application may have resulted in polymerization of low molecular weight labile into more stable compounds (Krull *et al*. 2003; de Leeuw *et al*. 2006) or enhanced organic-mineral interactions (Leinweber and Schulten 1993). Furthermore, increased microbial activity after compost application might lead to a transformation of originally labile compounds into the more refractory fractions of the SOM such as aromatics, phenolic rings and alkyl structures (Chefetz *et al*. 2002; Kiem and Kögel-Knabner 2003).

Compilation of findings from the two pyrolysis-MS methods can be summarized as follows. Compost application increased the C_{org} which was reflected by bulk ion intensity data such as sum of assigned compound classes in Py-GC/MS and TII in Py-FIMS, and it also altered the composition of SOM. Both methods indicated enrichments in lipids (more pronounced in Py-FIMS, **Table 4.3**) at the expense of carbohydrates in the compost fertilized compared to the mineral fertilized treatments. This is explained by an accelerated decomposition of carbohydrates due to stimulation of microbial activity by compost, in agreement with Franke *et al*. (2006) who reported a continuous decomposition of carbohydrates during repeated circulation of waste leachates along with increased proportions of lipids. Furthermore, the two methods agreed in showing constant proportion of almost all compounds classes in the compost fertilized soils sampled in the 19th and 34th year under grass as well as under arable use (**Tables 4.2 and 4.3**). This clearly indicates a long lasting stabilizing effect of compost on SOM, irrespective of the two contrasting soil managements. However, results from the two mass spectrometric techniques disagreed in the effect of compost on lignin building blocks. While Py-GC/MS indicated enrichment in the compost compared with mineral fertilized treatment, the opposite was indicated by Py-FIMS. A few papers indicated that compost application enriched lignin building blocks in soil (Leifeld *et al*. 2002; Ceccanti *et al*. 2007). Whereas our data confirmed Ceccanti *et al*. (2007), because they also used Py-GC, direct comparison to Leifeld *et al*. (2002) is more difficult because they reported increases in lignin building blocks following application of unmatured compost but pronounced decreases if mature compost was applied. The compost in the present study was mature as it was taken when the temperature remained stable and near ambient (17^{o}C) and it meets the requirement that C/N ratio should be less than 25 so that the Py-FIMS data confirm partially the results by Leifeld *et al*. (2002). On the other hand Py-FIMS enabled detecting higher molecular weight pyrolysis products, and thus, enrichments of the compost treatments in sterols, *n*-fatty acids and suberin which were hardly or not identified by Py-GC/MS. In other words, Py-GC/MS discriminated those high-molecular compounds which were particularly enriched by compost (Table 3) as also reported by Gonzalez-Vila *et al*. (1999). Thus, the preferential detection of low molecular-weight lignin building blocks by Py-GC/MS along with a smaller sum of total assigned compound classes may explain the different trends for phenols and lignin monomers observed by Py-GC/MS and Py-FIMS. Furthermore, Py-FIMS appeared to be more versatile because it showed enrichments in thermally stable phenols/lignin monomers, lipids and alkylaromatics in the compost treatments over time from year 19 to year 34 (Figure 2).

4.4 Conclusions

1. The combined application of two complementary analytical pyrolysis techniques for the first time enabled to investigate long-term effects of compost on the SOM composition at the molecular level under two contrasting managements with reduced aeration (permanent grass) or enhanced aeration (arable use). The disagreement in compost effects on phenols and lignin monomers calls for complementing the methodological approach by another lignin-specific method such as CuO-oxidation (Hedges and Ertel 1982).
2. The present results revealed that compost fertilization may result in enrichment of SOM with stable at the expense of labile compounds. This is conclusive from losses in compounds that are considered easily metabolized by microorganisms (e.g. carbohydrates as derived from Py-GC/MS and Py-FIMS) and gains in thermally stable proportions of some compound classes. However, these gains in thermally stable phenols, lignin monomers and alkylaromatics occur very slowly over time so that they were detected only 15 years after secession of compost application. Direct comparison of compost and mineral fertilizer at the same sampling date points to pronounced enrichments of labile lipids, fatty acids and sterols from compost or initiated microbial growth. Maintenance of the positive compost effects after conversion of soil from permanent grass to arable use points to the long lasting effect of compost on soil fertility and organic carbon sequestration in agricultural soils.
3. Therefore, compost application can be strongly recommended not only as a soil amendment but also as a measure to mitigate CO_2-enrichment in the atmosphere which should be considered by policy makers in developing systems for carbon credits. Forthcoming studies will be directed to disclose in more detail the complicated relationships between different kinds of compost and their direct and indirect effects on labile and stabile soil- and compost-derived organic molecules, and the kinetics of these complex reactions.

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References

Adani, F., Genevini, P., Ricca, G., Tambone, F. and Montoneri, E. 2007. Modification of soil humic matter after 4 years of compost application. *Waste Manage*., 27: 319–324.

Bustamante, M. A., Said-Pullicino, D., Paredes, C., Cecilia, J. A. and Moral, R. 2010. Influences of winery–distillery waste compost stability and soil type on soil carbon dynamics in amended soils. *Waste Manage*., 30(10): 1966-75.

Ceccanti, B., Masciandaro, G. and Macci, C. 2007. Pyrolysis-gas chromatography to evaluate the organic matter quality of a mulched soil. *Soil Till. Res.,* 97: 71–78.

Chefetz, B., Tarchitzky, J., Deshmukh, A. P., Hatcher, P. G. and Chen, Y. 2002. Structural characterization of soil organic matter and humic acids in particle-size fractions of an agricultural soil. *Soil Sci. Soc. Am. J.,* 66: 129–41.

de Leeuw, J. W., Versteegh, G. J. M. and van Bergen, P. F. 2006. Biomacromolecules of algae and plants and their fossil analogues. *Plant Ecol*., 182: 20–233.

Dignac, M. F., Houot, S. and Derenne, S. 2004. How the polarity of the separation column may influence the characterization of compost organic matter by pyrolysis–GC/MS. *J. Anal. Appl. Pyrol*., 75: 128–139.

Franke, M., Jandl, G. and Leinweber, P. 2006. Organic compounds in re-circulated leachates of aerobic biological treated municipal solid waste. *Biodegradation*, 17: 473–485.

Franke, M., Jandl, G. and Leinweber, P. 2007. Analytical pyrolysis of re-circulated leachates: Towards an improved municipal waste treatment. *J. Anal. Appl. Pyrol*., 79: 16–23.

Fortuna, A., Harwood, R. R., Kizilkaya, K. and Paul, E. A. 2003. Optimizing nutrient availability and potential carbon sequestration in an agro-ecosystem. *Soil Biol. Biochem*., 35: 1005–1013.

Gleixner, G., Poirier, N., Bol, R. and Balesdent, J. 2002. Molecular dynamics of organic matter in a cultivated soil. *Org. Geochem*., 33: 357–66.

González-Vila, F. J., Almendros, G. and Madrid, F. 1999. Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil. *Sci. Total Environ.*, 236: 215–229.

González-Vila, F. J., Lankes, U. and Lüdemann, H. D. 2001. Comparison of the information gained by pyrolytic techniques and NMR spectroscopy on the structural features of aquatic humic substances. *J. Anal. Appl. Pyrol*., 58: 349-359.

Gregorich, E. G., Monreal, C. M., Schnitzer, M. and Schulten, H. -R. 1996. Transformation of plant residues into SOM: chemical characterization of plant tissue, isolated soil fractions, and whole soils. *Soil Sci*., 161: 680–693.

Hedges, J. I. and Ertel, J. R. 1982. Characterization of lignin by gas capillarychromatography of cupric oxide oxidation products. *J. Anal Chem*., 54: 174–178.

Hempfling, R. and Schulten H.-R. 1988. Characterization and dynamics of organic compounds in forest humus studied by pyrolysis-gas chromatography/electron impact mass spectrometry and pyrolysis-(high resolution) field ionization mass spectrometry. *J. Anal. Appl. Pyrol*., 13: 319-325.

Hempfling, R. and Schulten, H.-R. 1990. Chemical characterization of the organic matter in forest soils by Curie-point pyrolysis-gas chromatography/ mass spectrometry and pyrolysis-field ionization mass spectrometry. *Org. Geochem*., 15: 131-145.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.,* 34: 139–162

Kiem, R. and Kögel-Knabner, I. 2003. Contribution of lignin and polysaccharides to the refractory carbon pool in C-depleted arable soils. *Soil Biol. Biochem*., 35: 101–18.

Krull, E. S., Baldock, J. A. and Skjemstad, J. O. 2003. Importance of mechanisms and processes of the stabilization of soil organic matter for modeling carbon turnover. *Funct. Plant Biol*., 30: 207–222.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science*, 304: 1623–1627.

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. *Eur. J. Soil Sci*., 53: 299–309.

Leinweber, P., Blumensteins, O. and Schulten, H.-R. 1996. Organic matter composition in sewage farm soils: Investigations by ^{l3}C-NMR and pyrolysis-field ionization mass spectrometry. *Eur. J. Soil Sci*., 47: 71-80.

Leinweber, P. and Schulten, H.-R. 1993. Dynamics of soil organic matter studied by pyrolysis-field ionization mass Spectrometry. *J. Anal. Appl. Pyrol.*, 25: 123-136.

Leinweber, P. and Reuter, G. 1992. The influence of different organic fertilization practices on concentrations of organic carbon and total nitrogen in particle-size fractions during 34 years of a soil formation experiment in loamy marl. *Biol. Fertil. Soils*, 13: 119–124.

Leinweber, P. and Schulten, H.-R. 1999. Advances in analytical pyrolysis of soil organic matter. *J Anal. Appl. Pyrol.,* 49: 359-383.

Lima, G., Diana, L. D., Santos, M. S., Scherer, W. H., Schneider, J. R., Duarte, C. A., Santos, B. H. E. and Esteves, I. V. 2009. Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, 150: 38-45.

Lorenz, K., Lal, R. and Shipitalo, M. J. 2006. Stabilization of organic carbon in chemically separated pools in no-till and meadow soils in northern Appalachia. *Geoderma*, 137: 205–211.

Marinari, S., Liburdi, K., Masciandaro, G., Ceccanti, B. and Grego, S. 2007. Humification-mineralization pyrolytic indices and carbon fractions of soil under organic and conventional management in central Italy. *Soil Till. Res*., 92: 10–17.

Nardi, S., Morari, F., Berti, A., Tosoni, M. and Giardini, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilizers. *Eur. J. Agron.,* 21: 357–367.

Negassa, W., Baum, C. and Leinweber, P. 2011. Soil amendement with agro-industrial products: Moluclar-chemical compostions and effects on soil biochemical activites and phosphrous fractions. *J. Plant Nutr. Soil Sci*., 174: 113-120.

Nierop, K. G. J., Pulleman, M. M. and Marinissen, J. C. Y. 2001. Management- induced OM differentiation in grassland arable soil: a study using pyrolysis techniques. *Soil Biol. Biochem.*, 33: 755–764.

Piccolo, A., Spaccini, R., Nieder, R. and Richter, J. 2004. Sequestration of a biologically labile organic Carbon in soils by humified organic matter. *Clim. Change,* 67: 329-343.

Quèdraogo, E., Mando, A. and Zombrè, N. P. 2001. Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. *Agr Ecosyst. Environ.,* 84: 259–266.

Reuter, G. and Kahle, P. 1992. Jahre Rostocker Dauerversuche. III. Entwicklung und Differenzierung physikalischer Bodeneigenschaften. *Arch. Acker. Pflanzenbau. Bodenkd.*, 36: 331-339.

Rivero, C., Chirenje, T., Ma, L.Q. and Martinez, G., 2004. Influence of compost on soil organic matter quality under tropical conditions. *Geoderma*, 123: 355–361.

Saison, C., Degrange, V., Oliver, R., Millard, P., Commeaux, C., Montange, D. and Le Roux, X. 2006. Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. *Environ. Microbiol*., 8: 247–257.

Schulten, H. -R. and Hempfling, R. 1992. Influence of agricultural soil management on humus composition, quality and dynamics. *Plant Soil*, 142: 259-271.

Schulten, H.-R. and Leinweber, P. 1991. Influence of long-term fertilization with farmyard manure on soil organic matter: Characteristics of particle size fractions. *Biol. Fertil. Soils*, 12: 81-88.

Schulten, H.-R., Leinweber, P. and Reuter, G.1992. Initial formation of soil organic matter from grass residues in a long-term experiment. *Biol. Fertil. Soils*, 14: 237-245.

Schulten, H.-R. and Leinweber, P. 1993. Pyrolysis-field ionization mass spectrometry of agricultural soils and humic substances: Effect of cropping systems and influence of the mineral matrix. *Plant Soil*, 151: 77-90.

Schulten, H.-R. 1992. Analytical pyrolysis of humic substances and soils: Geochemical, agricultural and ecological consequences. *J. Anal. Appl. Pyrol.,* 25: 97-122.

Six, J., Conant, R. T., Paul, E. A. and Paustian, E. A. P. 2002. Stabilization mechanism of soil organic matter: implication for C-saturation of soil. *Plant Soil*, 241: 155–176.

Sorge, C., Miiller, R., Leinweber, P. and Schulten, H.-R. 1993. Pyrolysis-mass spectrometry of whole soils, soil particle-size fractions, litter materials and humic substances: Statistical evaluation of sample weight, residue, volatile matter and total ion intensity. *Fresenius J. Anal. Chem.*, 346: 687-703.

Stewart, C. E., Neff, J. C., Amatangelo, K. L. and Vitousek, P. M. 2011. Vegetation Effects on soil organic matter chemistry of Aggregate Fractions in a Hawaiian Forest. *Ecosystems*, 14: 382–397.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Jamroz, E. and Kocowicz, A. 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biol. Biochem*., 39: 1294–1302.

5 Evaluation of plant available nutrient contents in differently aged composts and short term effects on soil fertility of a tropical Nitisol.

5.1 Introduction

Soils in many part of the world are increasingly stressed from long-term cultivation practices leading to inevitable degradation of soil organic matter and loss of soil fertility. Low soil fertility is a fundamental impediment in increasing food production and farm incomes in many parts of Africa (Sánchez et al., 1995; Shepherd and Soule, 1998). Limited data available at national level on soil fertility in Ethiopia suggested that these soils faced moderate to very severe fertility constraints affecting key farming regions (Stoorvogel and Smaling, 1990). This has more increased and likely worsened with the ongoing climate change, insufficient soil fertility management, high rates of soil erosion, increasing population density, farming without replenishing nutrients over time, and chemical imbalance issues (for example, acidity and salinity leading to fixation). Thus, there is an urgent need to improve soil fertility and nutrient management in the country.

Typical tropical Nitisols as used for this study constitute 12.2 % of the total area of Ethiopia. It is characterized as deep (>150 cm) reddish brown clays, highly weathered, acidic, high P-fixing and well drained soil. With proper fertilization and management it is inherently fertile and productive. But now its fertility has been depleted due to intensive cultivation, leaching and erosion (Nigussie and Kissi, 2012). However, under location specific management practice this depletion of soil fertility is minimized or reverted

Among the different management practices, composting has been adopted as a method of diverting organic waste materials from landfills while creating a product suitable for agriculture. The word “compost” often arouses positive associations, as it originates from nature it must be good for the plants. As reported by several authors, the addition of compost can be a feasible option to improve the soil physical, chemical and biological properties (Morlat and Chaussod, 2008; Lima *et al*., 2009). It is also evident that the incorporation of compost into soils results in an important supply of plant nutrient elements, such as nitrogen (N), phosphorus (P) and sulphur (S), exchangeable cations (calcium, potassium, magnesium and sodium) and micronutrients (Nardi *et al*., 2004;

Weber *et al*., 2007). Particularly in highly weathered soils, composts can also decrease the rate of micronutrient leaching and level of P fixation in a soil (Obi and Ebo, 1995). Likewise composts can increase pH in acidic soils (Crecchio *et al*., 2001; Haynes and Mokolobate, 2002) which enhances the availability of some nutrients and reduces the solubility of some toxic elements. All this possibly results in a reduction in the amount of mineral fertilizers to be used and related environmental problems (Grigatti *et al*., 2007). Moreover, compost application results in the stimulation of soil microbiological activity (Garcia-Gil *et al*., 2000; Ros *et al*., 2006; Saison *et al*. 2006; Bustamante *et al*., 2010) and soil structural stability (Albiach *et al.*, 2001; Goulet *et al*., 2004). Therefore, the application of compost may be beneficial for soil conservation, especially in degraded soils and those susceptible to erosion. On the other side, compost application has led to local limitations such as increase in the salt content, the toxicity of heavy metals, unnecessary nutrient enrichments and subsequent leaching, immobilization of N or emission of potent GHG's such as CH_4 and N_2O (McConnell *et al*., 1993; Inubushi *et al.*, 2000; Smith *et al.*, 2001). All these constraints questioned the feasibility of composting as a sustainable organic waste recycling technology. One way to avoid such problems is proper evaluation of compost effects on plant available nutrients (Bustamante, 2011). Furthermore, effective utilization of compost needs evaluation of changes in plant available nutrients during composting and characterization of its subsequent effect in different soil types. However, very few data on this particular characteristic of composts are available, and very likely those data need to be determined for each specific compost separately.

At present considerable amounts agro-industrial wastes such as coffee processing by-products like coffee pulp (40% of the wet weight) are generated in Ethiopia and other tropical regions, and become a source of severe environmental problems (Aranda *et al*., 2009). The annual coffee production in Ethiopia is estimated at 350,000 tons (Alemayehu *et al* 2007). Recently, interests to find out beneficial uses of this residue were started and proposed alternative uses for coffee pulp include direct use as a supplement for animal feed and soil amendment, as alternative energy resource, for the production of products (enzymes, citric acid, and flavoring substances, natural antioxidants), as a substrate for growth of mushrooms and uses as adsorbents (Pandey *et al*., 2000; Salmones *et al* 2005, Negassa *et al*., 2011). In Ethiopia, however, considerable amounts of these wastes are still mainly damped to water bodies, unsanitary landfills and cause freshwater eutrophication, health problems, require additional space for land filling as well as produce offensive odour and greenhouse gases when collected, transported and disposed. Moreover, the direct use of coffee pulp as a soil amendments and animal feed is restricted by the presence of toxic components such as polyphenols, tannins and caffeine as well as its adverse effects on soil properties such as induced anaerobic conditions and release of phytotoxic compounds impairing plant growth (Pandey *et al.,* 2000; Gouvea *et al*., 2009; Gezahegne *et al*., 2011; Negassa *et al*., 2011). On the other hand, the high organic matter, nitrogen and potassium content as well as low heating values (Orozco *et al*., 1996; Pandey *et al*., 2000; Orozco *et al.,* 2008) of coffee pulp opens up the chance for integrating agriculture and solid waste management by recycling the waste as compost for the improvement of soil fertility and as a mitigation measures of climate change.

Therefore, the objective of this study was to determine and compare the change in the available concentration of nutrients during composting and after its application in tropical Nitisol under controlled laboratory condition.

5.2 Materials and Methods

The soil was sampled from a coffee plantation farm located in the southwestern highlands of Ethiopia and belongs to the major Soil Unit “Nitosol” (FAO). The sampling area is situated $36^{o}36'$E longitude and $7^{o}56'$N latitude. The elevation varies between 1533-1548 m a.s.l. Mean maximum and minimum temperature range from 26 to 30 ^{o}C and 11 to 14 ^{o}C, respectively, and the mean of precipitation range 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 fruit and vegetable waste (dominated by fruit) and wet coffee pulp waste 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. Two piles were constructed for experimental purposes with duplicates each and monitored as treatment COM1 (where the main feedstock material was coffee pulp waste) and COM4 (prepared by mixing coffee pulp waste with fruit and vegetable waste in which the ratio was 50:50 by volume). About 5 % (w/w) soil and 10 % (w/w) of garden trimmings (dry leaves, and young tree branches) were added as additional source of microbial colonization and to improve the pile structure. The composting experiment lasted from November 2010 to February 2011. The moisture content of the compost was manually estimated on site by hand squeezing the compost tightly. Moisture contents above 40 % were indicated by a formation of a ball up on squeezing of the compost. Depending on the situation the piles were watered so as to always maintain > 40 % (w/w) moisture. Samples were taken once a week for pH and moisture content measurements in the laboratory. Temperatures were measured daily for the first two week and in a three-days-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 =COM-T), day

24/36 (mesophilic phase =COM-M, depending on the duration each treatment lasted in this phase), and 89/119 (final compost =COM-F, depending on the duration each treatment lasted in this phase). In this way composts of different age were obtained from two different composting piles. The samples were air dried and ground to pass through a 0.5 mm sieve. Main characteristics was given in **Table 5.1**

Table 5.1. Chemical properties of raw coffee pulp waste and compost samples from different phases of composting coffee pulp waste. Different letters indicate that samples are significantly different ($P < 0.05$) according to Fisher LSD test with in a column and values followed by the same letters with in a column were not significantly different.

Compost samples	pH_{H2O}	$C_{org.}$ (g kg^{-1})	N_{tot} (g kg^{-1})	S (g kg^{-1})	C/N	EC (ms cm^{-1})
RCPW	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
COM1-T	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
COM1-M	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
COM1-F	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
COM4 -T	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
COM4-M	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
COM4-F	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

COM – compost; T – thermophilic phase; M – mesophilic phase; F – final stage; RCPW - raw (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.

Aerobic incubation of the soil-compost-mixtures was done by mixing 10 g dry weight of sieved soil samples (<2 mm) with fresh and composted coffee pulp waste at desired application rates (48 t ha^{-1}) and placed in 100 ml incubation vessels in five replicates each. They were monitored as treatment S+RCPW, S+COM1-T, S+COM1-M, S+COM4-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 mixtures to keep the moisture at 60 % of water-holding capacity. 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 end of incubation for chemical analysis.

5.2.1 Chemical analysis of soil and compost

The concentrations of total organic carbon (C_{org}), total nitrogen (N_t) and total sulfur (S_t) were determined for soil, compost and amended soil samples using a CNS analyzer (Vario EL III; Elementar Analysensysteme, Hanau, Germany). Available phosphorus was determined using the double Ca-lactate (DCaL). This method is the standard method in Germany. Mehlich-I (1942) extraction method was used for determination of cation-exchange capacity (CEC) and exchangeable cations (Na, Ca, K, and Mg) in 0.2 N $BaC1_2$ extract buffered with triethanolamine to pH 8. They were determined by ICP-OES instrumentation.

5.2.2 Statistical analyses

Means and standard errors were calculated for chemical parameters. Data were subjected to One Way ANOVA test and Fischer LSD Test to compare mean results from amended and non-amended soils. Pearson's correlation coefficient was calculated to see how content of one nutrient added by the compost affect the availability of other nutrient in the amended soil. All statistics were computed using data analysis and graphic software (Origin 8.1G).

5.3 Results and discussion

As shown in the **Figure 5.1** the composts contained appreciable amount of CEC (>30 cmol kg^{-1}) and showed a significant increase during composting in the order RCPW< COM-T< COM-F. However, no significant difference was observed in the CEC between COM-M and COM-F. The increase in the CEC as composting process progresses indicated the degree of humification and the functional groups responsible for this may be the carboxyl and phenolic groups formed during oxidation and decomposition processes of the organic matter (Lax, 1986). The significant difference in CEC due to mixing of coffee pulp waste with equal proportions of fruit and vegetable waste become obvious by comparing the two composting mixtures COM1 (34.5 to 36.0 cmol kg^{-1}) and COM4 (37.3 to 38.9 cmol kg^{-1}) in a different phase of composting (**Figure 5.1**). This suggests that co-composting of coffee pulp waste with fruit and vegetable waste improves the humification process during composting.

The composts contained all the macronutrients that are necessary for plant growth (**Table 5.1, Figure 5.1**). The contents of the Mehlich 1-extractable nutrients in the compost were in the order K > Ca > Mg > Na (**Figure 5.1**). In addition, the significant difference ($P < 0.05$) with respect to exchangeable Ca, K and Na in the treatments COM1 and COM4 (**Figure 5.1**) resulted from mixing of equal proportion of fruit and vegetable wastes with the coffee pulp waste. This indicated that addition of fruit and vegetable waste enriched the compost with exchangeable Ca and decreased the content of exchangeable K and Na as their amounts were much higher in coffee pulp waste than fruit and vegetable waste (Orozco *et al*., 2008; Pandey *et al.,* 2000; Eshetu *et al*., 2008). However, a relatively larger content of exchangeable Ca in treatment COM4 (ranged from 23.1 to 27.0 cmol kg^{-1}) as induced by larger mineral content of fruit and vegetable waste (Garcia *et al*., 1993). This is of great interest as nutrient deficiencies in tropical acidic soils were occurred when Al has taken the buffering role over Ca up on its depletion (Harter, 2002).

An increasing trend with respect to K and Na was observed during composting whereas other nutrients did not show a defined trend during composting (**Figure 5.1**). Except Ca (which attain the highest value at early stage of composting), all macronutrients attained the highest concentration at the end of composting (**Figure 5.1**). This increasing trend of exchangeable cations likely resulted from the total weight loss from organic matter mineralization. Exchangeable Ca and Na contents were higher in the composts compared with the raw coffee pulp waste (**Figure 5.1**). However, reduction in exchangeable K by about 50% during composting as compared to its content in raw coffee pulp waste may have been related to a greater leaching of this nutrient.

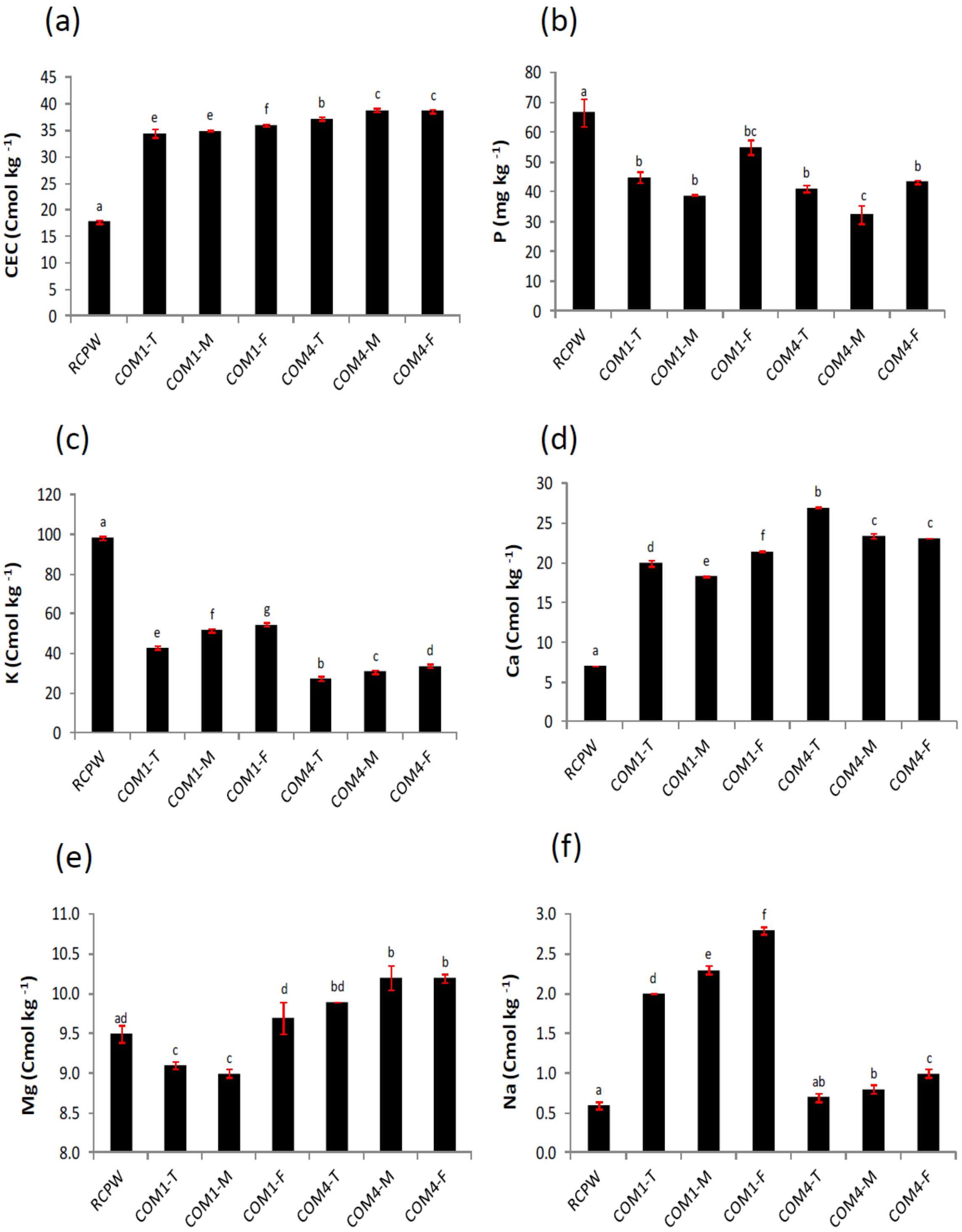

Figure 5.1. Cation exchange capacity (CEC), exchangeable cations (K, Ca, Mg and Na) and available P content in raw coffee pulp waste and compost samples taken in different phases of composting. Bars marked with different letters indicate statistically significant difference ($P < 0.05$) according to Fisher LSD test.

The contents of the exchangeable K, Ca and Mg were higher than the value reported in various composts such as in a vermicompost made up of coffee pulp as reported by Orozco *et al.* (1996), food waste compost (Farrell and Jones, 2010), coffee waste compost and kitchen waste compost (Ebid *et al*., 2007) as determined by extraction with ammonium Acetate (NH_4Ac). The vermicompost made of coffee pulp waste (Raphael *et al*., 2012) also contained lower amounts of exchangeable K and Ca, whereas no difference with respect to exchangeable Mg was observed. Nevertheless, except the exchangeable K, the CEC and exchangeable nutrients (Ca, Mg and Na) were lower than those reported in other composts such as composted vegetal residues (Herencia *et al.,* 2011), composted pruning waste (Benito *et al*., 2003) and composted urban refuses (Villar *et al*., 1993). Compost made of green waste and chopped wood contained higher CEC but lower exchangeable cations as reported by Liu *et al*. (2011). This might be due to composition difference/variability among the composted material, the extraction method used, and the poor distribution of macro and micro fauna in the compost piles as they are generally grown on the upper layer of the composting piles of coffee pulp (Pandey *et al*., 2000). However, vermi-composting with exploitation of different species of earth worms found in coffee pulp waste were recommended as the best solution to enhance exchangeable Ca and Na (Orozco et al., 1996; Aranda *et al.*, 1999).

The contents of available P (ranged from 32.5 to 55.1 mg kg^{-1}) in the composts showed no specific trend with variation in the age of the compost and composition of composting mixtures (Figure 5.1). However its content in RCPW was significantly higher than the contents of P in the composts, perhaps due to its utilization or immobilization by microorganisms during composting. Available P (DCaL-P) content in RCPW and composts (COM1 and COM4) are much less than a value reported by Orozco *et al*. (1996) using the Bray II method in a vermicompost, Olsen-P in green waste and catering waste and a compost made up of mixing catering waste with green waste (Farrell and Jones, 2010), and Bray II-P in a compost made up of coffee waste and kichen waste as reported by (Ebid *et al*., 2007). This difference in the concentration of available P may be primarily attributed to the differences in the extraction method. In addition, the higher pH of the compost samples in this study as compared to the others has favored P insolubility.

Table 5.2. Cation exchange capacity (CEC), exchangeable cations (cmol kg $^{-1}$) and available P content (mg kg^{-1}) in the control and amended soil at the end of incubation. Different letters indicate that samples are significantly different ($P < 0.05$) according to Fisher LSD test with in a column and values followed by the same letters with in a column were not significantly different.

Treatment	CEC	P	K	Ca	Mg	Na
Control	39.9 (0.65)a	BDL	1.1 (0.25)a	16.3 (0.1)a	3.7 (0.0)a	BDL
S+RCPW	37.5 (2.5)a	BDL	3.2 (0.05)b	17.1 (0.15)b	3.7 (0.0)a	BDL
S+COM1-T	36.9 (0.75)a	BDL	2.1 (0.1)c	18.2 (0.6)c	3.7 (0.0)a	1.9
S+COM1-M	39.3 (0.8)a	BDL	2.0 (0.05)c	18.1 (0.15)c	3.8 (0.0)ab	BDL
S+COM1-F	35.4 (3.1)a	BDL	2.0 (0.1)c	17.9 (0.05)c	3.9 (0.1)b	BDL
S+COM4-T	37.6 (1.75)a	BDL	2.3 (0.25)c	17.5 (0.05)bc	3.7 (0.0)a	0.01
S+COM4-M	39.5 (0.65)a	BDL	1.8 (0.0)c	17.1 (0.15)b	3.8 (0.0)ab	BDL
S+COM4-F	38.8(0.9)a	BDL	2.1(0.3)c	17.4(0.15)bc	3.9(0.05)c	BDL

S+COM – soil-compost mixture; T – thermophilic phase; M – mesophilic phase; F – final stage; S+RCPW – soil-raw coffee pulp waste mixture; BDL- below the detection level. Standard errors in brackets.

The main soil chemical parameters determined after four months incubation was shown in **Table 5.2**. In comparison to the control, all amended soils resulted in a significantly higher Ca and K content irrespective of the age of compost added. For instance application of COM 1 increased each of them by about 11% and 81%, respectively, while CEC was not significantly affected by any of compost amendments ($p<0.05$, **Table 5.2**). The plant available P and exchangeable Na contents were below the detection limit in all amended soil and the control. Usually the DCaL extraction lowers available P when it is applied in tropical soils. However, strong positive correlation ($r = 0.89$) between exchangeable Ca and available P (obtained by subtracting its concentration in the control from the concentrations in the amended soils) as observed in treatment S + COM 4. This indicated that the application of compost containing a larger concentration of exchangeable Ca (such as COM4-T) had a significant effect on the availability of P in tropical Nitisols. Furthermore, Kraus *et al*. (2003) reported that polyphenols, especially tannins which also exist in coffee pulp compost, may improve the P availability by interacting with metal oxides such as Al and Fe oxides that may otherwise fix P. No significant differences ($p<0.05$) in concentrations of the exchangeable cations and quantity of CEC were observed among the different compost treated soils **(Table 5.2)**. Despite of the composts had adequate CEC and Mg contents its application to soil did not increase above the level of the control. This may be due to the high level of CEC (40 cmol kg^{-1}) and Mg (3.7 cmol kg^{-1}) in the control requiring larger compost additions to bring a significant change.

Increase of CEC, exchangeable cations (Ca, K, Mg and Na) as well as availability of P following compost addition in particular and organic matter amendments in general were already reported in various studies (Haynes and Mokolobate, 2002; Wright *et al*., 2007, Weber *et al*., 2007; Smith, 2009; Liu *et al*., 2011; Ozores-Hampton *et al*., 2011). However it appears impossible to apply their results to the present problem because plants, type of vegetation, rate and method of application of the compost, and soil type were different.

5.4 Conclusions

In general, composting coffee pulp waste which is abundant in tropical region produce a valuable agricultural input and the product (compost) could supply all the macro-nutrients mainly K and N necessary for plant growth. However, exchangeable Ca, Mg and Na were not in high amounts as compared to other composts but they fit the minimum values. Furthermore, composting increased the agricultural value of coffee pulp waste as it raised the exchangeable Ca, Mg and CEC values. Extended composting beyond thermophilic phase may be not necessary as it does not significantly affect the nutrient content of the compost and their availability to the plant. On the other hand a relatively higher content of exchangeable Ca in relatively “fresh” compost has particularly important implications as its application in tropical Nitisols increase the availability of nutrients such as P that is otherwise strongly fixed by Al and Fe oxides. Further study would focus on optimization of the composting process so as to minimize nutrient leaching such as K as well as to increase P and other nutrient availability. Moreover, the controlled laboratory experiment has limitations to get sufficient information on the agronomic value of coffee pulp compost under field conditions and it needs to be addressed in the forthcoming study.

References

Albiach, R., Canet, R., Pomares, F. and Ingelmo, F. 2001. Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. *Bioresour. Technol.,* 76: 125-129.

Alemayehu, T., Esayas, K. and Kassu, K. 2007. Coffee development and marketing improvement plan in Ethiopia. In: Proceeding of national workshop. Four decades of coffee research and development in Ethiopia. Addis Ababa, Ethiopia, pp. 375-387

Aranda, A., Barois, I., Arellano, P., Irisson, S., Salazar, T., Rodriguez, J. and Patron J. C. 1999. Vermicomposting in the tropics In: Earthworm Management in Tropical Agroecosystems (Eds.) P Lavelle, L Brussaard and P Hendix, pp 253- 287.

Aranda, A., Duran, L. O. and Escamilla, E. P. 2009. Vermicomposting in coffee cultivation. In: Coffee growing, processing, sustainable production. Wintgens, J.N. (Ed). Wiley-VCH, Weiheim, pp 25-55.

Benito, M., Masaguer, A. Moliner, A., Arrigo, N. and Palma, R. M. 2003. Chemical and microbiological parameters for the characterization of the stability and maturity of pruning waste compost. *Biol. Fertil. Soils*, 37:184–189.

Bustamante, M. A., Said-Pullicino, D., Agullo, E. Andreu, J. Paredes, C. and Moral, R. 2011. Application of winery and distillery waste composts to a Jumilla (SE Spain) vineyard: effects on the characteristics of a calcareous sandy-loam soil. *Agric. Ecosyst. Environ.*, 140: 80–87

Crecchio, C., Curci, M., Mininni, R., Riccuti, P. and Ruggiero, P. (2001) Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. *Biol. Fertil. Soils,* 34: 311-318.

Ebid, A., Ueno, H. and Ghoneim, A. 2007. Nitrogen mineralization kinetics and nutrient availability in soil amended with composted tea leaves, coffee waste and kitchen garbage. *Int. J. soil sci.,* 2(2): 96-106.

Eshetu, B. 2008. A study on composting potential of source separated fruit and vegetable wastes.LAP LAMBERT academic publishing GmbH & Co. KG, Saarbrücken, Germeny

Farrell, M. and Jones, D. L. 2010. Food waste composting: Its use as a peat replacement field trials. *Tappi J.,* 72 (6): 199–206.

Garcia, C., Hernandez, T., Costa, C., Ceccanti, B., Masciandaro, G. and Ciardi, A. 1993. Study of biochemical parameters of composted and fresh municipal wastes. *Bioresour. Techno*l., 44:17-23

Garcia-Gil, J. C.; Plaza, C., Soler-Rovira, P. and Polo, A. 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biol. Biochem*., 32:1907-1913.

Goulet, E., Dousset, S., Chaussod, R., Bartoli, F., Doledec, A. F. and Andreux, F. 2004. Water stable aggregates and organic matter pools in a calcareous vineyard soil under four soil-surface management systems. *Soil Use Manage*., 20(3): 318–324.

Gouvea, B. M., Torres, C., Franca, A. S, Oliveria, L. S. and Oliveria, E.S. 2009. Feasibility of ethanol production from coffee husks. *Biotechnol Lett* , 31: 1315- 1319.

Grigatti, M., Giorgonni M. E. and Ciavatta, C. 2007. Compost-based growing media: influence on growth and nutrient use of bedding plants. *Bioresour. Technol.*, 98: 3526-3534.

Gezahegne, B. Fikre, L. and Mulatu, W. 2011. Exploring the suitability of coffee pulp compost as growth media substitute in greenhouse production. *Int. J. Agri. Res.,* 6(3):255-267

Harter, R. D. 2002. Acid soils of the tropics. ECHO Technical Note. 8pp. Accessible via http://echonet.org/tropicalag/technotes/Acidsoil.pdf (11 Jan 2009).

Haynes, R. J. and Mokolobate, M. S. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutr. Cycl. Agroecosyst.*, 59: 47–63.

Herencia, J. F., Garcia-Galavisa, P. A., Doradoa, J. A. R. and Maquedab, C. 2011. Comparison of nutritional quality of the crops grown in an organic and conventional fertilized soil. *Scientia Horticulturae*, 129: 882–888

Inubushi, K., Goyal, S., Sakamoto, K., Wada, Y., Yamakawa, K. and Arai, T. 2000. Influences of application of sewage sludge compost on N_2O production in soils. *Chemosphere*, 2: 329–334.

Kraus, T. E. C., Dahlgren, R. A. and Zasoski, R. J. 2003. Tannins in nutrient dynamics of forest ecosystems. A review. *plant soil*, 256: 41-46

Lax, A. 1986. A method for determining the cation-exchange capacity of organic materials. *Plant Soil*, 94: 349–355.

Lima, G., et al. 2009. Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, 150: 38-45.

Liu, J., Xu, X. -h., Li, H. -t., and Xu, Y. 2011. Effect of microbiological inocula on chemical and physical properties and microbial community of cow manure compost. *Biomass Bioenergy*, 35(8): 3433-3439.

McConnell, D. B., Shiralipour, A. and Smith, W. H. 1993. Compost Application Improves Soil Properties. *Biocycle,* 34: 61-63.

Mokolabate, M. S. and Haynes, R. J. 2002. Comparative liming effect of four organic residues applied to an acid soil. *Biol. Fertil. Soils,* 35: 79-85.

Morlat, R. and Chaussod, R. 2008. Long-term additions of organic amendments in a Loire valley vineyard. I. Effects on properties of a calcareous sandy soil. *J. Enol. Vitic*., 59: 353–363.

Nardi, S., Morari, F., Berti, A., Tosoni, M. and Giardini, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilisers. *Eur. J. Agron*., 21: 357-367.

Negassa,W., Baum,C. and Leinweber, P. 2011. Soil amendement with agro-industrial products: Moluclar-chemical compostions and effects on soil biochemical activites and phosphrous fractions. *J. Plant Nutr. Soil Sci*., 174: 113-120.

Nigussie, A and Kissi, E. 2012. Physicochemical Characterization of Nitisol in Southwestern Ethiopia and Its Fertilizer Recommendation Using NuMaSS. *J. Agri. Sci.,* 1(4): 066-073.

Obi, M. E. and Ebo, P. O. 1995. The Effects of Organic and Inorganic Amendments on Soil Physical Properties and Maize Production in a Severely Degraded Sandy Soil on Southern Nigeria. *Bioresou. Techno.,* 51: 117-123.

Orozco, A. L., et al. 2008. Biotechnological enhancement of coffee pulp residues by solid-state fermentation with Streptomyces. Py–GC/MS analysis. *J. Analy. Applied Pyrol*., 8: 247-252

Orozco, F. H., Cegarra, J. and Trujillo, L. M. 1996. Vermicomposting of coffee pulp using the earthworm Eisenia fetida: Effects on C and N contents and the availability of nutrients. *Biol Fertil Soils*, 22:162-166.

Ozores-Hampton, M.,. Stansly, P. A. and. Salame, T. P. 2011. Soil Chemical, Physical, and Biological Properties of a Sandy Soil Subjected to Long-Term Organic Amendments. *J. Sust. Agri*., 35(3): 243-259.

Pandey, A., Soccol, C. R., Nigam, P., Brand, D., Mohan, R. and Roussos, S. 2000. Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *J. Biochem. Eng.*, 6(2):153-162.

Raphael, K., Sureka and Velmourougane, K. 2012. Vermicomposting of Coffee Processing Wastes Using Exotic (*Eudrilus Eugeniae*) and Native Earthworm (*Perionyx Ceylanesis*) Species. DOI:10.1002/masy.201251008

Ros, M., Klammer, S., Knapp, B., Aichberger, K. and Insam, H., 2006. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. *Soil Use Manage*., 22: 209–218.

Salmones, D., Mata, G. and Waliszewski, K. N. 2005. Comparative culturing of Pleurotus spp. on coffee pulp and wheat straw: biomass production and substrate biodegradation. *Bioresou. Technol*., 96: 537-544.

Sánchez, P. A., Valencia, I., Izac A. M. and Pieri C. 1995. Soil fertility replenishment in Africa. Nairobi, Kenya: ICRAF.

Shepherd, K. D. and Soule, M .J. 1998. Soil fertility management in West Kenya: dynamic simulation of productivity, profitability and sustainability at different resource endowment levels. *Agri. Ecosyst. Environ.,* 71: 131–146

Smith, D. C., Beharee, V. and Hughes, J. C. 2001. The Effects of Composts Produced by a Simple Composting Procedure on the Yields of Swiss Chard (Beta vulgaris L. var. flavescens) and Common Bean (Phaseolus vulgaris L. var. nanus). *Scientia Horticulturae*, 91: 393-406.

Smith, S. R. 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.,* 35 (1): 142–156.

Stoorvogel, J. J. and Smaling, E. M. A. 1990. Assessment of soil nutrient depletion in Sub-Saharan Africa: 1983-2000. Report 28, DLO Winand Staring Center for Integrated Land, Soil and Water Research (SC-DLO), Wageningen, Netherlands.

Villar, M. C., Beloso, M.C., Acea, M. J., Cabaneiro, A., Gonzalez-Prieto, S. J., Carballas, M., Diaz-Ravina, M. and Carballas, T. 1993. Physical and chemical characterization of four composted urban refuses. *Bioresou. Technol*., 45 (2): 105-113.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Licznar, S. and Jamroz, E. 2007.Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts*. Soil Biol. Biochem., 39: 1294–1302*

Wright, A. L., Provin, T. L., Hons, F. M., Zuberer, D. A. and White, R. H. 2007. Soil micronutrient availability after compost addition to St. Augustine Grass. *Compost Sci. Util.,* 15(2):127-134.

6. Summary and conclusion

6 Summary and conclusions

Efficient recycling of organic waste as compost is essential: (i) to make use of the growing amount of agro-industrial by-products, (ii) for the rehabilitation and enhancement of the soil fertility and increase food production, (iii) reverse the impact of climate change by stabilizing soil organic matter (SOM) and contribute to C sequestration. Since in many tropical regions the soil fertility and SOM contents decreased due to degradation and poor waste management practices, they could profit significantly from well-adapted compost treatments. For the identification of well-adapted composts treatments a critical evaluation of the quality of composts, their transformation and moluclar-level effects on the SOM is essential.

Previous studies revealed the significant impact of compost on soil chemical, physical and biological properties in general and the content and quality of SOM in particular. A decreased mineralization of labile compounds of SOM, and increases in the proportions of humic acids (HA) compared to fulvic acids (FA) after compost applications were described. Furthermore, the related literature found different results that led them to conclude: (i) that addition of relatively stable compost always reduces the soil respiration and, thus, stabilizes SOM, (ii) the efficiency of composts for soil C conservation/SOM stabilization was independent on the degree of compost stability, and (iii) extended composting may led to losses in nitrogen and carbohydrate compounds rather than contributing to the formation stable compounds. Most of these results on effects of compost on SOM composition and C stabilization were based on general chemical information derived from chemolytic and FTIR, ^{13}C NMR and DRIFT spectroscopic methods, which do not yield detailed information on individual, molecular subunits. Moreover, none of these investigations was done on Nitisols, which cover over 200 million hectares world-wide and are a dominat soil type in the highlands of Ethiopia, Kenya, and Tanzaniya.

Therefore, the present study examined primarly the impact of addition of composts with different age and type on the molecular composition of SOM and its decomposition.

Effects of composts on the SOM composition and soil respiration

Amendments of soil with different age composts (equivalent to 48 t ha^{-1}) affect the soil respiration of tropical Nitisols in an incubation experiment for 14 weeks. In general, the cumulative CO_2-C respired was best explained by a first order plus linear model. Compost taken at the thermophilic phase caused a lower increase of soil respiration and thereby a lower loss of organic C than compost from later phases of composting. This possibly resulted from selective preservation of labile compounds that were mineralized with extended composting time. A significant enrichment of stable N-compounds during the incubation in all amended soils compared to the control was measured using Py-FIMS. Comparision among the amended treatments indicated that SOM that received the “thermophilic” compost was significantly enriched by carbohydrates, heterocyclic/nitrilic N-compounds and peptides compared to the other amended soils. Temperature-resolved volatilization curves for carbohydrates, N-compounds and peptides showed that the enriched compounds covered a wide range of thermal stability. Unlike that of the control, thermal volatilization curves of N-compounds and peptides showed a shift of peaks to higher pyrolysis temperature after 14 weeks of incubation in soil amended with compost. This can be explained by the selective accumulation of components with higher stability during decomposition and their physical protection through association with clay and soil aggregates.

The significant long-term effects of compost application on the molecular composition of SOM in a loamy soil were approved. The molecular chemical changes were determined by Curie point Pyrolysis-Gas Chromatography/Mass Spectrometry Cp Py-GC/MS) and Py-FIMS. The long-term effect of compost application on the composition of SOM was an enrichment of lipids with time at the expense of carbohydrates. Py-FIMS indicated higher proportions of labile *n*-fatty acids, lipids and sterols in the soils which were amended with compost than in soils with mineral fertilization only. Thermograms of volatilization indicated enrichments of stable (compounds volatilized in between 370 and 570°C) phenols/lignin monomers, lipids and alkylaromatics in compost fertilized soils between 19 and 34 years after the application. Permanent cropping of grass between years 19 and 34 revealed similar signal patterns, which is also maintained after conversion of soil from permanent grass to arable use. This confirmed consistience on the effect of compost even in two contrasting soil management practices. The decreased proportions of carbohydrates in SOM after compost application in the long term disagreed to the recent short term effects in other soils. It underlined the significant impact of the initial soil conditions on the impact of compost applications on the SOM.

Biochemical properties of compost in different age

The evaluation of phytotoxicity, cation exchange capacity (CEC) and available nutrient concentrations during composting of coffee pulp waste and its subsequent addition to tropical Nitisols proofed the advantages of composting. In a cress and radish biotest, the raw waste decreased the germination index (GI) to < 26% by phytotoxicity, compared to a GI > 80% after application of composts. Composts contained all macro nutrients for plants and the contents of the Mehlich 1-extractable nutrients in the compost followed the order K > Ca> Mg > Na. Content of exchangeable Ca attained the highest value at the thermophilic phase of composting, whereas other macronutrients and the CEC attained the highest value at the end of composting. Amendement of soils with composts resulted in a significantly higher Ca and K content irrespective of the age of compost added in comparison to the control. Composting increased the agricultural value of coffee pulp waste, as it raised the concentrations of exchangeable Ca, and the CEC values. Extended composting beyond the thermophilic phase was identified to be unnecessary, since it does not significantly affect the nutrient contents of the composts and their availability to the plants. Higher contents of exchangeable Ca in relatively “fresh” compost might be particularly valuable to increase the availability of P in tropical Nitisols, there P is rather strongly fixed by Al and Fe oxides, since the exchangeable Ca and available P in these soils were correlated (r = 0.89).

In general, recycling of organic waste as compost by application on agricultural soils increased the SOM content and improved the SOM quality of arable soils. This can contribute to increased soil fertility. Compost application in agricultural soil in general and its application from the thermophilic phase seems promising to compensate the SOM degradation by increasing the proportions of soil organic C compounds with higher stability in the long term. Comparison among composts of different age compost suggesting that extended composting beyond thermophilic phase may be not necessary: (i) as it does not significantly affect nutrient content of the compost and their availability to the plant; (ii) since extended composting may led to mineralization of compounds that are possibly stabilized by their interaction on the soil mineral surface and essential for the stability of native SOM by enhancing soil aggregate stability (such as Carbohydrates). After verification of this result in a varity of soil conditions, these findings may have considerable economic implications by the reduction of the time required for composting.

Therefore, future research activites in the area will be directed to optimizing the condition of composting which has significant impact on transformations at the thermophilic phase and disclose the effects of different composts on SOM of soils with diverse initial soil properties (e.g. texture, soil pH) leading to the subsequent development of efficient compost management strategies to sustain its application under different field conditions. Furthermore, our results based on small scale laboratory incubation experiment, however, are restricted to a single soil type and under exclusion of factors such as temperature/moisture fluctuations, meso- and macrofauna or plants and other field heterogeneity in general, so that further research in the field or using field replicates is recommended

7 Appendices

7.1 Tables and figures

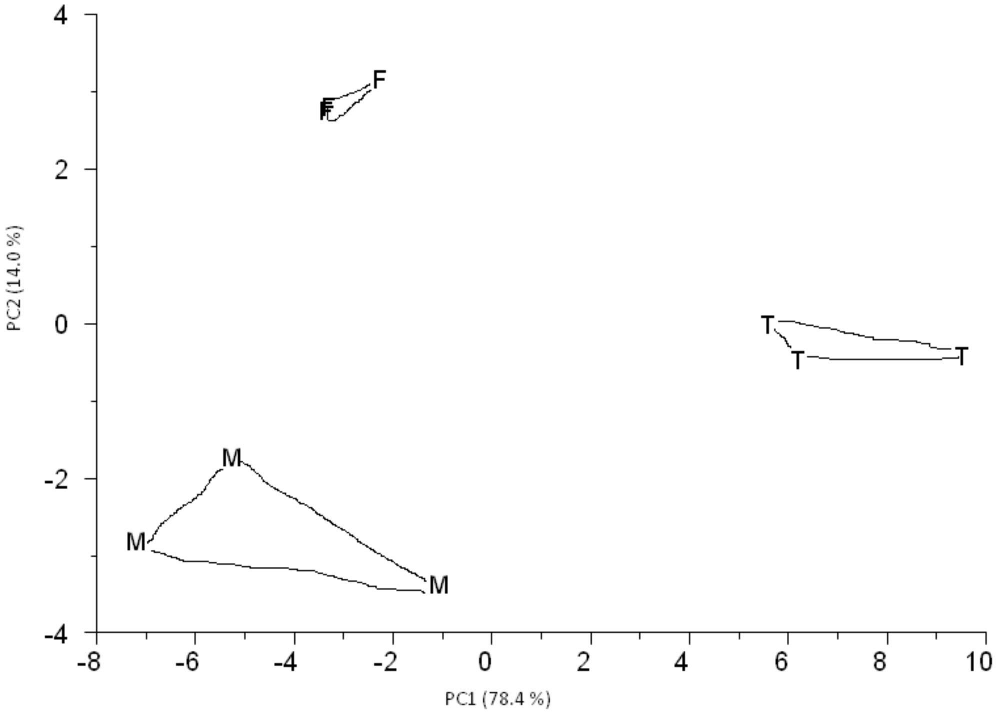

Figure 7.1.1. Plots of the principal component 1 vs 2 calculated from pyrolysis-field ionization mass spectra of samples taken at different phases of composting S+COM1-T, S+COM1-M and S+COM1-F (COM – compost; T – thermophilic phase; M – mesophilic phase; F – final stage).

Table 7.1.1 A list of major peaks (area greater than 0.45% of the spectra) identified by Py-GC/MS in compost fertilized soil and/or mineral fertilized soil.

	List of compounds	Compost fertilized soil	mineral fertilized soil
	derived from Carbohydrates		
1	Cyclopent-2-en-1,4-dione	ND	×
2	2(5H)-furanone	×	×
3	2-Furancarboxaldehyde	×	×
4	2-Furancarboxaldehyde, 5-methyl-	ND	×
5	Furan-3-carboxaldehyde	×	ND
6	2-Cyclopenten-1-one, 2-methyl-	Δ	ND
7	2-Cyclopenten-1-one, 3-methyl-	●	●
8	4H-Pyran-4-one, 3-hydroxy-2-methyl-	×	ND
9	Benzofuran, 2-methyl-	●	ND
10	Cyclohexane, 1,1,2-trimethyl-	×	ND
11	3-Furaldehyde	×	×
12	Benzofuran, 4,7-dimethyl-	ND	Δ
13	2-ethyl-5-methylfuran	ND	Δ
14	1-Ethyl-2-Methyl cyclododecane	ND	Δ
15	Benzofuran	×	ND
16	2,3-Dihydro-benzofuran	ND	×
17	2(3H)-Furanone, 5-methyl-	ND	Δ
18	2-Furancarboxaldehyde, 5-methyl-	×	×
19	Hydroxy dimethyl furanone	Δ	ND
20	2-Vinylfuran	×	×
	N-compounds(amino+nitriels+hetrocycles)		
21	1H-Indole, 3-methyl-	ND	●
22	Benzeneacetonitrile	×	×
23	6-Methyl-2,2'-bipyridine 1-oxide	ND	●
24	Pyridine, 3-methyl-	●	●
25	1H-Pyrrole-2-carboxaldehyde	×	×
26	1H-Indole, 5-methyl-	●	ND
27	Benzenepropanenitrile	×	×
28	4(1H)-Pyrimidinone	Δ	Δ
29	Benzoxazole, 2-methyl-	ND	Δ
30	1H-Pyrrole, 2-methyl-	×	×
31	1H-Pyrrole, 1-ethyl-	×	ND
32	7-Methylindole	Δ	ND
33	Pyridine, 4-methyl-	×	×
35	3-formylpyrrole	Δ	ND
36	1H-Indole	×	×
	Alkylaromatics(alkyl benzen+alkyl naphthalene +aromatic esters)		
37	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	ND	●
38	Benzaldehyde	×	×

39	1H-Inden-1-one, 2,3-dihydro-	×	×
40	Styrene	×	×
41	Benzene, 1,2,4-tripropyl-	ND	●
42	1,1,4,5,6-Pentamethyl-2,3-dihydroindene	ND	●
43	2-Ethyl-1,4-benzodioxin	●	●
44	Benzene, 1,3-dimethyl-	×	ND
45	Naphthalene	×	×
46	1H-Indene	×	×
47	Benzene, 1,2,5-trimethyl-	ND	×
48	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	×	×
49	1,4-Benzenediol	×	ND
	phenols and lignin monomers		
50	Phenol, 3-ethyl-	×	×
51	2-Methoxy-4-methylphenol	×	×
52	Phenol, 4-methyl-	×	×
53	4-vinyl-2-methoxy-phenol	×	×
54	Phenol, 2,5-dimethyl-	ND	●
55	1-Methoxy-2-(1-methyl-2-propenyl)benzene	×	×
56	Phenol, 3-methyl-	×	ND
57	Phenol, 4-methoxy-	×	ND
58	Phenol, 2-methoxy-4-(1-propenyl)-, (E)-	×	×
59	1-methoxy-1,3-cyclohexadiene	×	×
60	Phenol, 3,5-dimethyl-	Δ	ND
61	Phenol, 4-ethyl-	Δ	ND
62	Phenol, 4-ethyl-2-methoxy-	×	×
63	Benzene, ethoxy-	Δ	ND
64	Phenol, 2,6-dimethoxy-	×	×
65	Phenol	×	×
66	Phenol, 2-methoxy-	×	×
	Aliphatic compounds(alkanes+alkenes)		
67	1-pentadecene	×	×
68	Pentadecane	×	×
69	1-Hexadecene	×	×
70	Octadecane	×	×
71	1-Tetradecene	×	×
72	Tetradecane	×	×

Appeared at the experimental year of: ●, 19th only; Δ, 34th only; ×, both 19th and 34th; ND, not detected

7.2 Declaration of primary authorship

I declare that I have written the present thesis for doctorate and without of help of others. The present thesis is an original work not submitted either in Germany or outside of Germany for examination in the present or similar version. It is checked by Plagiatssoftware: Docoloc and problems were not found.

Rostock 21. 02. 2013

Eshetu Bekele Wondemagegnehu.

7.3 Theses / Thesen zur Dissertation

Compost effects on soil organic matter: transformation, composition and stabilization

Presented by

Eshetu Bekele Wondemagegnehu

I. Problem and rationale for research

- Recycling of organic waste as compost is an approach for sustainable land management: (i) for the use of the growing amount of agro-industrial by-products, (ii) to enhance the soil fertility and increase the food production, (iii) to reverse the impact of climate change by stabilizing soil organic matter and sequestering additional C in to the soil.

- It is known that compost addition has an influence on soil chemical, physical and biological properties, including the content and quality of soil organic matter (SOM). Most studies of compost effects on SOM composition and C stabilization were based on general chemical information derived from spectroscopic investigation do not yield detailed information at the level of molecular subunits. Such information, however, is essential to understand the mechanisms of the desired stabilization of compost and native C in soil.

- Furthermore, possible effects of compost maturation on the composition and stability of SOM in tropical soils are completely unknown, and therefore, a great challenge for organic soil chemistry.

II. Methodological approach

- The effects of differently stabilized composts on SOM can be investigated by incubating compost samples obtained from different composting phases in soil. Measuring the respiration (CO_2 release) and the molecular composition of the composts and compost-soil-mixtures by pyrolysis-mass spectrometry are well suited to disclose which composts have the best effects on SOM stability, and which compound classes contribute to the SOM enrichment and stabilization.

- The two complementary mass spectrometric methods, pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and pyrolysis-field ionization mass spectrometry (Py-FIMS) have potential to provide the most detailed molecular-level information about the parent materials of composts, their transformations during composting and their effects on soil organic matter.

III. Main results and new evidence

- Composting of raw coffee pulp waste led to an increased cation exchange capacity and of exchangeable cations except K. The CEC and all macronutrients except Ca reached their maximum concentrations at the end of composting. Addition of an equivalent of 48 t compost ha^{-1} to a tropical Nitisol significantly increased the Ca and K concentrations in the amended soils, irrespective of the age of the compost, while the CEC was not significantly affected ($p<0.05$). This is plausibly since the Nitisol had a large CEC similar to that of the compost because of the high clay content of the Nitisol.

- Raw coffee pulp waste is phytotoxic as low germination indices of 3% with cress and 26% with radish clearly show. A short composting that reaches the thermophilic phase overcomes the phytotoxicity to achieve germination indice over 80%.

- Soil amendment by addition of differently stabilized composts from three parent materials significantly increases the CO_2-C release. Between 35 % and 69 % of the total mineralized C is released in the first three of 14 weeks. The cumulative respiration curves of compost-soil-mixtures show two distinct phases, an initial very intensive phase followed by a slower second phase, which are best described by a first order plus linear model: $C_t = C_1\left(1-e^{-k_1 * t}\right)+K_2 t$.

- The C immobilization in soil is always largest in the treatments with relatively “fresh” compost taken immediately after the thermophilic phase of composting. This is derived from the lowest percentage decrease in the C_{org} concentration, ranging from 1 to 2 % compared to 5 to 10 % in soils amended with non-composted parent materials and longer matured composts.

- The Py-FIMS shows that a large portion of labile components of the compost material is transformed or degraded in the amended soil. This organic matter turnover in the compost-soil-mixtures is accompanied by enrichments in thermally stable carbohydrates, N-compounds and peptides which are most pronounced in treatments that received the “thermophilic” compost.

- Strong interactions of molecules that were formed during the thermophilic composting phase with reactive surfaces of the clay minerals from the Nitisol explain this unexpected result. Obviously, these reactions do not take place when the compost has undergone a longer maturation phase likely because the humic macromolecules formed are less reactive.

- In the Rostock long-term pot experiments on humus formation, compost application altered the SOM composition by enrichments in lipids at the expense of carbohydrates relative to the mineral fertilized treatments. Py-FIMS and Py-GC/MS revealed in good agreement unchanged proportions in all compound classes between the 19th and the 34th year. Since compost was not applied any longer after the 19th year and one treatment involved aeration by tillage, the constancy in SOM composition is a strong hint to the stability of compost-derived organic matter.

- This stability was clearly indicated by Py-FIMS through temperature-resolved volatilization curves for phenols/lignin monomers, lipids and alkylaromatics showing relative enrichments and shifts in their thermal evolution towards higher temperatures in the compost treatments over time.

IV. Conclusions & outlook

- The data from the compost incubation experiments provide clear evidence that a short thermophilc compositing phase is sufficient to sanitize coffee pulp waste and/or fruit waste and to get the maximum C sequestration in soil.

- The broad application of this evidence in developing countries contributes to (i) solving an urgent waste disposal problem, (ii) increasing the SOM content with all related benefits, and (iii) recycling plant nutrients that were previously taken up from the soil. It may have considerable economic implications because the composting periods can be greatly reduced from 6 to 8 months to three weeks.

- In forthcoming studies other soil types, mixtures of compost parent materials will be tested, and the experimental approach will be expanded to include field trials with typical crops grown in Ethiopia.

7.4 Curriculum Vitae

I. Personal Information

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II. Education

Since July 2010	Phd student of University Rostock at the chair of soil science, Germany
2006-2008	MSc degree in Environmental Science (chemistry stream) from Addis Ababa University, Ethiopia.
Sept. 2004-Feb.2005	Higher Diploma in professional teachers education from Addis Ababa University, Ethiopia
1997-2001	BSC Degree in Chemistry Alemaya University
1994-1997	Ethiopian School Leaving Certificate (E.S.L.C), Hailemariam Mamo, Ethiopia

III. Professional experience

Sept. 2002-2005	Graduate assistant at Adama University, Ethiopia.
Oct. 2003-July 2005	Department head of applied and natural sciences in Adama University
Feb. 2008-2012	Lecturer of environmental chemistry, Adama University, Ethiopia

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