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Lipid signature of the microbial community structure during composting of date palm waste alone or mixed with couch grass clippings

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ABSTRACT

The content of total lipids and fatty-acid methyl esters (FAMEs) identified by GC–MS analysis was followed during composting of date palm waste (DPW) alone or mixed with couch-grass clippings (DPGC). The DPGC compost contained more fats and FAMEs (FAs) than did the DPW compost. Total lipid content decreased during both composting processes with an increase towards the end of DPW composting. The initial fatty acid composition in both composts showed that the DPGC mixture was richer in microbial biomass than the DPW compost. The lignocellulose nature of the date palm substrate probably obstructed the proliferation and development of a broad category of microbial biomass. The appearance of a series of odd-numbered carbon and branched FAMEs was recorded only toward the end of DPW composting. This confirms the low level of biodegradation of date palm substrate rich in substrates that presented difficult access to micro-organisms, as confirmed by a slower degradation. DPW compost required longer to reach similar maturity to DPGC that of the mixture. Calculating the relationship between the various bacterial groups showed that bacteria strongly prevailed over fungi during both DPW and DPGC composting. The follow-up of these ratios in both composts showed that the stabilization phase was not complete in DPGC compost, but that it reached a very advanced stage during DPGC composting after the 8-month trial. An increase in G^+/G^- and G^+/fungus ratios toward the end of DPW composting indicated that composting had just started in the DPW. The variations in the levels of the different steroid compounds identified in the two composts showed that dehydration was stronger in the DPGC mixture than in DPW. An increase of the level of stigmastanol acetate (24-ethylcholestanol), methyl cholestadiene (campestadienes), and cholestanol acetate (cholestanol) in DPGC compost suggests the stimulation of microbial organisms and/or micro-fauna capable of performing the hydrogenation of the corresponding sterols. This microbial activity fluctuated during DPW composting following the bioavailability of substrate that was steadily degraded. The appearance of stigmastadienone toward the end of DPW composting confirmed that composting had just started after the full 8 months.

Introduction

The study of compost's organic matter composition has long been regarded as important in understanding how bio-waste can be processed. Several authors have studied and characterized humic substances (e.g. Sànchez-Monedero et al., 1999; Amir et al., 2006). This major fraction formed during the biotransformation of raw organic matter by composting provides useful information about the compost's maturity and stability. Lipids, which only constitute a minor fraction of the organic matter, also play an important role in soil processes (Stevenson, 1982). They affect the

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structural stability of amended soils and the water retention capacity and biodegradation-humification balance in compost and soil. These hydrophobic compounds are often co-extracted with humic substances (Réveillé et al., 2003). The lipids in soil are mainly of plant and microbial origin (Amblès et al., 1991). Analyses of these molecules can help to refine carbon turnover models and enhance our comprehension of the microbial community structure and associated nutrient flux. The microbial biomass is embryonic as it acts as a catalyst of major organic matter transformations and constitutes a nutrient pool with rapid turnover compared to soil organic matter (Bååth and Anderson, 2003). Several studies have focused on phospholipid fatty acid (PLFA) analysis to monitor the microbial community structure responsible for the biological breakdown that occurs during the co-composting process (Boulter et al., 2002; Barje et al., 2008; Jindo et al., 2012). Hachicha et al. (2009) studied the lipid fraction by examining the modifications of the fatty fraction during co-composting of sewage sludge with poultry manure and evaluated the quantity and quality of fats in the end product. Monitoring variations of different lipid structures (primarily FAMEs and sterols) during the humification process provides information about the state, maturity, and transformation of organic matter components (Dinel and Nolin, 2000). Bacteria and fungi are the principal microbial groups responsible for organic matter (OM) degradation. They are responsible for the rapid rise of temperature during the first days of composting (Abouelwafa, 2009). One of the methods used to estimate fungal and bacterial biomass is the measurement of the specific chemical components of these two microbial groups. Indeed, the composition of phospholipid fatty acids differs between bacteria and fungi (Tunlid and White, 1992). One major advantage of FAME measurements is that they enable the separation of large functional groups within the soil microbial community. Gram-negative bacteria contain more hydroxylated and cyclopropyl fatty acids (Zelles, 1997) as well as oleic acid (C18:1) (Fang et al., 2000; Bastida et al., 2008), while Gram-positive bacteria contain more branched fatty acids at iso and anteiso positions (Klamer and Bååth, 1998; Steger et al., 2003). Fatty acids—mainly linoleic acid (18:2)—have been used as a signature of saprotrophic and ectomycorrhizal fungi belonging to the phyla Zygomycota, Ascomycota, and Basidiomycota (Klamer and Bååth, 2004). However, a major inconvenience of phospholipid fatty acid (PLFA) analysis is that none of the FA is a fully specific indicator for certain microbial groups. As yet, we know relatively little about the PLFAs, the structure and complexity of compost's microbial populations, and the overall functioning of the complex microbial assemblages.

Sterols are another lipid fraction present in practically all eukaryotic cells. More than 100 sterols have been identified, including animal cholesterols, plant stigmasterols and b-sitosterols as well as fungal ergosterols (Volkman, 1986). Assaying for these components can indicate the state of advancement of composting of the organic substrates (Leeming et al., 1996; Réveillé et al., 2003; Jardé et al., 2005). In fact, many studies have reported changes in organic matter during composting (Réveillé et al., 2003; Jardé et al., 2005). The objective of this study is to achieve a better understanding of the microbial assemblages involved in lignocellulosic substrate composting and to estimate the diversity of the microbial community structures responsible for the biodegradation. This was accomplished by analyzing the composition of the lipid fraction and following qualitative and quantitative variations in the levels of different FAMEs identified during composting. The total lipid quantity was followed during the composting of date palm waste (DPW) alone or mixed with waste couch grass (DPGC) over a period of 14 months. The FAME profiles and sterol composition were established using GC/MS to assess the role of bacteria and fungi in the decomposition process.

Materials and methods

Work location

Marrakech, Morocco, is well known as a city of green spaces and date palms; the large quantities of green waste resulting from public and private gardens is estimated at 38% of the city's total waste (WRMTH, 2003). The waste has a negative impact because of its bad smell. Green waste is generally dumped with other wastes such as organic household refuse, which increases the impact of this waste on the environment. Together, the wastes contribute to an increase of the fermentable fraction and of the leachate load. Therefore this waste represents an important loss of value in terms of recycling and of production of an organic amendment by composting. The treatment of the waste by composting will reduce overloading of the municipal refuse tips and make available an organic soil amendment that can be used in the city's gardens and seedbeds, also reducing municipal expenditure on imported peat.

Composting trials

Two waste mixtures were prepared in the form of a windrow.

The first windrow, DPW (1), was composed of date palm waste (fronds + stem fragments), roughly chopped into small to medium-sized pieces (2–50 cm). Windrow 2 (DPGC), was composed of a mixture of 50% palm waste and 50% couch grass clippings.

The mixtures were moistened to 60% humidity, and covered with tarpaulins. The windrows were built on a composting platform of 2400 m^2 surrounded with a metal frame built in such a way as to prevent evaporation and drying of the windrows. Aeration was provided by regular manual mixing. Temperature was monitored daily at different levels in the windrows for a period of 8 months.

Sampling

The process was followed for 14 months. For analysis purposes, sub-samples were taken from 10 different points of the thoroughly mixed compost pile (bottom, surface, side, and center). The samples were refrigerated until analysis.

Lipid extraction

The lipids were extracted using an accelerated solvent extractor (Dionex ASE 100) that works under pressure with a dichloromethane/methanol solvent subsequently evaporated off to yield the lipids.

Methylation by the complex BF3–methanol

A known mass of lipid was dissolved in methanol and methanol–BF_3 was added at a ratio of 1 ml/100 mg lipid, according to Sakamoto et al. (2004). The reaction mixture was refluxed for 15 min at 70 °C under an inert atmosphere. It was then hydrolyzed with distilled water. The methylated acids were extracted with chloroform, washed with water, and neutralized with a solution of saturated sodium bicarbonate. The resulting chloroform solution was dried over magnesium sulfate and evaporated to dryness. The methylated lipids obtained were weighed.

Acetylation of alcohols (steroids)

The lipids were dissolved in excess acetic anhydride. A catalytic quantity of pyridine was added (3–4 drops). The acetylation reaction was initiated by heating for about 15 min at 70 °C. After stirring overnight at room temperature, the mixture was hydrolyzed, still