Applied Soil Ecology 75 (2014) 150–161

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Sewage sludge processing determines its impact on soil microbial community structure and function

S. Mattana[a,e,*], B. Petrovičová[b], L. Landi[c], A. Gelsomino[b], P. Cortés[d], O. Ortiz[a,e], G. Renella[c]

[a] CREAF, Cerdanyola del Vallès 08193, Spain
[b] Department of Agricultural Sciences, Mediterranean University of Reggio Calabria, Feo di Vito, I-89124 Reggio Calabria, Italy
[c] Department of Agrifood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 28, I-50144 Florence, Italy
[d] Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain
[e] Department of Animal and Plant Biology and Ecology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Spain

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 22 October 2013
Accepted 9 November 2013

Keywords:
Post-digestion treatments
Sludge amendments
Heavy metals
Organic contaminants
Microbial activity
Bacterial community structure

ABSTRACT

Composting and thermal drying are amongst the most commonly used post-digestion processes for allowing sanitation and biological stabilization of sewage sludge from municipal treatment plants, and making it suitable as soil conditioner for use in agriculture. To assess the impact of sludge-derived materials on soil microbial properties, fresh (LAF), composted (LAC) and thermally dried (LAT) sludge fractions, each resulting from a different post-treatment process of a same aerobically digested sewage sludge, were added at 1% (w/w) application rate on two contrasting (a loam and a loamy sand) soils and incubated under laboratory conditions for 28 days. Soil respiration, microbial ATP content, hydrolytic activities and arginine ammonification rate were monitored throughout the incubation period. Results showed that soil biochemical variables, including the metabolic quotient (qCO_2), were markedly stimulated after sludge application, and the magnitude of this stimulatory effect was dependent on sludge type (precisely LAT > LAF > LAC), but not on soil type. This effect was related to the content of stable organic matter, which was lower in LAT. Genetic fingerprinting by PCR–DGGE revealed that compositional shifts of soil bacterial and, at greater extent, actinobacterial communities were responsive to the amendment with a differing sludge fraction. The observed time-dependent changes in the DGGE profiles of amended soils reflected the microbial turnover dependent on the sludge nutrient input, whereas no indications of adverse effects of sludge-borne contaminants were noted. Our findings indicate that composting rather thermal drying can represent a more appropriate post-digestion process to make sewage sludge suitable for use as soil conditioner in agriculture.

1. Introduction

Use in agriculture of organic residues from a range of human activities can be regarded as an increasingly attractive strategy to reduce landfill disposal and maintain soil fertility (Ajwa and Tabatabai, 1994; Soe et al., 2004; Singh and Agrawal, 2008). In agricultural soils, organic wastes promote the formation of stable aggregates and improve soil aeration, water-holding capacity, cation exchange capacity, and enhance soil organic C storage (Lal, 2004). Amendment with organic residues can be of great significance to contrast the decline in soil fertility especially in European semi-arid Mediterranean croplands, which are characterized by soils prone to decline in the organic matter content (Zdruli et al., 2004; Larchevêque et al., 2006; Diacono and Montemurro, 2010).

In EU, the increasing production of sewage sludge from municipal wastewater treatment plants is nowadays considered a question of public concern. Sewage sludge may be disposed by incineration or land filling, or maybe transformed into a resource and recycled as soil conditioner for land reclamation or use in agriculture. However, it is known that sewage sludge produced in municipal treatment plants constitutes a highly heterogeneous matrix containing a large variety of potentially harmful pathogens and pollutants. For safe use in agriculture as soil conditioner, sewage sludge must comply with current mandatory limits: the EU Directive 86/278/EEC on sludge (European Commission, 1986) compels the raw sludge stabilization and states the upper limits of heavy metal content. Moreover, the latest EC Working Document on sludge (3rd draft; European Commission, 2000) has revised the EC Directive 86/278/EEC to include the mandatory limits for

* Corresponding author. Tel.: +34 93 5814851; fax: +34 93 5814151.
E-mail address: s.mattana@creaf.uab.es (S. Mattana).

0929-1393/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsoil.2013.11.007

organic pollutants (AOX: absorbable organic halogens; LAS: linear alkylbenzene sulphonate; DEHP: diethylhexylphthalate; NPE: nonylphenol ethoxylates; PAH: polyaromatic hydrocarbons; PCB: polychlorinated biphenyls; and PCDD/PCDF: dioxins/furans).

In these wastes, the occurrence of compounds potentially harmful to human health or to the environment has promoted the search of adequate technologies for post-digestion treatment able to decrease their intrinsic toxicity. Composting and thermal drying are among the most commonly used post-digestion processes for allowing concentration, sanitation and biological stabilization of aerobically digested sewage sludge. In particular, composting represents an effective strategy for transforming and recovering a wide range of biological wastes in agricultural products (Keener et al., 1993). Thermal drying at relatively high temperatures is also considered a feasible technique to reduce the waste volume, thus saving transport costs over a broad area (Stasta et al., 2006). Literature on use of sewage sludge as soil conditioner is somewhat vast (Ortiz and Alcañiz, 1993; Bååth et al., 1998; Bramryd, 2001; Quemada and Menacho, 2001; Petersen et al., 2003). The environmental impact of composted sewage sludge on soil microbial activity was found to be lower but more persistent than that of non post-treated sludge (Bernal et al., 1998; Mattana et al., 2010). Further, the effects of thermally dried sewage sludge on soil physical and chemical properties, plants, soil fauna and selected microbiological activities have also been studied, and differences with simply digested sludge were correlated with changes in the degradability of organic materials during the thermal treatment (Ojeda et al., 2006; Domene et al., 2008; Ramírez et al., 2008). In particular, the impact of sewage sludge addition on soil microorganisms has been investigated at field and laboratory scale, by use of culture-dependent or culture independent methods (Pascual et al., 1998; Crecchio et al., 2001; Ros et al., 2006; Gomes et al., 2010; Mattana et al., 2010). Using a PLFA (phospholipid fatty acid) profiling approach, Bastida et al. (2008) showed that in soil amended with sewage sludge or sludge-based compost the molecular structure of microbial communities changed considerably. This finding was also supported by Ros et al. (2006), who used a PCR–DGGE approach to investigate molecular changes in microbial diversity after soil incorporation of different types of compost. In general, contrasting microbial responses (i.e. stimulation vs. inhibition) have been observed in sludge-amended soils and they were found to be related to the increased mobility of nutrients or harmful compounds, which was primarily promoted by the post-digestion treatment process (Böhme et al., 2005; Renella et al., 2007b; Gomes et al., 2010). In previous soil incubation experiments, Domene et al. (2008) and Mattana et al. (2010) have observed marked (but transient) changes in soil microbial activity immediately following amendment with an aerobically digested sewage sludge obtained from a wastewater treatment plant and further processed by different post-digestion treatments. However, these works focused on general ecotoxicological aspects of treated sludge fractions and no information on selected microbial responses has been provided.

Aims of this work were to investigate the biochemical responses and compositional changes of bacterial community structure in two contrasting soils soon after amendment with three sewage sludge fractions obtained after aerobic digestion (LAF), aerobic digestion + composting (LAC) or aerobic digestion + thermal drying (LAT) stabilization processes. A special emphasis has been given to soil Bacteria and Actinobacteria, which are intimately involved in most nutrient turnover and organic matter decomposition and whose compositional shifts are widely regarded as an early indicator of environmental impact (Piao et al., 2008). We hypothesized that post-treatment processes can significantly affect sludge properties (**H1**); sludge addition to soil stimulate microbial responses and compositional shifts in the bacterial community structures which are dependent on single sludge properties (**H2**); the degree of stability of the sludge organic matter plays a major role in regulating soil microbial responses and bacterial community structure (**H3**).

2. Materials and methods

2.1. Soils

Soil samples were collected from the top layer (0–20 cm) at two field sites located in Catalonia (Spain), named La Granadella (Granadella soil) (Lleida) and Serra de Prades (Prades soil) (Tarragona). Granadella soil is classified as a Hypercalcic Calcisol (IUSS Working Group WRB, 2006), is presently being cultivated with olive tree (*Olea europea* L.), and is characterized by a mean annual rainfall and air temperature of 500 mm and 15 °C, respectively. Prades soil is classified as a Haplic Regosol (IUSS Working Group WRB, 2006), is currently kept under grassland management, and is characterized by a mean annual rainfall and air temperature of 725 mm and 12 °C, respectively. After sampling, soils were sieved at <2 mm and stored at 4 °C for 10 days prior to use. Main soil physical-chemical properties were determined by standard methods (Sparks, 1996).

2.2. Sewage sludge fractions, origin and properties

Sewage sludge fractions used in the present research (fresh, LAF; composted, LAC; thermally dried, LAT), all derived from the same aerobically digested batch of a municipal wastewater treatment plant located in Banyoles (Northern East Spain) and further processed by different post-digestion treatments. Precisely, fresh sludge (LAF) was as it was from the treatment plant. Composted sludge (LAC) was obtained by composting LAF with pinewood splinters as bulking agent (1:4.5, w/w) under forced aeration and controlled moisture conditions in bioconversion tunnels for 15 days, during which the temperature of the biomass reached 60 °C. Soon after the thermal phase had ceased, the mixture was sieved at <10 mm (90% pine splinters were recovered) and then allowed to mature for further 3 months (curing phase). Thermally dried sludge (LAT) was produced by thermally treating LAF for 45 min in a rotating cylinder heated with injected hot (130–150 °C) air until a solid product with a granular structure and a moisture content equal to approximately 14% had been obtained. Main chemical properties of LAF, LAC and LAT fractions are shown in Table 2.

Chemical stability of the organic fraction occurring in LAF, LAC and LAT was assessed by calculating the percentage of organic amount resistant to acid hydrolysis according to Rovira and Vallejo (2002) with minor changes. Briefly, an amount of finely ground sludge (equivalent to 1.5 g dry weight, dw) was initially treated with 10 mL of H_2SO_4 (72%, w/w) at room temperature for 3 h and after the addition of 400 mL of distilled water the mixture was boiled with reflux at 420 °C for further 5 h. Following centrifugation, the aqueous supernatant was discarded and the precipitate containing the acid resistant organic faction recovered for further analysis. Organic C content was analytically determined by a dry combustion procedure (560 °C, 4 h) using a 1.724 conversion factor to calculate the organic matter content. Each analysis was done in triplicate.

Elemental analysis of P, K, Cd, Cr, Cu, Hg, Ni, Pb and Zn was carried out by ICP–MS according to ISO 11885 (1996). Polychlorinated dibenzodioxins and dibenzofuranes (PCDD/F) were measured by HRGC-HRMS, polychlorinated biphenyls (PCB) by HRGC-ECD, di-(2-ethylhexyl) phthalate (DEHP) and nonylphenols (NPE) by HRGC–MS. Polycyclic aromatic hydrocarbons (PAH) and linear alkylbenzene sulphonates (LAS) were determined by HPLC equipped with a fluorescence and UV detector, respectively. Values for each pollutant group were reported as suggested in