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Sewage sludge addition modifies soil microbial communities and plant performance depending on the sludge stabilization process

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ABSTRACT

Despite the widespread use of sewage sludge as an organic amendment to improve soil stability and plant productivity, relatively little is known about how the different sludge stabilization processes affect the microbial composition and diversity of the sludge and the soil microbial populations as well as plant performance. In this study, the effects caused by addition of thermophilic aerobic (ATAD) and mesophilic anaerobic (MAD) sludge and inorganic fertilization on soil microbial community structure and diversity was assessed by pyrosequencing of 16S and 18S rRNA genes. Melon (*Cucumis melo* L., cv. Giotto) was used as model crop and its performance (growth and physiological state) was monitored together with changes in soil chemical parameters. Our results showed that the stabilization process of sewage sludge determined the feasibility of the final by-product as an organic amendment by altering in different manner the soil environment and modifying the soil microbial community structure and functioning. Changes in soil microbial community were related more to changes in the soil chemical environment rather than to the introduction of sludge-borne microorganisms. We also have shown that changes in a single physicochemical parameter (electrical conductivity) due to sludge application are associated with a pronounced shift in microbial community structure and activity as well as in plant performance. Along these lines, we showed that the application of ATAD sludge into soil resulted in less pronounced changes in its chemistry and microbial community structure, while enhancing soil microbial activity and plant performance. This study shows, therefore, that ATAD sludge could be applied as an excellent alternative to MAD sludge or inorganic fertilization.

1. Introduction

More than ten million tons of sewage sludge (dry matter) are produced in Europe annually, of which approximately 37% are used in agriculture (Milieu et al., 2010). In Spain, over one million tons per year (83% of the sludge produced) are applied to land (MARM, 2011; Milieu et al., 2010). Land application of sewage sludge, which is encouraged by the Council Directive 91/271/EEC (European Commission, 1991), provides nutrients and organic matter that confer significant positive agricultural benefits (Insam et al., 2015; Singh and Agrawal, 2008), and is of special interest in the Mediterranean region due to a widespread lack of soil organic matter (García et al., 2000). The quality of sewage sludge is strongly dependent on three main factors: (1) the original inputs into the sewers; (2) the wastewater treatment process; and (3) the subsequent sludge stabilization process (Milieu et al., 2010). Autothermal thermophilic aerobic digestion (ATAD) is listed as an “advanced” treatment in the Proposal for a Directive of the European Parliament and of the Council on spreading of sludge on land (European Commission, 2003), as well as a technology capable of producing Class A biosolids (USEPA, 2003). Mesophilic anaerobic digestion (MAD), on the other hand, is one of the most widely used processes for the stabilization of sludge in wastewater treatment plants (Ahn and Forster, 2000) and is considered a “conventional” treatment and a technology capable of producing Class B biosolids (European Commission, 2003; USEPA, 2003).

The presence of oxygen in ATAD produces a more efficient mineralization that reduces the concentration of labile carbon (C)

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and bioavailable nutrients (Bernal et al., 1998; Odlare et al., 2011). Likewise, higher process temperatures result in larger stabilization rates and a higher efficiency of solids' degradation (Juteau, 2006). Aerobiosis, temperature and physical-chemical parameters are the main factors controlling microbial communities in organic residues (Han et al., 2011; Hayes et al., 2011; Insam et al., 2010; Kim et al., 2002; Liu et al., 2010; Piterina et al., 2006; van Lier et al., 1993), and thus it is to be expected that the sludge microbiota are ultimately determined by the stabilization process.

The addition of sewage sludge alters the physical and chemical properties of soils due to organic matter input. This addition may significantly modify soil structure, increase soil moisture and porosity, and enhance humus content and cation exchange capacity (Barzegar et al., 2002; Korentajer, 1991). Furthermore, the application of sludge to soil leads to a reduction in pH and an increase in electrical conductivity (EC) (Antolín et al., 2005; Pascual et al., 2009). Overall, the use of organic amendments has been associated with an acceleration of microbial development and activity (Bailey and Lazarovits, 2003), together with changes in microbial community composition (Marschner et al., 2003). These shifts are likely associated with changes in the functional capabilities of soil microbial communities (Fierer et al., 2012), and can in turn alter soil fertility and crop production (Kelley et al., 1984; Min-Jian, 1997; Singh and Agrawal, 2008). Finally, elevating nitrogen (N) inputs, as with sludge amendments, might increase plant performance (LeBauer and Treseder, 2008).

Here, we integrated the effects of sewage sludge addition on soil chemical properties, microbial genetic and functional profiles, as well as plant performance, in a comparative study of two different sludge stabilization processes (ATAD vs MAD). Specifically, we aimed to evaluate: (1) how the distinct stabilization processes impacted the sludge microbial community; and (2) the effects of the addition of ATAD and MAD sludge separately on the soil physical-chemical properties, soil microbial community structure and activity, and plant growth and physiology, in relation to the addition of mineral fertilization. The stabilized sludges were produced from the same original incoming raw sludge and their application into an agricultural soil was performed at a microcosm scale. We hypothesized that: (1) the sludge stabilization process would shape its chemistry and its microbial community composition; and (2) different nutrient sources (ATAD and MAD sludge versus inorganic fertilizer) would influence the soil microbial community as well as plant performance. We postulated that due to the nature of the different stabilization processes, amending soils with ATAD sludge would affect to a lesser extent the soil chemical properties, having therefore a reduced impact on the soil microbiota (in terms of changing community structure, activity and metabolic profiles) but leading to a decreased crop production relative to the application of MAD-treated sludge.

2. Material and methods

2.1. Soils, organic amendments, experimental layout and growth conditions

Soils were collected from an agricultural land in Murcia (Southeast Spain). The topsoil layer (0–20 cm) was sampled and sieved (<3 mm). Both types of sewage sludge were collected from the Molina de Segura wastewater treatment plant (WWTP) (Murcia, Spain). The sludges were produced after: (1) ATAD digestion with thermal treatment as described by Lloret et al. (2012); and (2) MAD digestion (Table 1). Both sludges and soils were collected and stored at 4 °C until the set-up of the microcosm experiment. Four experimental treatments were performed in 2.8 L pots: (1) none (control soil); (2) mineral fertilizer; (3) ATAD sludge; and (4) MAD sludge. The mineral fertilizer (NH_4NO_3 and KH_2PO_4) and sewage sludges were applied at a rate of 1.8 g N kg^{-1} and 0.13 g P kg^{-1} soil (dry weight). After an equilibration period of 24 h, one melon seedling (*Cucumis melo* L., cv. Giotto) was transplanted into each pot. All plants were grown in a controlled environment greenhouse (28 °C) and harvested after 66 days when fructification became the predominant process. Pots were irrigated as needed to adjust soil water content to 60% water holding capacity (WHC) throughout the experiment. Soil samples were taken at the beginning and at the end of the experiment (before planting and after harvesting, respectively). One subsample from each pot was air-dried and used for chemical analysis. Another subsample was stored at 4 °C and used for soil microbiological parameters and another stored at −80 °C until molecular analysis. A full description of the soils, the organic amendments, the experimental layout and the growth conditions are shown in the Supplementary material.

Table 1
Chemical characteristics of ATAD and MAD sewage sludge (values on dry weight basis).

Parameters[a]	ATAD sludge	MAD sludge	UE limits[b]
Dry matter (%)	1.2	1.4	
pH	9.5	7.8	
EC (dS m^{-1})	8.9	14.0	
TOC (g kg^{-1})	346	315	
Total N (g kg^{-1})	183	129	
Total P (g kg^{-1})	16.7	14.3	
Total K (g kg^{-1})	8.0	8.0	
Total Cd (mg kg^{-1})	0.80	2.2	10
Total Cr (mg kg^{-1})	25.0	57.1	1000
Total Cu (mg kg^{-1})	208	314	1000
Total Fe (g kg^{-1})	14.8	29.1	
Total Ni (mg kg^{-1})	41.7	157	300
Total Pb (mg kg^{-1})	33.3	96.3	750
Total Zn (mg kg^{-1})	825	550	2500

[a] EC: electrical conductivity; TOC: total organic carbon. Both sludges were applied to soil at the same rate.
[b] Heavy metal limits for soils (European Commission, 2003).

2.2. Soil chemical and microbiological analysis and community-level physiological profiles

Soil chemical analyses were performed at the start and at the end of the experiment. The chemical parameters that were analyzed included EC, pH, C and N content and other macro- and micronutrients as well as heavy metals. The analysis of microbiological parameters included basal respiration and microbial biomass C (C_{mic}). Soil basal respiration was measured as CO_2 evolution from moist soil samples using continuous flow infrared gas analysis (IRGA) (Heinemeyer et al., 1989). From basal respiration, organic C and C_{mic}, the microbial quotient (C_{mic}/C_{org}) and the metabolic quotient (qCO_2, $\mu g\ CO_2–C\,g^{-1}C_{mic}\,h^{-1}$) were calculated. The CLPPs were assessed by using a micro-respiration technique (MicroResp^{TM}) which is a convenient and sensitive method to perform catabolic assays to characterize the functional potentials of the communities (Campbell et al., 2003). The different C sources consisted of six carbohydrates, five amino acids, and four carboxylic acids. Further details of the chemical and microbiological analysis and community-level physiological profiles (CLPPs) can be found in the Supplementary material.

2.3. DNA extraction and pyrosequencing of SSU rRNA gene

Total nucleic acids were extracted from 0.1 g of ATAD and MAD sludges and from 0.5 g of soil samples taken at the end of the experiment. Sludges were freeze-dried and soils ground prior to DNA extraction. DNA extraction was performed according to the protocol described by Ivanov et al. (2009) including some