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Original article

Effects of municipal solid waste compost, farmyard manure and chemical fertilizers on wheat growth, soil composition and soil bacterial characteristics under Tunisian arid climate

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ABSTRACT

The use of municipal solid waste compost (MSWC) as soil organic amendment is of an economic and environmental interest. However, little is known about the effectiveness of MSWC application on agricultural soil in northern Africa arid climate. We assessed the impact of five years' applications of different organic and mineral fertilizers on wheat grain yields and soil chemical and microbial characteristics. Soils were treated with MSWC at rates of 40 (C1) and 80 (C2) Mg ha^{-1}, farmyard manure at a rate of 40 Mg ha^{-1} (M), chemical fertilizers (Cf) and the combinations (C1Cf, C2Cf, MCf). Wheat grain yield was enhanced with all amendments. Parallel increases of heavy metal levels and faecal coliform were also recorded except for Cf treatments. Based on wheat grain yield, heavy metal and faecal coliform data, we determined the treatment effectiveness index (E_{xx}), calculated by dividing the pollutant increase ratio by the grain yield increase ratio. The treatment effectiveness index E_{C1} indicated lower faecal and heavy metal pollution with positive gains in wheat yields. Despite polluting effects on soil determined by the different treatments, no significant differences between treatments were observed in total bacterial count and soil bacterial community structure, as shown by 16S rRNA gene PCR-denaturing gradient gel electrophoresis banding patterns and 16S rRNA gene Length Heterogeneity-PCR analysis. According to the collected data, the use of MSWC at a rate of 40 Mg ha^{-1} might be recommended.

1. Introduction

In Tunisia considerable attention has been focused on municipal solid waste compost (MSWC), in order to reduce the volumes to be disposed in landfill and to provide a new organic amendment to compensate for the shortage of farmyard manure in the Tunisian farming system.

A potential environmental problem in the use of MSWC is the heavy metal pollution. This pollution may induce changes in the soil microbial community structure [34], which may

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influence plant yields and quality [41]. Moreover, the application of MSWC was shown to increase fecal coliform bacteria content and may cause the contamination of agricultural products [12]. Some studies have been conducted in African semi-arid climates showing soil morphological and chemical properties' improvement under MSWC treatment [28] and crop production enhancement when MSWC was combined with nitrogen fertilizer [29,39]. However, in the Tunisian arid climate, few preliminary studies have been conducted to assess the impact of MSWC application as an organic amendment and to define the best fertilizer rates. These studies were mainly focused on the effects on soil microbial biomass, plant yields, and heavy metals in different parts of the plant. Hamdi et al. [17] showed that after two farming seasons, the best wheat grain yield was obtained with the application of MSWC at a rate of 80 $Mg\,ha^{-1}$ plus chemical fertilizer. However, compost treatment led to the most important heavy metal concentrations either in soil or in different parts of the wheat plant with decreasing gradient from roots to fruit. Bouzaiane et al. [4] showed that, after two years, soil microbial biomass C and N increased when organic amendments were combined with chemical fertilizers, as compared to organic amendments alone, but decreased significantly when compost amount increases from 40 to 80 $Mg\,ha^{-1}$.

Systematic long-term investigations on the plant growth as well as on heavy metal and fecal bacteria concentrations and on the stability of the bacterial community structure in response to amendments are still lacking. To evaluate the response of the soil bacterial community structure, several methods have been proposed, like community-level physiological profiling [11] and fatty acid methyl ester analysis [44] or nucleic acid-based molecular techniques [22,32,31,13,9]. Some nucleic acid-based techniques such as denaturing gradient gel electrophoresis (DGGE) of amplified bacterial 16S rRNA gene were shown to be sensitive enough to detect significant changes in the microbial community structure in response to soil management practices and environmental conditions [37,16].

Our aim was to investigate the best amendments to use in the Tunisian dry climate that would enhance wheat grain yield yet limit pollution. We compared the effects of treatments based on MSWC, farmyard manure and chemical fertilizers, on wheat grain yield, soil heavy metal content and fecal coliform and total bacteria counts in a loamy-clay soil in the arid climate of Tunisia. For assessing effects on the soil bacterial community structure we compared bacterial 16S rRNA gene LH-PCR (Length Heterogeneity-PCR) and DGGE profiles from different treated soils.

2. Materials and methods

2.1. *Site description, experimental design and soil sampling*

The field trial was laid out in 1999 in Mornag (20 km south of Tunis 36°50′N 10°9′E, Tunisia) to investigate different management systems on wheat (*Triticum turgidum* subsp. Durum, var. Karim) cultivated soils. The climate is semi-arid with annual precipitation average varying between 200 and 400 mm and annual mean temperature of 18.6 °C. The field had been cropped with wheat without fertilization. The soil was classified as a clayey-loamy, Vertic Xero Fluvent, with 27% clay, 62% silt and 11% sand. Total K and Mg in the top soil surface (0–20 cm) were about 5650 and 3380 $mg\,kg^{-1}$, respectively. Exchangeable K, P and Ca were about 440, 25.02 and 9650 $mg\,kg^{-1}$ respectively. The parcel design was a randomized complete block with four replications. MSWC was obtained from sorted municipal solid wastes by aerobic composting process for 120 days in the composting plant at Beja (100 km northern of Tunis). Seven treatments were applied annually in autumn by including MSWC at rates of 40 (C1) and 80 (C2) $Mg\,ha^{-1}$, farmyard manure at 40 $Mg\,ha^{-1}$ (M), chemical fertilizers (Cf; 0.3 $Mg\,ha^{-1}$ NH_4NO_3, and 0.1 $Mg\,ha^{-1}$ P_2O_5), compost C1 plus chemical fertilizers (C1Cf), compost C2 plus chemical fertilizers (C2Cf) and farmyard manure plus chemical fertilizers (MCf). Plots without amendment were used as controls (T). All the organic residues were incorporated into soil by ploughing. Soils were sampled in 2005, after wheat harvest in five randomly selected positions at a depth of 0–20 cm. Collected samples were thoroughly mixed to give one homogenous sample and analyzed within 2 h for bacterial counts. For bacterial DNA analyses, 2-mm-sieved soil subsamples from each plot were stored at −20 °C.

2.2. *Chemical analysis of soil and organic residues and crop yield determination*

Soil and organic residues' pH were determined with a glass electrode pH meter in 1:2.5 soil to water ratio. Total nitrogen was determined by the Kjeldahl method as recommended by Brookes et al. [5]. Organic amendments and soil samples' organic C content was determined by dry combustion [43]. Heavy metal contents (Cu, Zn, Ni, Pb, Cr and Cd) were determined by atomic absorption spectrophotometer after acid digestion (nitric acid and chloridric acid, 3:1 ratio) according to Pauwels et al. [30]. A summary of the soil and organic residues' characteristics is reported in Table 1. After harvest, wheat grain yields were collected and weighed. Values were expressed in megagram per hectare ($Mg\,ha^{-1}$).

2.3. *Total bacteria and fecal coliform counts*

Cultivable populations of bacteria were estimated by plating serial dilutions (in sterile distilled water) of 10 g of soil onto TSA (Bio-Rad, France) containing 100 μg cycloheximide ml^{-1} to inhibit fungal growth and plates were incubated at 26 °C for three days. Enumeration of fecal coliforms was estimated by using the multiple-tube fermentation direct test as previously described [15]. Tube contents were incubated at 37 °C for 24 h. Positive tubes presenting gas in the inverted Durham tubes were used to inoculate another serial dilution of tubes at 44 °C for 48 h. The most probable number (MPN) was estimated by using an MPN table. Data from replicate readings were expressed as colony forming units (CFU) g^{-1} of dry soil.

2.4. *Bacterial community structure analysis*

2.4.1. PCR-DGGE analysis

Total DNA was extracted from MSWC, farmyard manure and soils treated with different fertilizers. DNA extraction was performed using the Fast DNA Spin Kit for Soil