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Earthworm community and soil microstructure changes with long-term organic fertilization

Running head: Soil health assessment in organic fertilization

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Alcira Sunilda Valdez[a,b] , Àngela Dolores Bosch-Serra[a,*], María Rosa Yagüe[a], Rosa Maria Poch[a], Elena Puigpinós[c]

[a]Department of Environment and Soil Sciences, University of Lleida, Av. Alcalde Rovira Roure 191, E-25198, Lleida, Spain.
[b]National University of Asunción, Faculty of Agricultural Sciences, San Pedro de Ycuamandyyu, PY-020101, Paraguay.
[c]Department of Agriculture, Livestock, Fisheries and Food, Generalitat de Catalunya. Av. Alcalde Rovira Roure 191, E-25198, Lleida, Spain.
*Corresponding author. E-mail address: angela.bosch@macs.udl.cat

ORCID identifiers

Alcira Sunilda Valdez-Ibañez.   0000-0002-6658-9415
Àngela D. Bosch-Serra.   0000-0003-0428-2036
María Rosa Yagüe-Carrasco.   0000-0002-5296-2688
Rosa Maria Poch-Claret.   0000-0001-8639-4204
Elena Puigpinós.   0000-0002-4794-6085

Abstract

The aim of this study was to evaluate the effects of sludge compost (SC) in two rates and pig slurry (PS) on soil quality, in the framework of a field experiment (19-year-old) in a Mediterranean rainfed system. The treatments were compared with mineral fertilization (MF) plus a control treatment (no N fertilization). Soil microstructure and types of voids, earthworm community and its bioturbation were studied using micromorphological methods. Two earthworm species, Koinodrilus roseus and Nicodrilus trapezoides were identified; the latter was not present in the SC treatments. Earthworm abundance and biomass were not affected by fertilization. Pig slurry increased bioturbation associated with earthworm activity, improved soil microstructure (crumb type) and increased the biopore presence (compound packing voids). The control and MF plots showed a platy to massive microstructure with an absence of faunal chambers. In SC plots, non-mixed soil-organic materials were observed and soil vughs were not visible. Composition differences between SC and PS and the total amount of OM applied may have had an impact on the activity and species of earthworms; such changes can be an early indicator of further potential impacts on soil quality however, further contaminant studies are needed to validate this initial assessment.

Keywords: dryland agricultural system, pig slurry, sludge compost, soil bioturbation, soil micromorphology.

Abbreviations: CO, control; DM, dry matter; MF, mineral fertilizer; OM, organic matter; PS, pig slurry; SC, sewage sludge compost.

Introduction

In semiarid Mediterranean agricultural systems, the application of organic fertilizers has focused on strategies to improve productivity, particularly on the efficient use of nitrogen (Bosch-Serra et al. 2015). However, the use of urban and agricultural/livestock organic wastes should be based on objective criteria to maintain soil quality (Kibblewhite et al. 2008) and its productive potential as part of sustainable agriculture (Singh 2018). Therefore, it is necessary to consider that repeated applications of organic fertilizers in long term together with different quality or characteristics (e.g. C:N ratio, nutrient content, heavy metals and/or organic pollutants) could affect the quality of the soil (Gómez-Muñoz et al. 2017).

Spain is the first producer of pork in the European Union (European Union 2018, 30 million pigs yearly). The high volumes of pig slurry (PS) obtained are mainly used as fertilizer. The PS is characterized by high nutrient content, mainly as ammonium-N; a low C:N ratio and some amounts of heavy metals (mainly Cu and Zn) that can vary according to the feeding and sanitary status of the animal in intensive production (Sánchez and González 2005; Yagüe et al. 2012).

The present and future increase of human population goes hand in hand with the availability of a solid sewage sludge resulting from different treatment processes (Sharma et al. 2017). This sewage sludge for agricultural use purposes could face technical problems or have a limited use because of its pollutant load (i.e. pathogenic microorganisms, organic compound and heavy metals) (Alvarenga et al. 2015, 2017), since its agricultural use has been regulated (86/278/EEC, European Union 1986). The sewage sludge is commonly used as a co-substrate composted with others organic residues derived from forestry or agricultural wastes (i.e. tree bark, sawdust, wood chip), namely sludge compost (SC). This composting process improves its quality as it reduces its water content, decreases contamination with pathogenic microorganisms by stabilization of the product and dilutes heavy metal content (Gomez 1998;

Alvarenga et al. 2015; Onwosi et al. 2017; Asses et al. 2018). The SC is an organic fertilizer with high C content (Gómez-Muñoz et al. 2017; Głąb et al. 2018) and its application increases organic matter in soil (Paetsch et al. 2016). Nevertheless, Renaud et al. (2017) demonstrated that contaminant concentrations required by current legislation might not be properly translated into the expected potential effects on soil quality (e. g. soil biology effects).

The evaluation of soil quality is complex. Bioindicators can be used, such as soil earthworms (Shepherd et al. 2008), because of their limited mobility and high sensitivity to changes in the soil properties which are generated by modifications in agricultural practices or by the introduction of contaminants (Paoletti 1999). Application of organic fertilizers generally affects the biological activity of the soil (Yagüe et al. 2016; Sharma et al. 2017) and in particular the earthworm population (Pérès et al. 2011; Murchie et al. 2015). The earthworms have a key role in the sustainability of the agrarian system (Singh 2018). This fact is mainly because they intervene in the decomposition of organic matter, in the flow of water, nutrients and gases in the soil, besides their contribution to the formation of structure and their improvement of soil porosity: size, distribution and pore network connectivity and its morphology (Blouin et al. 2013; Schon et al. 2017). The reciprocal relationship between fauna and soil structure has been well recognized (Kooistra and Pulleman 2001) and its study using micromorphological techniques, by the microscopical analysis of thin sections, has been applied for a long time (Bal 1970; Kooistra 1991). These studies include the description and quantification of different excremental types (from enchytraeids, oribatid, beetles, diptera larvae and earthworms) and other pedofeatures (plant fragments and void space) showing its utility to the interpretation of structural changes associated to faunal activities (Bruneau et al. 2004, 2005; Davidson et al. 2002, 2004; Davidson and Grieve 2006). Others authors estimated the soil porosity, either qualitatively (VandenBygaart et al. 2000) or quantitatively (Piron et al. 2012; Sauzet et al. 2017) as a result of the earthworm activity at soil micromorphology scale in thin sections. Recently, Domínguez-Haydar et al. (2018) combined the quantification of different taxonomic groups at a class level (e.g. Oligochaeta, Chilopoda and Diplodopa) and soil macroaggregate types, and related them with changes on the observed bioturbation (no quantified) in thin sections from rehabilitated coal mine technosols revegetated with diverse native plants and grasses. On the other hand, at the profile scale, Piron et al. (2012, 2017) developed a method for visual and morphological description of soil structure patterns produced by earthworm bioturbation in soils. This bioturbation is related to two types of biostructures: the burrows (formed by excavation or by the redistribution of the soil material caused by its own displacement) and the excrements or casts (which are ovoid granules produced by the ingestion of soil and organic material that subsequently are excreted on the surface or below it) (Lee and Foster 1991). Both bio-structures create different pore morphologies (Stoops 2003): compound packing pores (i.e. voids between elementary soil particles or aggregates), cavities in the form of stars, channels and chambers. The degree of alteration is influenced by the functional role of earthworms in the soil according to ecological groups: epigeic, endogeic and anecic (Lamandé et al. 2003; Pérès et al. 2014). Pirón et al. (2017) indicated that additional to the main indicators currently used to describe soil structure (type of porosity and the size and appearance of aggregates), these new typologies seemed relevant and complementary to the typical indicators of the abundance and activity of earthworms. The use of bioindicators linked to the earthworm communities (e.g. abundance, biomass, species richness, diversity) are sensitive to management changes and can provide information on soil quality (Paoletti 1999; Ponge et al. 2013). In particular, management practices as the incorporation of organic wastes containing specific compounds, when applied as fertilizers for a long term, could bring about the accumulation of such compounds with threshold risks for the soil organisms. The combination of both methodologies: the use of bioindicators linked to earthworm communities (abundance, biomass and classification at species and functional group level) and its effect on soil bioturbation (observation of thin sections at micromorphological scale) can be a new procedure to evaluate biological activity and soil quality. Indeed, the current trends for the assessment soil quality promotes the use of innovative indicators that complement the use of analytical, visual and digital diagnostic tools (Bünemann et al. 2018).

A recent study conducted by D'Hose et al. (2018) shows that the evaluations of agricultural practices that affect soil biota, in Mediterranean conditions, have focused on soil tillage. Meanwhile, Bertrand et al. (2015) mention the scarcity of field data describing the response of earthworm populations to different organic amendments. This fact contrasts with wide information in relation with biological indicators as microorganisms and enzymatic activities after organic fertilizer application in reviews by Diacono and Montemurto (2010) and Sharma et al. (2017), using standard methodologies.

The hypothesis of this work assumes that the use of these biological indicators can be strengthened if they are combined with visible changes in the structure of the soil, specifically with the description of areas with biostructures produced by the activity of earthworms (burrows and casts) and the types of associated pores. This research aims to evaluate the influence of the long term application of organic fertilizers in the maintenance of soil quality, through the use of the earthworm community and soil microstructure as indicators.

Materials and methods

Experimental framework

This study is included in a long term fertilization research study that started in 1997, and was carried out during the cropping season (October–June) 2015/16. The experimental site is located in Agramunt, Lleida, Spain (41° 46' 31.7"N, 1° 5' 40"E). The area has a dry Mediterranean climate according to Papadakis classification. During the 2015/16 period

(August 2015–July 2016), the average mean temperature was 14 °C (standard deviation, SD ± 7 °C), the accumulated precipitation was 346 mm and the reference evapotranspiration was 1085 mm yr^{-1} (Figure 1). This period was a representative one in terms of weather conditions. The characteristics of the top soil (0–0.25 m) were: loam texture (182 g clay kg^{-1}, 435 g silt kg^{-1}, 383 g sand kg^{-1}, Robinson pipette method), and the average gravimetric soil water content (Gupta and Wang 2007) at permanent wilting point (–1500 kPa) and at field capacity (–33 kPa) were 6 ± 0.9% (± SD) and 18 ± 0.9% (± SD) (w/w), respectively. The soil is non-saline (electrical conductivity, EC 1:5 w/v; 0.18 dS m^{-1}), with a basic pH (potenciometry, pH 1:2.5 w/v) 8.2, and it is calcareous (Bernard calcimeter method, 270 g $CaCO_3$ kg^{-1}). The soil was classified as Typic Xerorthent (Soil Survey Staff 2014).

The experimental field includes an annual rotation of winter cereals: wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), except in the 2013/14 season when the crop was rapeseed (*Brassica napus* L.) under rainfed conditions. Grain yield averages in the last twelve cropping seasons were similar for MF, PS and SC treatments, and they oscillated between 3150 and 3500 kg ha^{-1}. Seeding is done in October–November and harvest is June–July. Straw is removed from fields according to farmers' practice. The agricultural practices correspond to a rainfed agricultural system, which includes the incorporation of fertilizers (disc-harrowing, ~ 0.15 m depth) within 24h after their application.

Description of the experiment

Barley (*Nuria* variety, two-row barley) was sown in the 2015/16 season. Plots from five different N fertilization treatments were randomly assigned to three blocks (replications) at the start of the experiment and they were always maintained in the same position from 1997 onwards. The treatments were: control with no N added (named CO), mineral N fertilizer applied at a rate of 80 kg N ha^{-1} (named MF; 38% N applied before sowing and the rest as topdressing at a V6–V8 Zadok cereal physiological stage), pig slurry applied at a rate of 96 kg N ha^{-1} equivalent to 27 t ha^{-1} (named PS) and two rates of sludge compost: 88 and 174 kg N ha^{-1} equivalent to 4.4 and 8.7 t ha $^{-1}$ (named 4SC and 8SC, respectively). Thus, the criterion for the organic fertilizer dose to be applied was based on N supply. The rates applied with the organic fertilizers guarantee PK availability for the crop. The PS and SC were provided by pig farms and by a commercial officially licensed composting plant close to the experimental site and these were applied before sowing and buried within 24 h after application. The compost was obtained by mixing one part of municipal wastewater sludge combined with three parts of the agro-industrial and forest waste. The proportion of sludge was always the same; however, the proportion of agro-industrial versus forest waste depended on their availability. The compost always met the legal regulations for its agricultural use. In the 2015/16 cropping season, just before fertilization, a composite sample from each of these fertilizer products was taken and their dry matter (DM, gravimetric method at 105 °C) and organic matter (OM, ignition at 550 °C) were analysed. Dry matter values were 4.3% (w/w) for PS and 72% (w/w) for the sludge compost, and OM content was 64% over dry weight for PS (equivalent to 0.74 t OM ha^{-1} yr^{-1}) and 45% for the SC in two rates for both components expressed over dry weight (equivalent to 1.42 t OM ha^{-1} yr^{-1} and 2.84 t OM ha^{-1} yr^{-1} for 4SC and 8SC respectively). As a consequence of the long duration of the experiment (19 years), a gradient in soil organic matter content (Walkley and Black method) developed according to the different treatments. At the end of the 2015/16 cropping season, soil OM average values were 1.5, 1.7, 1.8, 2.1 and 2.3% for CO, MF, PS, 4SC and 8SC, respectively. The heavy metal content of SC in the 2015/16 season was: Co = 2.1, Cu = 83, Zn = 293, Cr = 28, Cd = 0.49, Ni = 20, Pb = 19 and Hg = 0.3 all of them expressed in mg kg^{-1} of SC dry matter.

Earthworm sampling and community characterization

Earthworm sampling was carried out on April 11 and 12, 2016 (~ 6 months after the last pre-sowing organic fertilization and 2 months after the last mineral fertilizer topdressing). The sampling date took into account the Pérès et al. (2014) recommendation about best samplings between the end of winter time and the beginning of spring time, where conditions are favourable for earthworm activity. Considering the low rainfall and high evapotranspiration in the study area during the summer period (Figure 1), samplings at the beginning of autumn, before sowing, cannot be recommended as the earthworm population at that season might be affected by low soil moisture content. The average water content in the soil (14 ± 1%, w/w, gravimetric humidity) was measured during sampling. Soil monoliths (0.25 m x 0.25 m) were excavated to a depth of 0.20 m in each plot with a spade, transferred to the laboratory and hand-sorted for earthworms within 24 h after collection (n = 3, each treatment). The earthworms were immediately fixed with formaldehyde and stored in ethanol (70%). Furthermore, in the field, in each pit hole, different volumes of formalin were poured at different concentrations (0.2%, 0.750 mL two times and 0.4%, 1 L one time) for periods of 45 minutes but applied at intervals of fifteen minutes to expel residual earthworms which might have escaped to deeper layers (AENOR 2009). The infiltration of the formaldehyde solutions was slow, because a compacted layer was found at that depth (0.20–0.25 m depth), and no earthworms were detected below that level.

The parameters associated with the earthworm community were used as bioindicators following AENOR (2009): total and juvenile abundance, considering the whole earthworm body - and if it was cut, only the fore part (i.e. with head) was counted; and earthworm biomass, considering the weight of specimens preserved in ethanol and earthworm richness (number of species). Besides, species identification, and species dominance (%) were also studied.

Earthworm bioturbation and micromorphological soil traits

An undisturbed soil prism sample, with a rectangular shape (0.06 m height, 0.09 m width, 0.19 m length), was taken on January 27, 2016 from each treatment and with two replicates (blocks). The rectangular prisms (n = 10) were dried at room temperature and impregnated with polyester resin with a fluorescent dye (Uvitex©). One vertical thin section (0.05 m height, 0.13 m length was obtained from each prism. All thin sections (10 in total) were scanned with a high resolution Epson scanner to obtain digital images which were processed with the 3200 dpi (dots per inch) option and with a 24 bit spectral resolution ("true colour"). Thus, images with a dimension of 14,126 x 5,461 pixels (equivalent to an area of 4,860 mm^2) were obtained. The microstructure and the abundance of the different shapes of pores were described, following Stoops (2003) and Zaiets and Poch (2016). At the image scale, three types of bioturbation were quantified as a percentage of total area. The measurements of bioturbated areas were processed with Olympus Stream image analysis software (Olympus 2013). The classification of earthworm bioturbation, adapted to the micromorphological technique from the visual description of the soil profiles by Piron et al. (2012, 2017), was included (Table 1).

Data analysis

All statistical analyses were performed using SAS V8.2 statistical software (SAS Institute 2001). When differences, according to the analyses of variance (ANOVA), were considered significant ($p < 0.05$), Least Statistical Difference (LSD) was computed to compare pairs of means at the 0.05 probability level. Earthworm abundance data were normalized, before the ANOVA analysis, using the log-transformation. A qualitative scale was used to describe the abundance of the different types of pores (from "not observed" up to "abundant").

Results

Earthworm abundance (total and juvenile specimens) and biomass did not show significant differences between the fertilizer treatments (Table 2). The total abundance values were in the interval between 101 to 176 individuals m^{-2} and earthworm biomass ranged from 17.3 to 34.4 g m^{-2}. Two earthworm species were recovered in the experimental site (Table 3), the endogenic earthworm Koinodrilus roseus (Savigny 1826) and the anecic species Nicodrilus trapezoides (Dugés 1828). The second of these was not present in the SC treatments (4SC and 8SC) (Table 3). Their dimensions agree with general descriptions of the species, as Koinodrilus roseus is characterized by a length between 25 to 75 mm and by a width between 2 to 4 mm; Nicodrilus trapezoides has a length between 55 to 148 mm and a width between 3 to 5 mm.

Micromorphological differences (microstructure and types of voids) were observed (Figure 2 and Table 4). The control and the MF treatments had a platy to massive microstructure, moderately separated (i.e. aggregates are, in general, horizontally elongated and separated by planar voids; no separated areas with a few visible voids are also present). The sludge compost treatments had a sub-angular blocky microstructure weakly separated (i.e. aggregates separated by short planar voids on all or in most sides) while the PS treatment had a crumb microstructure, highly separated by rugose packing pores (Figure 2 and Table 4). Pores related to biological activity were more abundant when organic fertilizers were applied, mainly in PS. Vughs were not present in SC treatments, which had planar voids distributed randomly in vertical and horizontal forms, linked to microstructure type (Figure 2). By contrast, in CO and MF treatments, planar voids were mainly horizontally distributed. In the PS treatment (Table 2 and Figure 3), the presence of earthworm bioturbation Type 1 (4%; burrows) and Type 2 (42%, casts) were significantly higher. Channels and pores associated with the accommodation of the fresh casts, welded or compacted (compound packing voids and star-shaped vughs) linked to earthworm bioturbation predominated with PS application. The percentage of casts in SC treatments was higher than in CO and MF treatments (Type 2), without differences in the presence of burrows (Type 1). Also, the presence of non-mixed fresh vegetal material was observed in the SC treatments, while in the PS treatments the organic material was more intimately mixed and was distributed homogeneously throughout the observation area (Figures 4(a) and (b)). The earthworm bioturbated area accounted for an average 47 and 22% in PS and SC treatments respectively (Figure 3).

Discussion

Earthworm abundance agreed with other observations made under a semiarid Mediterranean climate, where less than 200 individuals m^{-2} were found (Rutgers et al. 2016). The abundance of juvenile specimens can be explained by the sampling period, as spring samplings capture higher percentages than autumn samplings (Murchie et al. 2015). The absence of differences between treatments in other parameters such as earthworm biomass might be interpreted as these parameters are insufficiently sensitive to differences between fertilization materials and the decline in earthworm abundance can only be expected under heavier impacts as the ones present in contaminated soils (Pérès et al. 2011).

The biodiversity was low, in accordance with Baldivieso-Freitas et al. (2018) who only found five earthworm species in an agricultural land under Mediterranean climate. The two species collected in this work live in field crops and forests although endogeic species such as K. roseus predominate in field crops (Lamandé et al. 2003), and anecic earthworms such as N. trapezoides predominate in forests (Kooch et al. 2008). The absence of epigeic earthworms can be explained by the lack of litter in the cropland (Koblenz et al. 2015), common in rainfed areas where straw is removed from the fields. Furthermore, N. trapezoides was not present in SC treatments. The absence of anecics is directly associated with the characteristics of the organic material due to their saprophagic habit, as endogenics have a geophagic habit (Piron et al. 2017). This finding agrees with Coors et al. (2016) who observed a decrease of anecic species with sludge applications. Besides, N. trapezoides shows a low susceptibility (changes in abundance) to mineral fertilization (Reinecke and Visser 1980). Sludge compost increases soil organic carbon stocks but it is a strongly stabilized source of OM and it might contain substances potentially toxic to soil organisms (Fernández et al. 2007a, 2007b; Rigby et al. 2016). Our results indicate that the presence of earthworm species should be monitored to assess potential environmental risks (Renaud et al. 2017) when sludge compost is applied for a long-term. Although SC satisfies legal limits, in the framework of this long-term fertilization experiment, the accumulation of other non-identified compounds could be harmful to a specific earthworm species. It is known that sewage sludge contains organic and inorganic contaminants not regulated by law i.e. emerging contaminants or nanoparticles; thus its compliance is not a reliable guarantee of lack of toxicity (Aparicio et al. 2009; Fijalkowski et al. 2017). Furthermore, the processes undergone by the sewage sludge (composting, anaerobic digestion, even thermal carbonization) do not ensure that the final product has a high quality and does not contain contaminants (in fact only a lower amount by a dilution effect). These facts should be investigated in future studies as other authors also pointed out (Fijalkowski et al. 2017). Nevertheless, differences in the total amount of OM applied by PS and SC might play a role apart from OM composition.

Differences in feeding habits and specific physiological characteristics of species make them useful as bioindicators of disturbed or contaminated soils (Nahmani et al. 2003): N. trapezoides, according to our results, could be a sound bioindicator candidate, in the context of the experiment might probably be related to OM over-fertilization.

The earthworm community is sensitive to agricultural management (Biau et al. 2012; Yagüe et al. 2016) but the influence is reciprocal. In practice, the soil influences the development of living organisms and the latter influence the development of porosity as stated by Kooistra and Pulleman (2001). In our experiment, earthworm bioturbation differed according to the treatment applied, and in turn soil microstructure and its shape (Figure 2) evolved from a platy-massive (CO, MF), to a subangular blocky (SC) and finally to a crumb microstructure (PS). The application of sludges decreased the amount of large horizontal cracks when compared with CO and MF, but was not able to increase neither the abundance of biopores (i.e. channels, chambers, compound packing voids and especially vughs, Table 4) nor earthworm bioturbation (Figure 3, Type 1 and 2) compared with PS treatment. Overall, the application of slurries increased the activity of earthworms due to their nutritional value (D'Hose et al. 2018). According to Domínguez-Hydar et al. (2018), when the soil has an advanced state of homogenization by a high faunal activity, the soil microstructure evolves from subangular blocks to more rounded peds, as it has been observed under the PS treatment. This microstructure development (crumb) is typically linked with large compound packing voids and results from the disturbance of former voids or their refilling with various materials like parts of burrow walls and casts from earthworms (VandenBygaart et al. 2000; Bruneau et al. 2004; Sauzet et al. 2017). In agreement with our findings, Adesodun et al. (2005) observed an increase in excrement-produced by soil fauna (included earthworms) with two SC applications (every two years) in relation to control (without OM application) but they observed an evident negative effect of SC on microbial biomass. This may be due to a damaging effect on the soil ecosystem as a whole, even when the applications are not long-term. The negative effect of SC on microorganisms and enzymes of the soil is indeed very well documented (Charlton et al. 2016; Sharma et al. 2017). Specifically, the lowest bioturbation of Type 1 in the CO and MF treatments might be associated to a massive microstructure, while in SC it is attributed to the absence of anecic species because non-mixed organic material was visible in soil thin sections (Figure 4(a)). The microstructure and fissures observed in CO and MF (Figure 2) are typical of agricultural systems without external inputs of organic matter (Bosch-Serra et al. 2017) and possible compaction problems (Pagliai 2003). Anecic activities are important for Type 1 bioturbation as they build and live in relatively permanent vertical burrows (channels or tubular voids), feed on decomposed litter and mix litter fragments with mineral particles (Lamandé et al. 2003; Piron et al. 2012, 2017). Our results support the conclusions of Lamandé et al. (2013) in the sense that in order to improve our understanding of earthworm and structure interaction it is necessary to consider the functional diversity of earthworms, as well as their abundance. The highest earthworm bioturbation in the PS treatment was mainly associated with two biostructures (Figure 3): burrows (Type 1) and casts (Type 2). In general, Type 2 bioturbation corresponds to an important part of soil aggregates and is more abundant in soils with favourable physical conditions (Lee and Foster 1991; Pulleman et al. 2005). The microstructure improvement and the biopore presence have a potential and positive functional impact, favouring gaseous exchanges, the movement and retention of soil water and root penetration in soil (Lamandé et al. 2003; Peigné et al. 2013).

Earthworms significantly contribute to many of the ecosystem services provided by the soil, but they are also indicators of unknown but harmful soil components. The analysis of earthworm roles, apart from abundance and diversity of species, must include their activity (Birkas et al. 2010) through the evaluation of soil bioturbation (soil microstructure description) in order to properly assess the impact of a long term use of wastes as fertilizers, as it is proved in our conditions. Our results support the recent trend towards integrative approaches in soil health assessment combining qualitative and quantitative tools (Pirón et al. 2017; Bünemann et al. 2018). Current works mention the need for monitoring the soil ecosystem when SC is used as fertilizer (Fijalkowski et al. 2017) and the increase interest in taking preventive measures before the load of pollutants that entails the use of SC can affect soil quality, and compromise the circular economy (Rigueiro-Rodríguez et al. 2018).

The integrated analysis of micromorphological features and earthworm traits is an useful tool for the record of past (structures casts, burrows) and present soil fauna (abundance, biomass, diversity) and it makes possible to monitor changes in soils associated to the effect of management agriculture practices, as long term organic fertilization.

Conclusions

Earthworm numbers (abundance and biomass) were not useful to detect differences between fertilization treatments while the species identification and the analysis of soil bioturbation did. The bioturbated area was lower than 9% for the control and MF treatments. The application of PS resulted in increased bioturbation (up to 47%). Specifically, when compared with sludge compost, PS improved the indicators of soil fertility: physical (microstructure), and biological (number of earthworm species and activity). No chambers were observed in the control and in the mineral fertilized plots, while vughs were absent in SC treatments. Globally, bio-structures developed by earthworms (presence of burrows and casts), were more abundant when PS was applied. Composted sludge treatments compared with PS reduced the percentage of area bioturbated by 47% and the earthworm community to one species: Koinodrilus roseus. The lack of Nicodrilus trapezoides (present in the rest of the treatments) points to this species as an indicator of the risk in soil biological quality reduction in this dryland agricultural systems, which in our experiment coincided with a long-term sludge compost application. Hence, these insights about the possible causes for the absence of Nicodrilus trapezoides under sludge compost fertilization should be investigated in future studies as well as if it implies negative impacts on an integrative soil quality concept.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Legend of figures

Figure 1. Monthly rainfall, reference crop evapotranspiration (ET_0, FAO Penman–Monteith equation), and monthly average of air temperature during the cropping year (August 2015 – July 2016). Data were obtained from the Tàrrega meteorological station, located 15 km from the experimental site (UTMX, UTMY: 347015.00, 4614430.00).

Figure 2. Scanned images of thin sections used for the description of the microstructure, types of voids and micromorphological earthworm bioturbation (Type 0, absence of bioturbation: anthropogenic and unidentified processes; Type 1, burrows; Type 2, casts) from different fertilizer treatments. Treatments were: CO, control; MF, mineral N fertilizer; SC, sludge compost, numbers indicate the applied rate of 4 or 8 t ha^{-1}; PS, pig slurry applied at a rate of 27 t ha^{-1}.

Figure 3. Presence (%) of the different earthworms' bioturbation types in the different fertilization treatments. Mean values followed by different letter were significantly different at the 0.05 probability level based on the Fisher's Least Significant Difference test. Codes for biotubation were: Type 0, absence of bioturbation: anthropogenic and indefinite processes; Type 1, burrows; Type 2, casts. Codes for treatments were: CO, control; MF, mineral N fertilizer; SC, sludge compost, numbers indicate the applied rate of 4 or 8 t ha^{-1}; PS, pig slurry applied at a rate of 27 t ha^{-1}.

Figure 4. Fresh plant material in soil. a) fresh material in treatments with sewage compost (SC) attacked by soil organisms other than earthworms (oribatids). b) mixed soil materials due to earthworm bioturbation in pig slurry (PS) treatment.

Table 1. Classification of the earthworm bioturbation and the micromorphological description of the associated soil structure.

Type of earthworm[a] bioturbation	**Micromorphological characteristics**
Type 0	Absence of earthworm bioturbation. Inclusion of soil anthropogenic processes: - Soil compaction: area without discernible porosity, low roughness and larger cracks. - Soil tillage: aggregate assemblages of various sizes and shapes. High porosity and roughness. Unidentified processes: apedal microstructure without an aggregative aspect.
Type 1	Burrows, mainly empty.
Type 2	Packing of homogeneous (in shape and size) cast aggregates, individually distributed and not welded. Packing of welded cast aggregates. Packing of strongly welded casts (compacted).

[a] Adapted from Piron et al. (2012, 2017).

Table 2. Analysis of variance of earthworm bioindicators: total abundance (individuals m^{-2}), juvenile abundance (individuals m^{-2}), earthworm biomass (g m^{-2}), and earthworm bioturbation (% of total soil section area) classified in three types: Type 0 (absence), Type 1 (burrows) and Type 2 (casts).

Earthworm bioindicators		**Total abundance**		**Juvenile abundance**		**Biomass**	
Source	**DF**[a]	**MS**	**p**	**MS**	**p**	**MS**	**p**
Between treatments	4	0.1	0.96	0.1	0.92	130.0	0.77
Between blocks	2	1.0	0.09	0.9	0.17	590.3	0.20
Residual	8	0.3		0.4		293.1	
SE		0.3		0.4		9.9	
Earthworm bioturbation		**Type 0**		**Type 1**		**Type 2**	
Source	**DF**	**MS**	**p**	**MS**	**p**	**MS**	**p**
Between treatments	4	526.3	0.002	4.4	0.009	437.9	0.002
Between blocks	1	3.5	0.66	0.6	0.21	1.2	0.76
Residual	4	5.6		0.3		11.9	
SE, SED, LSD[b]		2.8, 4.0, 11.0		0.4, 0.5, 1.4		2.4, 3.5, 9.6	

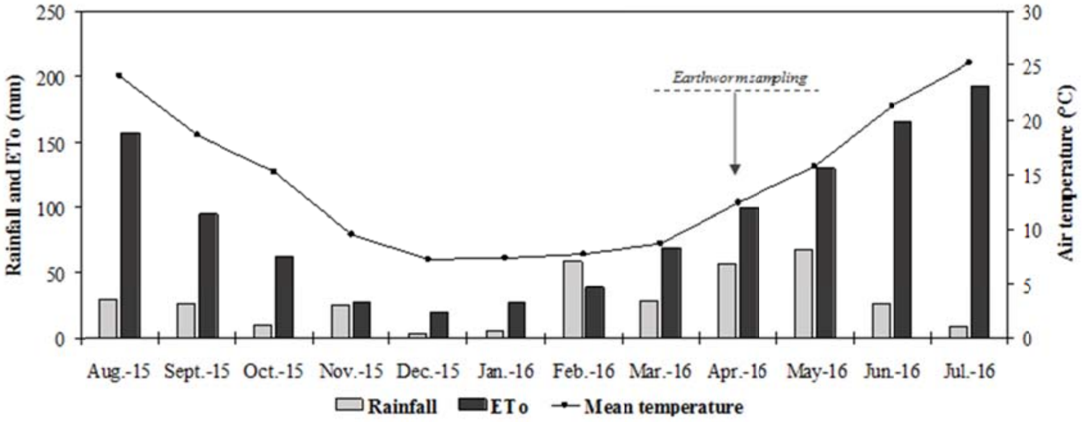
[a] DF: degrees of freedom; MS: mean square.

[b] SE, standard error of the mean; SED, standard error of a difference; LSD, least significant difference test (p = 0.05).

Table 3. Average values (n = 3) of total and juvenile earthworm abundance, earthworm biomass and distribution of the earthworm community for the different fertilizer treatments.

	Abundance (individuals m^{-2})		**Biomass ($g\ m^{-2}$)**	**Community composition (%)**	
Treatments[a]	**Total**	**Juvenile**		**Koinodrilus roseus**	**Nicodrilus trapezoides**
CO	139	101	29.5	83	17
MF	112	69	34.4	63	38
4SC	176	155	22.2	100	0
8SC	117	106	17.3	100	0
PS	101	80	26.5	75	25

[a] CO: control; MF: mineral N fertilizer; SC: sludge compost, numbers indicate the applied rate of 4 or 8 t ha^{-1}; PS: pig slurry applied at a rate of 27 t ha^{-1}.


250
200
150
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50
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Rainfall and ETo (mm)
30
25
20
15
10
5
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Air temperature (°C)
Earthworm sampling
Aug.-15
Sept.-15
Oct.-15
Nov.-15
Dec.-15
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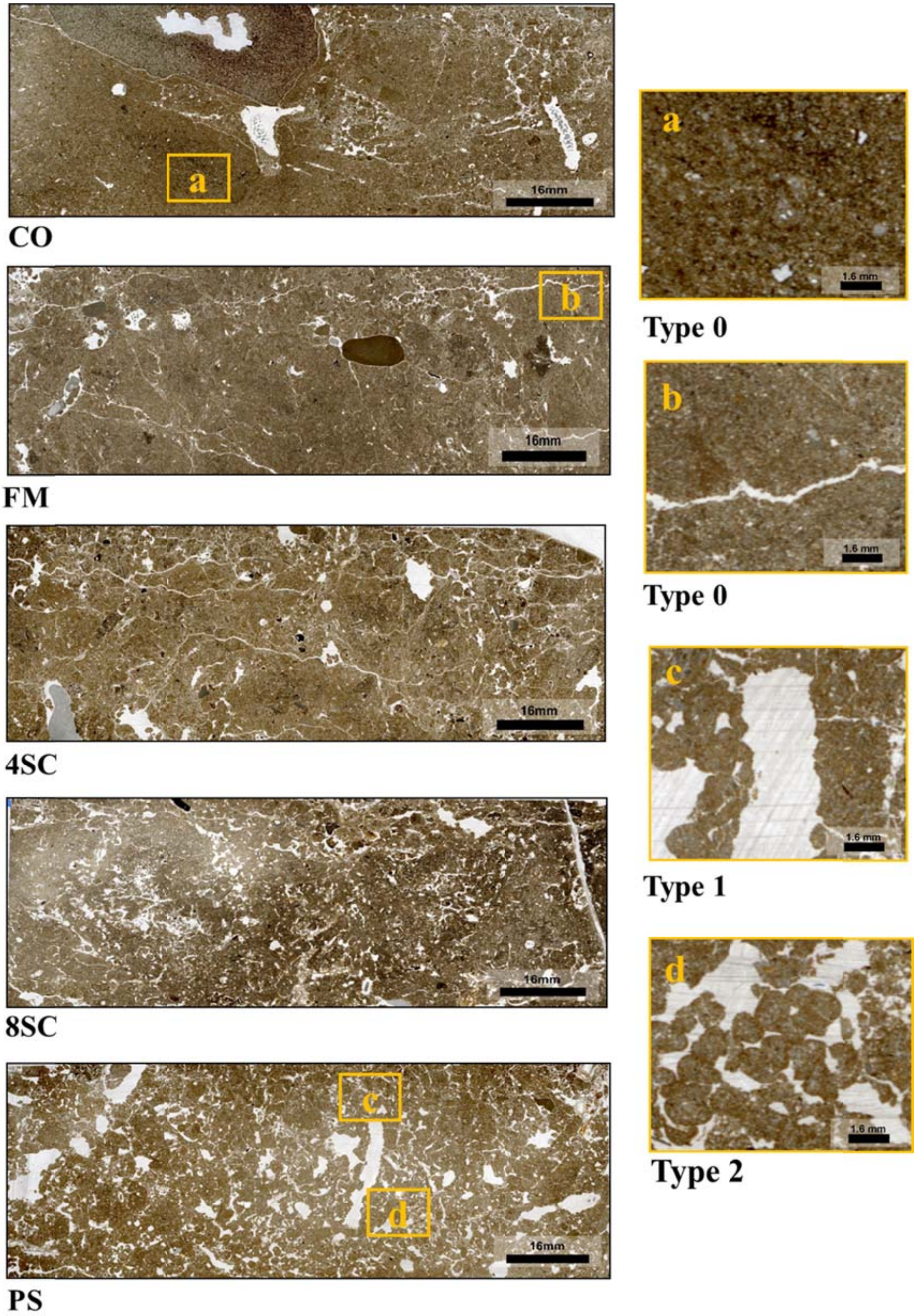
Feb.-16
Mar.-16
Apr.-16
May-16
Jun.-16
Jul.-16
Rainfall
ETo
Mean temperature

Figure 1.


a
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CO
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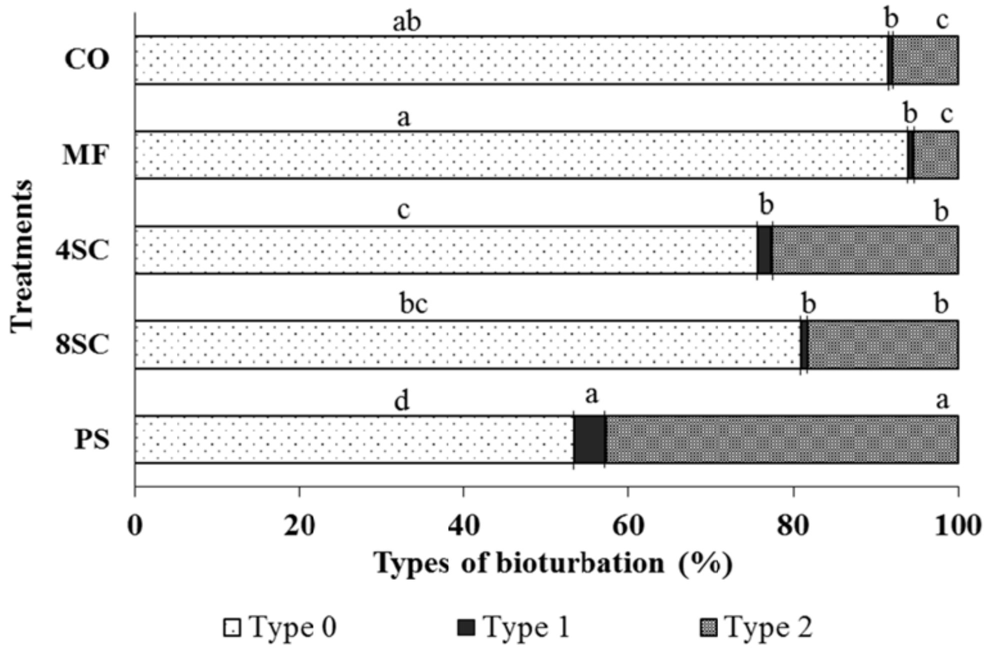
Type 2
c
d
16mm
PS

Figure 2.



Treatments
CO
MF
4SC
8SC
PS
ab
b
c
a
b
c
c
b
b
bc
b
b
d
a
a
0
20
40
60
80
100
Types of bioturbation (%)
Type 0
Type 1
Type 2

Figure 3.

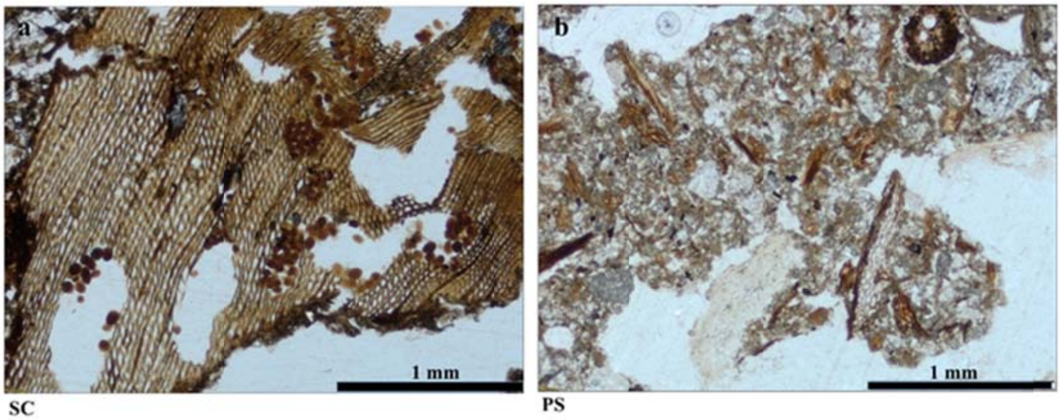


Figure 4.