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Effects of long-term sewage sludge application to a calcareous soil structure

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Abstract

Soil degradation is a growing challenge to global agriculture and the United Nations' Sustainable Development Goals (SDGs). This has prompted calls for less use of mineral fertilizer and greater reliance on organic fertilizers. However, we need to understand better the long-term effects of organic fertilizer usage on soil structure to guide soil management practice, as many soil functions are sensitive to pore morphology and connectivity. In this study, we characterized topsoil (0–30 cm) pore architecture in relation to soil physical properties in a long-term experiment (LTE) site where calcareous soil had received 25 years of sewage sludge application. Two dosage rates (SS_a, 20 and SS_e, 80 Mg ha^{-1}) were compared to mineral fertilization treatment and a control (no fertilization) in a random factorial block design. Soil microstructure and the types of pores were characterized using micromorphological methods and image analysis, in soil thin sections. Long-term sewage sludge SS_a application improved soil microstructure (crumb and sub-angular-blocky type) and increased the presence of biopores, while mineral fertilized soil showed a platy to apedal microstructure, with more elongated pores and lower faunal activity. Mineral fertilized soil had the lowest total porosity values, with differences found in the aspect ratio of pores of equivalent diameter 100–200 μm. These findings suggest a relation between the different types of fertilization and soil pore shape and network. Further exploration of these changes in soil functioning is needed for a complete assessment of the consequences of SS application.

KEYWORDS

calcareous soil, image analysis, sewage sludge, soil micromorphology, soil porosity, soil quality

1 | INTRODUCTION

Agricultural soils face a major threat as consequence of decades of conventional agricultural practices (Pagliai & Vignozzi, 2002). Long-term depletion of soil organic matter (SOM) leads to physical degradation (Jensen et al., 2019; Kopittke et al., 2020), as the soil is more vulnerable to erosion and compaction and less able to stabilize SOM.

Organic wastes, such as sewage sludge (SS), are a resource that can be converted to fertilizer (Metcalf &

Eddy, 2003; Singh & Agrawal, 2008). Long-term studies are important to understand the impact of applying SS, as it presents several risks to the environment by the accumulation of trace metals (TM) (McBride, 2003; Sharma et al., 2010; Zaragüeta et al., 2021) and resistance to antibiotics (Urra et al., 2019). Amending soils with SS generally improve some soil properties, such as SOM and nutrient contents, soil porosity, bulk density, aggregate stability or available water holding capacity (Annabi et al., 2011; Roig et al., 2012; Singh & Agrawal, 2008; Skowrońska et al., 2020; Soria et al., 2021). These soil properties directly affect crop production as they regulate, among others, root penetration (Lal, 1991), water flows and storage (Franzluebbers, 2002) and soil faunal diversity (Bottinelli et al., 2015; Nunan et al., 2017). Other authors, however, have reported adverse effects on these properties (Yu et al., 2020) suggesting a soil- site- and SS-quality-dependent effect.

Structure a key property of soil (Banwart et al., 2019), several approaches have been developed for assessing the quality of soil structure and its relation to soil management (Abiven et al., 2009). These include the study of aggregate stability, soil hydraulic conductivity or pore space characterization. Rabot et al. (2018) highlighted the relevance of soil porosity, pore distances and pore connectivity to assess soil functions. The study of soil thin sections allows a visual classification of voids (pores) based on their origin (Skvortsova & Utkaeva, 2008). They provide information on the complex pore network at microscales, in which the processes and reactions responsible for the characteristics and composition of the soil solution and the soil matrix occur (Pagliai & Vignozzi, 2002). In the context of potentially toxic materials, such as SS, soil micromorphology also applies to the behaviour of sensitive organisms, such as earthworms (Valdez et al., 2020; Yagüe et al., 2016), for their limited mobility and high sensitivity to change in soil properties (Alvarez et al., 2021; Pulleman et al., 2005; Valdez et al., 2020). By providing a better understanding of these processes in the soil, micromorphology can guide soil management practice. In addition, morphological characteristics based on undisturbed samples can incorporate quantitative information on soil structure into soil models (Kravchenko & Guber, 2017).

Recent work on organic fertilization, carried out in the context of agriculture in the Ebro Valley in NE Spain, has shown the usefulness of micromorphology to assess soil quality. Yagüe et al. (2016) reported on a well drained Oxiaquic Xerofluvent soil with maize (*Zea mays* L.) irrigated monoculture fertilized with dairy cattle manure at different rates and mineral fertilization (MF). They found an increase in pores with diameter >400 μm without modifying their shape. Domingo-Olivé et al. (2016), under the same conditions comparing pig slurry, cattle manure and MF, reported a significantly higher porosity in the 65–400 μm size range in treatments with slurry, comparing with MF. The opposite was detected for pores larger than 400 μm. Valdez et al. (2020) reported on a calcareous xeric Entisol supporting an annual rainfed rotation of winter cereals. They observed that the application of composted SS decreased the amount of large horizontal cracks when compared with MF. The consequences of these changes and their relation to other soil properties and functionality remain poorly studied. Image analysis of soil thin sections is not a routine soil analytical technique, but represents a promising approach to understand changes related to soil management.

The objectives of this study were (i) to determine the effect of different SS application doses on soil physical status and pore architecture of a tilled layer of calcareous soil after 25 years of SS application in Navarre (NE Spain) and (ii) to identify relationships between these changes and other soil properties related to the soil physical condition. Following previous studies on trace metal accumulation (Zaragüeta et al., 2021), soil biological diversity (Urra et al., 2019) and the sensitivity of several soil quality indicators (Simoes-Mota et al., 2021) at the same experimental site, we hypothesized that long-term use of SS would lead to soil structure better suited for soil functioning in terms of water movement and biological activity.

2 | MATERIALS AND METHODS

2.1 | Site and experimental design

A long-term experimental field site was established in Arazuri, Navarra, NE Spain (42°48′N, 1°43′W, 396 m a.s.l.) in 1992 to assess the effect of the continuous application of SS on agricultural soil quality and productivity (Figure 1). The area is temperate Mediterranean (Papadakis, 1961), with a xeric soil water regime (Soil Survey Staff, 2014). Mean annual precipitation is 750 mm year^{-1}, and mean annual Thornthwaite's evapotranspiration is 687 mm year^{-1} (Gobierno de Navarra, 2021).

The soil is calcareous (approx. 20% of calcium carbonate in the tilled layer) with a clay-loam topsoil (31% clay, 30% silt, 39% sand) (Gee & Bauder, 1986). It has been classified as a Calcaric Cambisol (FAO, 2014) and is well drained and has no salinity problems. The experimental design consists of a random factorial block design with eight treatments and three replicates ($n = 3$), each plot with an area of 35 m^2 (10 m × 3.5 m). For this study, agronomic (SSa) and extreme (SSe) SS doses were chosen according to regional practice, being 20 t SS ha^{-1} per year established as the agronomic dose in the area (SSa) and 80 t SS ha^{-1} per year the extreme dose chosen to follow

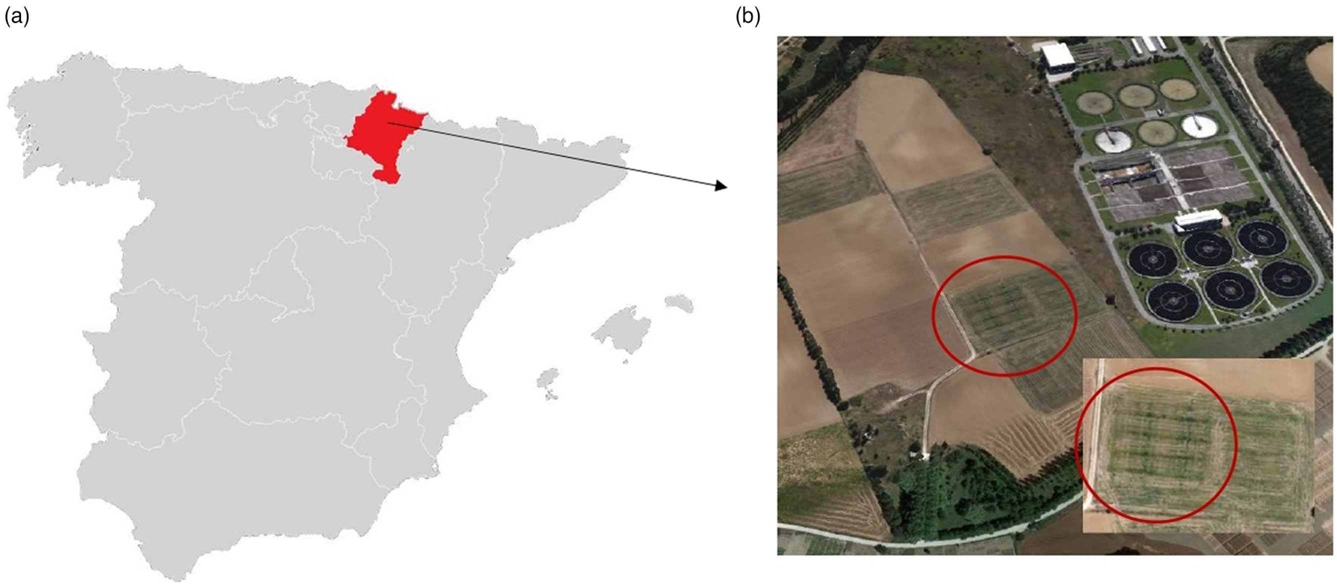

FIGURE 1 Ampling site context. (a) Arazuri, Navarre, North Spain; (b) The experimental field localization. Four out of eight treatments were sampled for this study (20ss, 80ss, MF, C).

TABLE 1 Physical and chemical properties of the sewage sludge

Sewage sludge physical and chemical properties	
pH	8.16 ± 0.03
Electric conductivity (μs cm^{-3})	1795 ± 28
Dry material (%)	18.1 ± 0.4
Volatile matter (% of dry substance)	62.8 ± 1.9
C/N	5.35 ± 0.08
Total N	5.85 ± 0.13
Ammonium-N	0.75 ± 0.02
Phosphorus (P_2O_5)	5.59 ± 0.22
Potassium (K_2O)	0.62 ± 0.05
Iron (Fe)	1.68 ± 0.04
Calcium (CaO)	7.98 ± 0.29
Cadmium (Cd)	0.88 ± 0.09
Copper (Cu)	187 ± 11
Nickel (Ni)	32.1 ± 0.77
Lead (Pb)	39.0 ± 1.2
Zinc (Zn)	874 ± 0.003
Mercury (Hg)	0.003 ± 0.003
Chromium (Cr)	58.3 ± 3.2

Note: Values are given as the mean ± *SD* ($n = 3$).

TABLE 2 Physical and chemical properties of the soil tilled layer (0–30 cm) for the control plots

Soil physical and chemical properties	
pH (water 1:2:5)	8.67 ± 0.03
Electrical Conductivity (μs cm^{-3} at 25°C) (soil: water extract 1:2.5)	169 ± 10
Bulk density (g cm^{-3})	1.59 ± 0.08
Carbonates (%)	16.0 ± 2.1
Clay (%)	27.7 ± 1.03
Organic Carbon (%) (Walkley-Black)	1.35 ± 0.02

Note: Values are given as the mean ± *SD* ($n = 3$).

the crop and soil response to SS application above the recommendations (Zaragüeta et al., 2021). In addition, mineral fertilization (MF) treatment and the unfertilized control (C) were studied. The SS came from a municipality wastewater treatment plant, involving primary and secondary treatments, and stabilized through anaerobic digestion and mechanical dewatering. Industrial waste is not processed in this plant. Sewage sludge characteristics, as described by (Zaragüeta et al., 2021), are summarized in Table 1. The equivalent annual NPK fertilization doses corresponding to MF, SSa and Sse were 180 Kg N ha^{-1} + 22 Kg P ha^{-1} + 0 Kg K ha^{-1}, 227 Kg N ha^{-1} + 95 Kg P ha^{-1} + 17 Kg K ha^{-1} and 908 Kg N ha^{-1} + 380 Kg P ha^{-1} + 68 Kg K ha^{-1}, respectively (Irañeta et al., 2013).

The soil main physical–chemical characteristics in the tilled layer (0–30 cm) at the control plots (treatment C) are summarized in Table 2.

The crops consist of a rotation of wheat (*Triticum aestivum* L.), with another extensive crop sunflower (*Helianthus annuus* L.), canola (*Brassica napus* L.) or peas (*Pisum sativum* L.), every 3 years. All treatments are managed with an annual tillage using a 30 cm deep mouldboard plough, and application of phytosanitary products according to the crops' needs each year. Twenty-five years after the onset of the experiment, wheat in each field replicate was harvested with a plot-scale combine and grain yields were recorded. Grain weights were taken directly from the combine and grain samples were collected to analyse their water content, to get yield data on a dry-mass basis.

2.2 | Soil sampling and analysis

Soil sampling was carried out at each treatment and replicated, 25 years after the onset of the experiment. Disturbed soil samples were collected using an Edelman type auger (Ø = 5 cm) or a shovel. Three subsamples were collected per plot and combined to obtain a composite sample. Part of the sample was gently sieved (5 mm). Aggregates were air dried and used for aggregate stability determinations. The remainder of the soil was air-dried and sieved to 2 mm. Undisturbed core samples were collected in triplicate using bevel-edged steel rings (Ø = 5 cm, total volume = 100 cm^3) to determine soil bulk density (ρ_b) and water retention characteristics.

Undisturbed soil samples as horizontal prisms were collected using Kubiëna boxes for thin section analysis (13 × 5 cm) at the soil upper depth (0–15 cm). Soil thin sections were prepared as described in Benyarku and Stoops (2005) and described following Stoops (2003) guidelines using a petrographic microscope Olympus BX51 connected to a Olympus SC20 camera. Image analysis was used to determine parameters related to macroporosity. Scanned images were obtained per thin section under two light conditions: parallel polarizers and crossed polarizers. Images were processed using Image J (Rasband, 2008) to obtain digital binary images. From each binarized thin section, five random images (10 × 10 mm) were selected using an adaptation of the method used by (Virto et al., 2013), where a grid of 27 squares (1 cm^2 each) was placed in each scanned section from which the eligible squares were chosen using a random number generator. Using ImageJ, total porosity and morphological descriptors data were determined for the whole surface of each 1-cm^2 square. The parameters used were those described by Ferreira & Rasband (2012): Perimeter (which represents the length of the pores outside boundary), Feret diameter (the longest distance between any two points along the pore), circularity (with a maximum value of 1 indicating a perfect circle), aspect ratio (the ratio of major-to-minor axes of the pore) and solidity (area of the pore/convex area of the pore). Pores overlapping the square boundaries were excluded from the analysis.

Pore-size distribution analysis was based on a mathematical algorithm available at the Quantim4 library (Vogel, 2008). The area occupied by pores within each 1-cm^2 square was divided into five intervals according to the pore's apparent diameter: <100 μm; 100–200 μm; 200–400 μm; >400 μm. The morphological study of the proportion of area (equivalent to volume proportion over total soil volume) occupied by pores with diameters <400 μm was selected for this study, because of their special relevance when describing structure (size of planar voids or fissures) and also because these pores can result from the activity of mesofauna (FAO, 2020).

Soil indicators were selected according to their relevance for the pore network quality. Bulk density was determined with the core method (Carter, 1993). For wet aggregate stability, a constant shower-like flux (6 $L\,min^{-1}$) of distilled water was applied from the top of the same set of sieves while sieving 4 g of 1–2 mm sieved soil (60 strokes min^{-1}, 60s). A mechanical sample divisor (Retsch GmbH & Co.) was used to ensure that the initial distribution of aggregates was similar among replicates. Aggregate size-distribution and stability were expressed as the mean weight diameter (MWD) after wet sieving (Bosch-Serra et al., 2017). The stability of the aggregates was also evaluated using the mass proportion of water-stable aggregates (WSA) >0.25 mm (Franzluebbers, 2002).

Soil water retention at −33 kPa was determined on intact soil cores and sieved (<2 mm). Soil samples were used for water retention assessment at −1500 kPa, using pressure plate extractors (Soil Moisture Equipment Corp.). Available water-holding capacity (AWHC) was calculated as the difference between volumetric water content at field capacity (−33 kPa) and wilting point (−1500 kPa).

Soil organic C (SOC) was determined by wet oxidation on air-dry sieved (<2 mm) samples (Nelson & Sommers, 1982). As other studies in the region have shown that earthworms can respond differently to different management strategies involving organic C inputs into the soil (Murchie et al., 2015; Valdez-Ibañez et al., 2019; Virto et al., 2007), earthworms were sampled in the fall season. Earthworms were collected from 20 × 20 × 30 cm soil blocks, which were crumbled by hand, placed in a glass jar and weighed to obtain a fresh weight for each field replicate (Baker & Lee, 1993). This allowed us to determine the total biomass (g per m^{-2}), the abundance (number of individuals per m^{-2}) and the average size (g per individual).

Crop sampling was carried out at physiological maturity. Crop samples were oven-dried at 50°C for 7 days. Once dry, they were shelled with a 6 mm sieve to separate the grain from the straw and ground separately with an agate ball mill (Zaragüeta et al., 2021).

2.3 | Statistical analysis

For to the analyses of variance (ANOVA) among treatments (SSa, SSe, MF and C), differences were considered significant when $p < .05$, unless otherwise indicated. All statistical analyses were performed using IBM SPSS Statistics 27.0 (SPSS Inc., 2021).

3 | RESULTS

3.1 | Micromorphological description

The groundmass composition of all thin sections was similar, due to the calcareous nature of the soil in the experimental field (Figure 2). The coarse/fine material (c/f) limit was set at 20 μm; the c/f ratio was 3:2 and the c/f related distribution pattern was single to close-spaced porphyric. The coarse material was composed of fine to very coarse sand (40%) made of subangular quartz grains; fine to medium sand (35%) of sparite; medium to very coarse sand of quartzite grains (10%); medium to very coarse sand (10%) of rounded limestone grains; and small amounts of shell fragments, medium to coarse and very sand of calcareous sandstone grains (2.5%) (percentages are based on visual estimations). In the thin sections corresponding to the SSa treatment, biogenic (earthworms) calcite nodules and some charcoal fragments were present. The micromass consisted of a brownish speckled mixture of clay, fine silt, micrite and amorphous organic matter (OM), showing a crystalitic micritic *b*-fabric.

The qualitative description of the different typologies and characteristics of soil pores in the studied layer (0–15 cm) showed that SS application induced changes related to the soil pore structure (Table 3). It was observed that long-term SS application changed the soil microstructure (crumb and sub-angular-blocky type) and the abundance of compound packing voids and channels, while MF-treated soil showed a platy to apedal microstructure with lower faunal activity. The control treatment showed a crumb to apedal channel microstructure, with abundance of faunal activity (compound packing voids, vughs and channels).

Fertilization affected soil porosity (Figure 3): Mineral fertilization showed the lowest percentage of total porosity of pores >25 μm. Regarding the pore size-distribution over total porosity, no significant differences were, however, observed between treatments. A high variability was detected in some pore size-ranges.

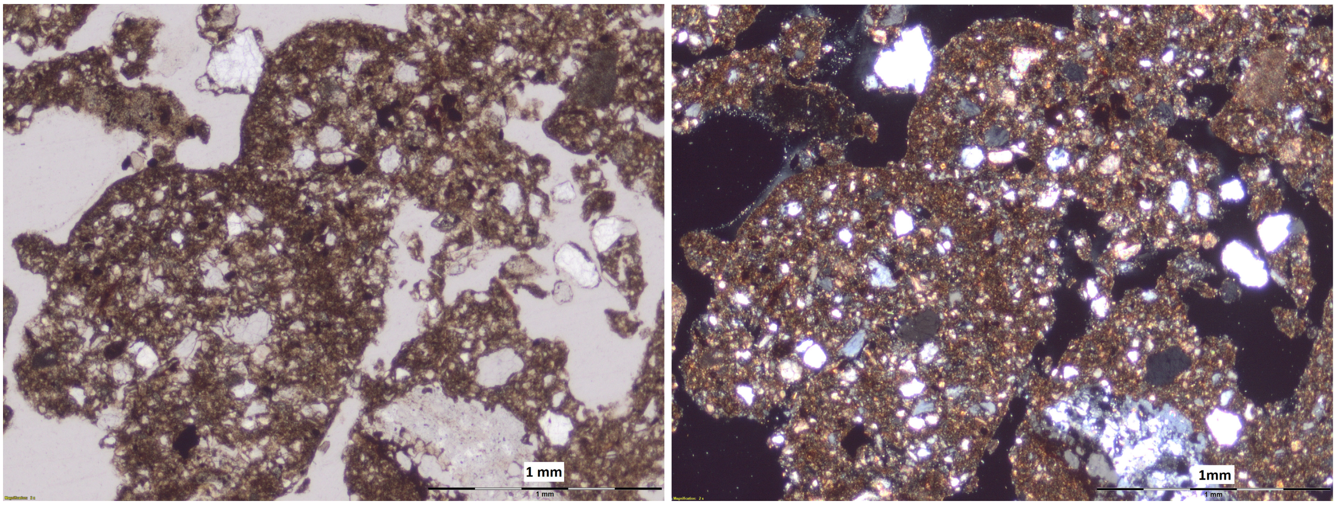

FIGURE 2 Crystalitic micritic b-fabric and evidence of biofaunal activity in a SSa thin section. Left PPL; right xPL. Red line represents 1 mm scale.

TABLE 3 Microstructures' description

Treatment	Microstructure	Types of voids
SSe	Sub-angular blocky (Strongly to moderately separated); Crumb; Apedal Channel	Compound packing voids; Channels; Infilled Channels ~1 mm; Vughs
SSa	Sub-angular blocky (Weakly separated); Crumb; Apedal Channel	Compound packing voids; Channels
MF	Platy	Compound packing voids; Fissures
C	Crumb; Apedal Channel	Compound packing voids; Channels; Vughs

Abbreviations: C = Control (n = 3); MF, Mineral Fertilizer (n = 3); SSe, SS 80 t ha^{-1} per year (n = 3); SSa, SS 20 t ha^{-1} per year (n = 3).

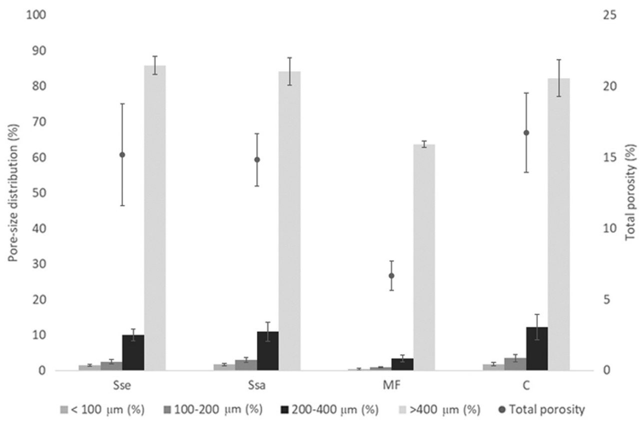

FIGURE 3 Total porosity >25 μm (right axis) and pore size-distribution (in % over total porosity, left axis) by pore diameter range on the pores >25 μm. C, Control ($n = 3$); MF, Mineral Fertilizer ($n = 3$); SSe, SS 80 t ha^{-1} per year ($n = 3$); SSa, SS 20 t ha^{-1} per year ($n = 3$). Treatments with the same letters are not statistically different $p < .05$. Standard error bars. Each value is the average of five measurements.

The morphological characteristics of pores analysed through image analysis are summarized in Table 4. The morphology of the pores <100 μm, did not show significant differences between treatments. On the diameter range 100–200 μm, treatments showed significant differences for the aspect ratio: mineral fertilizer displayed a smaller aspect ratio than in the other treatments (SSa, SSe and C). The treatment without fertilization (C) had an intermediate behaviour between MF and those receiving SS.

The most relevant observation in the pore size range 200–400 μm is that no pores within this size range were found to fit the 1-cm^2 squares in MF. As such, it was not possible to determine it shape parameters. Although morphological differences between the other treatments were not found, SSe treatments exhibit a trend of intermediate values between C, SSa.

3.2 | Soil indicators

The fertilizer treatments induced changes among different indicators (Table 5). Significant differences were found on the gravimetric AWHC, separating SSa, with a lower value, from the other treatments. Regarding MWD, it was significantly smaller in SSe-treated soil than the other treatments. The SOC content differentiated SSe from SSa and both SS treatments from MF and C. For the earthworm indicators, C treatment was significantly different than the others, with higher biomass, abundance and average size, except for SSa on earthworm's average size, with no differences from treatment C. Regarding yield, SSa and MF had similar values, and higher yield than SSe. The control treatment displayed the lowest value of all.

4 | DISCUSSION

4.1 | Microstructure and porosity

Sewage sludge application induced changes in the soil physical structure. Thin section analysis was able to detect these changes by enabling a qualitative evaluation of the soil pore network. Rabot et al. (2018) highlighted the efficiency of image analysis techniques to assess soil aggregate microstructure, porosity and the type of pores existing, adding an important value when soil functions are under study.

We observed that long-term SS application improved the soil microstructure for root growth and faunal activity (crumb and sub-angular-blocky type), which is characterized by the presence of biopores (compound packing voids, channels), while MF-treated soil showed a platy to apedal microstructure, with lower faunal activity. A crumb microstructure can be directly related to biological activity, as it usually appears mostly in the upper horizon of natural soils with its genesis on fine roots, organic debris, fragments of faecal material, particularly earthworms (Fitzpatrick, 1993).

The observation of higher earthworm abundance in treatments receiving SS than in MF (Table 5) supports this finding as well as the information about pore morphology issued from image analyses. The lowest observed earthworm abundance (Table 5) was in MF, the only treatment without data regarding pores 200–400 μm (Table 4) which correspond to one of the intervals of mesofauna. The subangular blocky type of structure develops mostly from wetting and drying (Fitzpatrick, 1993). This type of structure allows good water movement and facilitates root growth (Pagliai & Vignozzi, 2002), and when strongly separated, as in our case for SSe, it represents a stable soil structure, which is also of interest in agricultural soils.

We found that aggregate stability, as expressed by WSA, was not different among treatments, which can be related to the abundance of Ca in this calcareous soil granting resistance to soil aggregates to the action of water in all treatments (Rabot et al., 2018). However, data on MWD after submission to water sieving indicated that the highest dose of SS (SSe) resulted in smaller stable aggregates than in the treatments with a lower dose (SSa) or without SS (MF and C). This implies that the observed structural traits might not be directly related to aggregate stability in this case and supports the idea that the potential benefits of SS for soil structure are limited to their use within a certain dose range.

TABLE 4 Morphological pore measurements by pore equivalent-diameter interval

	Perimeter (μm)	Feret (μm)	Circularity	Aspect ratio	Solidity
<100 μm					
SSe	1.958 ± 0.11	0.731 ± 0.03	0.561 ± 0.00	2.089 ± 0.10	0.677 ± 0.01
SSa	0.207 ± 0.07	0.762 ± 0.02	0.549 ± 0.02	2.097 ± 0.04	0.676 ± 0.01
MF	0.197 ± 0.07	0.745 ± 0.00	0.554 ± 0.02	2.146 ± 0.02	0.687 ± 0.01
C	0.218 ± 0.15	0.800 ± 0.03	0.520 ± 0.02	2.212 ± 0.02	0.663 ± 0.01
	NS	NS	NS	NS	NS
100–200 μm					
SSe	10.42 ± 1.64	2.759 ± 0.30	0.215 ± 0.04	2.213 ± 0.26 B	0.524 ± 0.04
SSa	8.858 ± 0.85	2.527 ± 0.18	0.254 ± 0.04	2.316 ± 0.15 B	0.567 ± 0.03
MF	7.126 ± 1.70	2.014 ± 0.02	0.416 ± 0.00	1.569 ± 0.02 A	0.641 ± 0.09
C	9.225 ± 0.51	2.498 ± 0.01	0.251 ± 0.00	1.980 ± 0.13 AB	0.573 ± 0.03
	NS	NS	NS	**	NS
200–400 μm					
SSe	15.110 ± 7.58	3.739 ± 1.90	0.101 ± 0.05	1.442 ± 0.89	0.355 ± 0.19
SSa	17.284 ± 4.68	4.004 ± 0.84	0.214 ± 0.07	1.585 ± 0.37	0.515 ± 0.12
MF	ND	ND	ND	ND	ND
C	20.703 ± 2.65	4.733 ± 0.06	0.126 ± 0.03	2.637 ± 0.32	0.444 ± 0.03
	NS	NS	NS	NS	NS

Note: Treatments with the same letters are not statistically different.

Abbreviations: C, Control ($n = 3$); MF, Mineral Fertilizer ($n = 3$); ND, not determinate; SSe, SS 80 t ha^{-1} per year ($n = 3$); SSa, SS 20 t ha^{-1} per year ($n = 3$).

*$p < .1$; **$p < .05$; ***$p < .001$.

In relation to total porosity, the MF treatment was the one with the lowest total porosity >25 μm (Figure 2). This can be explained by the size and shape of pores within this size range, which were bigger in average than in the other treatments and included mostly large, elongated pores corresponding to horizontal fissures and compound packing pores (Table 1). As such, the pore area was probably larger than the 1 cm^2-square used for the description, and the pore measurements could not be properly made (Mateo-Marín et al., 2021). Long-term MF can be therefore associated to the development of a less functional structure (laminar structure, with horizontal pores limiting infiltration and seed emergence) than SS addition or even no fertilization (C treatment). Other studies also show organic fertilization displaying a more diverse, connected and complex pore system than in MF soils (Dal Ferro et al., 2013; Schjønning et al., 2002).

Under an annually-tilled agricultural management type, connected pores >100 and <400 μm have an important role for soil functioning, as they represent the transmission pores where water moves through and where the roots grow into (Pagliai & Vignozzi, 2002). In our study, within this apparent pore diameter interval, results suggested that SS addition stimulated the development of bigger pores (Figure 2).

Within the size range 100–200 μm, the aspect ratio was higher within SS treatments compared to MF, with C displaying an intermediate behaviour (Table 4). Aspect ratio translates the elongation of pores, and within this size-range, more elliptical pores correspond to the sections of channels and assure a good network for water transmission. In comparison, rounded pores in MF-treated soil are sections of more spherical and, therefore, isolated pores, which are characteristic of poorly aggregated soils. The same trend was found (without significance) by Yagüe et al. (2016), on a Oxiaquic Xerofluvent soil in a dry Mediterranean climate, where the treatments fertilized with dairy cattle manure showed a higher aspect ratio than MF. In contrast, in a study made on a Typic Xerofluvent soil also in a dry Mediterranean climate, but using pig slurry (Mateo-Marín et al., 2021), control plots showed higher AR than the fertilized plots. The different results found in these studies highlight the site-dependency relevance on the changes in soil structure, as the three studies were each conducted in different textured soils. Additionally, the nature of the exogenous sources of organic C (in our case SS) seems pertinent in relation to the response of soil structure to their addition.

Finally, SS treatments tended to have a higher proportion of pores in all categories (not significant difference),

TABLE 5 Soil physical and biological indicators (0–15 cm)

	Treatments			
Soil indicators	**SSe**	**SSa**	**MF**	**C**
AWHC (g g^{-1})	0.102 ± 0.01 B ***	0.048 ± 0.00 A ***	0.105 ± 0.01 B ***	0.091 ± 0.00 B ***
Bulk density (g cm^{-3})	1.46 ± 0.07 NS	1.47 ± 0.03 NS	1.56 ± 0.05 NS	1.52 ± 0.02 NS
WSA (%)	85.8 ± 0.82 NS	84.6 ± 0.77 NS	85.9 ± 1.11 NS	83.8 ± 1.21 NS
MWD (mm)	0.707 ± 0.01 A **	0.792 ± 0.01 B **	0.773 ± 0.01 B **	0.820 ± 0.02 B **
Organic C (gC kg^{-1})	18.2 ± 0.24 C **	15.7 ± 0.14 B **	14.5 ± 0.47 A **	13.949 ± 0.40 A **
Earthworms' biomass (g m^{-2})	13.3 ± 4.5 A **	32.0 ± 16.5 A **	5.97 ± 2.50 A **	194.4 ± 82.3 B ***
Earthworms' abundance (ind m^{-2})	45.8 ± 20.8 A **	65.4 ± 25.4 AB **	12.5 ± 7.22 A **	145.8 ± 34.1 B **
Earthworms' average size (g ind^{-1})	0.345 ± 0.09 A **	0.679 ± 0.23 B **	0.199 ± 0.10 A **	1.687 ± 0.37 B **
Yield (*Triticum aestivum* L, kg ha^{-1})	6470 ± 730 B ***	8268 ± 320 C ***	8877 ± 266 C ***	3505 ± 475 A ***

Note: Treatments with the same letters are not statistically different.

Abbreviations: AWHC, available water capacity; C = Control ($n = 3$); MF, Mineral Fertilizer ($n = 3$); MWD, mean weight diameter; SSe, SS 80 t ha^{-1} per year ($n = 3$); SSa, SS 20 t ha^{-1} per year ($n = 3$); WSA, water stable aggregates.

*$p < .1$; **$p < .05$; ***$p < .001$.

based on their apparent pore diameter compared to MF-treated soil (Table 4). However, the reverse was seen on total porosity >25 μm (Figure 2), where MF had the highest porosity. These contrasting results point towards a different origin and functionality of the pores under different fertilization managements (organic vs. mineral), which corresponds to the differences observed in terms of earthworm abundance (Table 5). The fact that no pores were identified in MF within the 200–400 μm size range, supports the idea that this treatment favoured the development of more elongated pores or fissures >1 cm long, not detectable with the approach used.

These observations together allowed to understand that changes resulting in an overall higher porosity when using MF vs different amounts of SS for fertilization, or even no fertilization (treatment C) were also related to the development of a different kind of porosity. Mineral fertilization would result in a higher abundance of (horizontal) fissures and cracks, whilst with SS application a structure including different categories of pores, more related to faunal and root activity was found. This suggests that, despite annual tillage taking place in all treatments, a known factor of soil structure disturbance, mineral fertilization and organic fertilization displayed positive effect on soil porosity and pore shape morphology.

4.2 | Soil physical indicators, earthworms and yield

The indicators chosen for this study are commonly related to soil porosity and generally used to assess the soil physical condition and biological activity in agricultural soils. Fertilization with organic materials frequently results in an increase in SOC stock (Antón et al., 2021), which can positively affect the development of stable aggregates (Abiven et al., 2009), and result in more efficient carbon and water storage. All these effects can be directly or indirectly related to the quantity and quality of soil pores. As a consequence, in other experiments, long-term addition of SS was observed to have a positive effect on the soil biological activity (Yagüe et al., 2016), particularly, on earthworm populations (Murchie et al., 2015; Pérès et al., 2011; Valdez et al., 2020).

In our experiment, however, some observations indicated that the relationship between SS addition and the improvement of physical and biological indicators was not straightforward. In relation to physical indicators, significant differences were found between treatments only for gravimetric AWHC and MWD, for which the dose seemed to make a difference in the SS-amended treatments (Table 5). Regarding AWHC results, SSa had significantly lower values than the other treatments. This observation can be related to the slightly distinct characteristics of 200–400 μm pores in SSe, compared to SSa, suggesting a different effect of SS addition or MF in the soil microporosity than in the pores 200–400 μm (Table 4). This would imply that the changes induced by different fertilization types in the studied soil relate to water infiltration and transfer more than water retention.

For MWD, SSe resulted in a less stable structure than the other treatments (Table 5). This suggests that the higher dose disturbed the stability of aggregates but without effect on the water holding capacity compared to the use of MF or no fertilization, while the opposite happened with SSa. This lack of correlation between the observed results in SOC and aggregate stability can be related to the nature of the amendment (Dal Ferro et al., 2013). The addition of SS, a relatively labile source of OM, which can be easily mineralized, could result in the positive effects that OM inputs can have on soil structure not being pronounced over time. This was also observed by Annabi et al. (2011) with five fertilization times within a 9 year field trial, using different organic amendments In addition, the possible incorporation of other compounds present in SS that may destabilize soil structure, such as soluble salts, can result in long-term degradation of the soil structural stability. Other studies have found a link between the application of SS and higher electrical conductivity values (Cai et al., 2010; Chong* & Purvis, 2004), which was observed previously at our study site (Simoes-Mota et al., 2021).

Earthworm abundance and biomass are biological indicators to assess the response of soil fauna to contamination (Pérès et al., 2011). These results agreed with the observations from the micromorphological study, both descriptive (Table 3) and pore sizes and shapes (Table 4). The SS treatments had better values in both parameters than MF, which is in accordance with other studies (D'Hose et al., 2018; Spiegel et al., 2018). However, in our study, the earthworm population was more abundant in the control plots (treatment C), than in SSa and SSe, whereas a positive correlation between OM addition and earthworm population was expected. This finding suggests a negative effect from SS application, especially in excessive doses as earthworms avoided the SSe treatment despite the high carbon input. The characteristics of SS, as well as the potential accumulation of metals and other pollutants (Urra et al., 2019; Zaragüeta et al., 2021), may explain this behaviour (Rastetter & Gerhardt, 2017). Other reports on the effect of SS application on earthworm populations on farmlands are inconsistent (Barrera et al., 2001). Nevertheless, the narrow link between the micromorphological approach and earthworms assessment in this study reaffirms the positive effect earthworm activity has on soil porosity (Schon et al., 2017; Valdez et al., 2020).

Soil organic C concentrations were similar in MF and C plots, despite the former gaining yields twice as higher as those in the latter (Table 5). Different aspects, such as the quality of crop residues (i.e., C/N ratio [Chen et al., 2018; Grzyb et al., 2020; Lu et al., 2011]) or the mineralization of these residues induced by MF (Cheng et al., 2020) can at least partially explain this observation (Liang et al., 2022). Also, the calcareous nature of the soil, which can promote long-term SOC stabilization, can explain a more efficient overall SOC protection in this soil, resulting in C treatments maintaining SOC stocks in the long term (Rasmussen et al., 2018; Rowley et al., 2018). Identifying mechanistic processes is beyond the scope of this work. However, it is suggested that higher the earthworm activity in C plots resulted in a more crumbly structure compared to MF (Table 3), which increased the incorporation of crop residues into the soil matrix, which may explain the absence of difference in SOC. Finally, wheat yield results also support the idea that the dose of SS plays a role in soil fertility, as the maximum yields were obtained with MF and SSa, while SSe displayed an intermediate value between those treatments with fertilization and the unfertilized control.

The lower SS dose (SSa) was as efficient as MF (Jaber et al., 2005; Obriot et al., 2016), while SSe had a deleterious effect on yield (Albornoz, 2016). In relation to the observations on porosity, yield results also showed a decoupling between physical and biological condition and biomass productivity, as the two most contrasting treatments in terms of porosity and earthworms' activity (MF and SSa) resulted in the highest yields. In terms of soil functioning, this is as an example of yield not always being the best indicator of soil condition, as the nutrient availability can compensate a poorer physical condition. To address with accuracy the state of soil quality condition, holistic approaches are needed to move towards productive and sustainable cropping systems (Salomé et al., 2016; Simoes-Mota et al., 2021).

5 | CONCLUSIONS

In this study, micromorphology analysis provided valuable information not found with other indicators. For example, image analysis detected subtle changes in soil structure more adequately than bulk density. In addition, it offers the possibility to understand to what extent these changes correspond to the morphological characteristics of pores, extending the information given by AWHC measurements. In general, we found that SS fertilization at agronomic doses improved the structure and the pore network compared to mineral fertilization. Conversely, long-term mineral fertilization, although as efficient as organic fertilization in supporting crop yields, resulted in greater soil structural degradation than fertilization with SS at agronomic doses or even no fertilization at all. The use of extreme doses of SS needs further examination, as it may have deleterious effects in terms of both soil structure and yields. Overall, the long-term application of an exogenous source of OM at agronomic doses had a better effect on protecting soil from physical degradation than mineral fertilization in the studied soil. Exploring the actual consequences of these observations in terms of soil functioning is needed for a complete assessment of SS application in this type of soils and agronomic conditions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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