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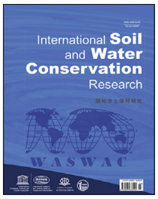

Original Research Article

Monitoring the variation of soil quality with sewage sludge application rates in absence of rhizosphere effect

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ABSTRACT

Agricultural soils in semi-arid regions have frequently been degraded due to adverse climatic conditions, organic matter depletion, and poor farming practices. To enhance soil quality, this study examines the reuse of sewage sludge (SS) as an available source of organic matter in a typical Mediterranean sandy-loam soil. Accordingly, we studied the cumulative effect of two annual applications of 40, 80 and 120 tons of sludge per ha on soil quality in absence of vegetation. The dose-dependent improvement of organic matter content was the most significant event that reflected sludge application rates, and consequently influenced other soil properties. Accordingly, soil structural stability increased by 13.3%, 28.8% and 59.4% for treatments SS-40, SS-80 and SS-120 respectively as compared to unamended control. Structural stability improvement was also confirmed by the dose-dependent variation of other edaphic factors including calcium content, the microbial quotient as well as Welt and C:N ratios. These parameters are involved in cementing soil aggregates by cation bridging, the formation of microbial mucilage, and clay-humic complexes. Soil magnetic susceptibility (SMS) was measured in situ as a possible rapid tool to evaluate soil condition. SMS showed significant correlation with sludge dose and stability amelioration testifying to the aggregation role that can play Al_2O_3 and particularly Fe_2O_3 minerals added by the hematite-rich sludge. Besides, analytical results and field observations revealed no trends of soil salinization or acidification by excessive sludge amounts. By avoiding the rhizosphere effect, outcomes could reflect the resilience and intrinsic capacity of the soil to cope with excessive sludge loads.

1. Introduction

Degradation of productive croplands has been of a great economic concern due to its impact on current and future production and implications on food security. Global estimates of total degraded lands vary from less than 1 billion ha to over 6 billion ha, with equally wide disagreement in their spatial distribution (Gibbs & Salmon, 2015). In any case, improving soil quality contributes to minimize arable land degradation and allows for better sustainable agricultural practices. On the other hand, semi-arid soils in particular are more vulnerable to degradation because they store little organic carbon (Janzen, 2004); whereas these soils possess a great potential for carbon sequestration after organic amendments (Lal, 2009). Therefore, enriching soils with exogenous organic matter is a major practice that restores degraded soils in semi-arid regions (Masciandaro et al., 2013; Zoghlami et al., 2016).

It has historically been proved that the valorization of agricultural wastes such as farm manures or plant residues improves the fertility of soils by enhancing their structure, nutrient content, hydrodynamics properties, and biological activities (Adediran et al.,

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2003; Zoghlami et al., 2016). In addition, nutrient inputs from organic wastes to croplands is a way to reduce the need for chemical fertilizers (Siebielec et al., 2018). In recent years, agricultural intensification has led to greater input requirements, which resulted in high demand for traditional farm manure and raised the need of seeking organic matter supply from unconventional sources. For instance, urban sewage sludge (SS) is an organic by-product continuously generated during domestic wastewater treatment. Urban sludge is naturally rich in organic carbon, N, P, and micronutrients, which gives it unique fertilizing benefits (Fytili & Zabaniotou, 2008). Sludge addition to croplands has been a promising practice for farmers in semi-arid regions because it equilibrates soil humic balance, supplies nitrogen and phosphorus at lower costs, and most importantly, copes with farm manure shortages (Lu et al., 2012; Siebielec et al., 2018). While the improvement of crop yield and the changes of soil chemical properties including contaminant accumulation have extensively been studied (Delibacak & Ongon, 2016; Siebielec et al., 2018; Tejeda & Gonzalez, 2007), less is known about changes in other soil properties following repetitive or mismanaged SS applications. In this regard, soil physical degradation may occur depending on sludge quality, applied dose, amendment frequency, and pedo-environmental conditions (Hamdi et al., 2007; Singh & Agrawal, 2008).

Physical degradation has generally been monitored by addressing soil pH and electrical conductivity, which reflect acidification and salinization risks after sludge application (Eid et al., 2018; Hamdi et al., 2007; Singh & Agrawal, 2008). These factors affect soil aggregate stability resulting in dispersive soils with poor structure (Odeh & Onus, 2008). On the other hand, most of studies that monitor sludge effect on soil have been conducted under cropped conditions (Delibacak & Ongon, 2016; Eid et al., 2018; Kayikcioglu et al., 2019). Consequently, the intrinsic capacity of soils to cope with degradation is likely to be influenced by plant roots referred to as the rhizosphere effect (Kobierski et al., 2018). Therefore, the aim of this study was to monitor soil indicators that reflect changes in global soil quality after two annual amendments with increasing rates of sewage sludge. Conducted in complete unvegetated soil conditions, this study could be then carried out by ascertaining the effect of sewage sludge addition on: (i) soil organic matter quality, (ii) soil structural stability, (iii) soil pH and salinity, and (iv) whether these properties were influenced or not by moderate to excessive rates of sludge. Accordingly, our hypotheses were as follows: (i) appropriate sludge reuse is a suitable practice for the restoration of semi-arid agricultural soils by improving soil fertility, (ii) the quality of organic matter (fulvic and humic acids) would affect structural stability, and (iii) the absence of vegetal cover would ultimately reflect soil resilience and intrinsic capacity to cope with excessive sludge loads.

2. Materials and methods

2.1. Experimental design

The field study was conducted in an Agricultural Experiment Station located in the city of Nabeul (northeastern Tunisia, 36° 27′ 15″ N, 10° 44′ 5″ E). The region has a southern semi-arid Mediterranean climate characterized by prolonged dry summers, warm winters, moderate and irregular precipitation (350–400 mm). The experimental soil is typical light-textured agricultural soil of the region classified as sandy loam, having moderate organic matter and low N contents (Table 1). Soil X-ray diffraction (XRD) pattern shows a dominance of quartz and calcite minerals (71%), while the smaller clay fraction (~12%) is composed mostly of kaolinite and illite. Consequently, the soil has a relatively small specific surface area of 27 $m^2 g^{-1}$ (Table 1). The mean topsoil annual temperature is 18 °C but varies largely with seasons (9.7–31.7 °C). Aerobically digested SS was directly collected from the drying beds of a close urban wastewater treatment plant. It was further air dried at the experimental farm for three weeks to reach a final water content of about 10%. The urban sludge had a C:N ratio of 15 and complied with Tunisian guidelines (NT 106-20) for biosolids reuse in agriculture.

Table 1
Physico-chemical properties of experimental soil and sewage sludge.

	Soil (sandy loam)	Sewage sludge
Sand (%)	70.9	–
Clay (%)	11.9	–
Silt (%)	17.2	–
SSA ($m^2 g^{-1}$)	27	–
Bulk density ($g cm^{-3}$)	1.31	–
pH (1:2.5)	7.72	7.7
EC ($\mu S cm^{-1}$) (1:5)	155	1702
TOC (%)	0.76	18.5
OM (%)	1.30	31.8
N (%)	0.071	1.18
C:N	10.7	15.7
P Olsen ($mg kg^{-1}$)	14.1	220
K ($mg kg^{-1}$)	58.8	9.54
Na ($mg kg^{-1}$)	19.6	1231
Ca ($g kg^{-1}$)	9.56	113.5
Fe_2O_3 (%)	0.95	2.08
$CaCO_3$ (%)	1.8	11.8
Al_2O_3 (%)	1.91	4.41

All values are given on dry weight basis.

The experimental protocol consisted of four soil treatments with four replicates distributed in four completely randomized blocks. Each replicate plot had a surface area of 4 m^2 (2 m × 2 m) separated from other neighbouring plots by a pathway of 2 m. Treatments were: control C (without sludge), and SS added at moderate to excessive rates calculated in equivalent metric ton per ha per year as follows: SS-40 (40 $t ha^{-1} year^{-1}$), SS-80 (80 $t ha^{-1} year^{-1}$), and SS-120 (120 $t ha^{-1} year^{-1}$). Since 2012, sewage sludge has been yearly added during the fall season (October–November) by uniform spreading onto amended plots followed by incorporation into the topsoil (~10 cm). Control plots were hand-hoed similarly without SS addition. Outcomes described in this study represent changes in soil quality after two annual successive amendments. To this end, soil samples were collected from each replicate plot at 0–20 cm depth, composited and then stored until analysis. Throughout the experimental period, emerging weeds had regularly been removed to avoid the rhizosphere effect on soil dynamics. Consequently, changes in soil properties were exclusively influenced by sludge dose and prevailing climatic conditions.

2.2. Physico-chemical analysis

Physico-chemical properties were determined using air-dried soil samples sieved through 2 mm mesh. Soil pH and EC were measured in soil-water slurries of 1:2.5 and 1:5, respectively. Aluminium and iron oxides were analysed by X-ray fluorescence (XRF). Total organic carbon (TOC) was determined using dichromate oxidation method (Walkley & Black, 1934). Total nitrogen was analysed by the Kjeldahl method (Bremner, 1996). Extractable phosphorus was determined according to the Olsen method (Olsen et al., 1954). Exchangeable bases were extracted from the soil with ammonium acetate (1 M) and analysed by flame photometer (Pauwels et al., 1992, p. 180).

2.3. Soil structural stability

Soil structural stability was determined by the Henin and Monnier method (1956) using aggregate size fraction ≤ 2 mm. The proportions (%, w/w) of stable Ag, Ag_a and Ag_b aggregates (corresponding to untreated, alcohol-treated, and benzene-treated aggregates, respectively) were calculated, and the instability index (Is) was obtained using the following equation:

$$Is = \frac{(\% < 20\mu m)max}{\frac{(Ag+Aga+Agb)}{3} - 0.9\ (\%Cs)}$$

where numerator indicates the largest proportion of suspended particles < 20 μm for each soil sample, and $\%C_S$ is the largest proportion of coarse sand fraction (0.2–2 mm) forming part of the stable aggregates (Tejeda & Gonzalez, 2007).

2.4. Biomass C and N

Microbial biomass C and N (BC and BN) were estimated by the fumigation-extraction of fresh soil samples (Vance et al., 1987). To this end, a moist soil sample was divided into two portions equivalent to 10 g of oven-dry soil. One portion was fumigated for 24 h at 25 °C with ethanol-free $CHC1_3$. Following fumigant removal, the soil was extracted with 40 mL K_2SO_4 (0.5 M) by horizontal shaking for 60 min (Joergensen & Brookes, 1990). The non-fumigated portion was extracted similarly at the time fumigation had started. After organic C and N analysis in extracts, microbial biomass C or N were calculated as follows:

$$\text{BC or BN (mg kg}^{-1}) = E/ke$$

where E = (organic C or N extracted from fumigated soils) - (organic C or N extracted from non-fumigated soils)

ke = 0.45 (Joergensen & Brookes, 1990).

2.5. Humic substances

Humic compounds in soil samples were analysed according to Shnitzer (1982) modified by Fourti et al. (2010). Accordingly, 10 g of soil were extracted with 100 mL of NaOH (0.1N) for 24 h until the calcium-containing humic compounds are exhausted. The mixture was separated by centrifugation 4000 rpm for 20 min, then sulphuric acid (2N, pH = 1) was added to the supernatant. After breather for 24 h at ambient temperature and centrifugation of 4000 rpm for 20 min, the collected supernatant represented fulvic acids (FA) and the pelleted fraction humic acids (HA).

The quality of soil organic matter was estimated using a colorimetric method described by Shnitzer (1982). As such, before the final extraction of FA and HA, absorbance of the mixture (pH 7–8) was measured at two wavelengths (E_4 = 465 nm; E_6 = 665 nm). The rate of E_4/E_6 known as the Welt ratio reflects organic matter quality and represents the humification index (Chen et al., 1977).

2.6. Magnetic susceptibility

Soil magnetic susceptibility (SMS) was measured in situ onto the surface of each replicate plot by means of a shirt-pocket size magnetic susceptibility meter (SM-20; ZH instruments, Czech Republic). This device operates at a frequency of 10 kHz and measures SMS on an average depth of 10 cm. Readings are displayed as values x 10^{-3} SI and indicate the content of ferromagnetic minerals in soil (Yoshida et al., 2003).

2.7. Statistical analysis

As previously mentioned, the experimental protocol consisted of four completely randomized blocks, each containing four treatments (C, SS-40, SS-80 and SS-120). ANOVA analysis with *post hoc* Duncan's multiple range test ($P \leq 0.05$) was used for quadruplicate mean separation (SPSS statistics 17.0, SPSS Inc., Chicago, USA). Relationships between different measured parameters were estimated with Pearson product–moment correlation coefficients ($P \leq 0.05$).

3. Results and discussion

This study aimed to examine the interaction effect of sewage sludge dose and intrinsic soil properties on soil quality under semi-arid pedo-climatic conditions. In particular, soil fertility is characterized by the influence of three soil components namely, (i) physical properties (ii), chemical properties, and (iii) biological properties (Delgado & Gómez, 2016). Accordingly, two annual successive SS applications increased soil TOC in a dose-dependent manner (Table 2). Therefore, the highest significant TOC content was observed in treatment SS-120 (1.88%) with respect to the rest of treatments and unamended soil (0.86%). Moreover, all sludge-treated soils had a soil organic matter (SOM) content greater than 2% (Table 2), a reference value considered as the highest in semi-arid Mediterranean croplands (Ryan & Pala, 2007). It has already been proven that the agricultural reuse of sewage sludge improves SOM and enriches amended soils with macro and micronutrients (Masciandaro et al., 2013; Zoghlami et al., 2016; Kayikcioglu et al., 2019). This is related to the richness of urban sludge with organic carbon (18.5%) and its stability (maturity) at the time of application (C:N = 15, Table 1). In this regard, soil carbon to nitrogen ratio (C:N) is one of the most important parameters reflecting soil quality and ecological functions. For instance, Bird et al. (2002) found that this ratio was the best predictor of aggregate stability in a semiarid rangeland in New Mexico, USA. Besides, C:N reflects carbon and nitrogen cycling and nutrition balance in soils (Sun et al., 2017). In this study, C:N ratio decreased significantly with SS dose reaching the lowest value of 9.9 in treatment SS-120 as illustrated in Table 2. This value matches C:N ratios of stabilized humus (10), which allows for beneficial slow releases of bioavailable forms of nitrogen (Bengtsson et al., 2003). Consequently, increasing SS dose to 120 t ha^{-1} $year^{-1}$ created better conditions in terms of N mineralization under the described pedo-climatic conditions. In contrast, unamended control showed the highest C:N ratio (29) among treatments, which also significantly increased with respect to that calculated for the experimental soil (13.5) as illustrated in Table 1.

Table 2

Variation of soil properties after two annual successive amendments with urban sewage sludge (SS).

Treatments	C	SS-40	SS-80	SS-120
pH	8.17^a	8.19^{ab}	7.99^b	7.88^b
EC (μS cm^{-1})	313^a	480^{ab}	650^{bc}	722^c
TOC, %	0.86^a	1.21^{ab}	1.30^b	1.88^c
SOM, %	1.48^a	2.08^{ab}	2.24^b	3.23^c
N, %	0.04^a	0.09^b	0.12^b	0.19^c
C/N	21.5	13.4	10.8	9.9
P (mg kg^{-1})	14.77^a	15.54^b	18.19^c	20.02^d
Na^+ (mg kg^{-1})	48.2^a	55.5^b	63.5^c	75.7^d
Ca^{2+} (g kg^{-1})	6.08^a	6.47^{ab}	7.01^b	7.14^b
Fe_2O_3, %	1.15^a	1.15^a	1.16^{ab}	1.19^b
Al_2O_3, %	2.52^a	2.51^a	2.51^a	2.56^b

Numbers associated to treatment names represent SS application rates in t ha^{-1} $year^{-1}$. C: unamended soil control. For each soil parameter, means with the same lowercase letters are not statistically different at $P \leq 0.05$.

Soil depletion in absence of organic amendments is in direct connection with degradation caused by “N-hunger” and the subsequent incapacity of microorganisms to mineralize organic carbon (Atkins et al., 1989).

Depending on prevailing conditions, organic matter mineralization affects directly soil physical properties (Hamdi et al., 2007; Abdollahi et al., 2014). The observed pH decrease and EC increase with sludge application rates were previously reported in a sandy soil under the same experimental conditions (Zoghlami et al., 2016). Under oxic and warm temperature environments that characterize light-textured topsoils in semi-arid regions, the aerobic biodegradation of organic matter has always been reported to be rapid (Rey et al., 2008). Part of the released CO_2 reacts with H_2O of the soil solution to form a weak acid called carbonic acid (H_2CO_3) (Hamdi et al., 2007). The latter will release H^+ protons by double dissociation causing gradual soil pH decrease over time (Stevenson, 1994, p. 496). Despite the slight dose-dependent decrease of soil pH in the current study, values remained within neutral range (7.88–8.17) as shown in Table 2. This indicates that SS-amended soils were still able to buffer against acidification resulting from TOC transformation and cycling over two years (Bolan et al., 2005; Hajnos, 2011). In addition, it is likely that the prevailing semi-arid conditions of the region did not allow for maintaining adequate soil moisture that accelerates the formation of H_2CO_3. In fact, particulraly low and irregular pluviometry of 263 and 370 mm was respectively recoreded during the two years of study.

After two successive SS amendments, soil salinity increased significantly with application rates as reflected by the variation of EC and Na^+ (Table 2). However, salinization did not occur since EC values and exchangeable Na^+ concentrations remained low enough to cause substantial soil degradation even for the highest sludge dose of 120 t ha^{-1} (722 µS cm^{-1} and 76 mg kg^{-1}, respectively) as compared to control (313 µS cm^{-1} and 48 mg kg^{-1}, respectively) (Yan & Marschner, 2013). In addition, no visible signs of saline/sodic soils were ever observed on the surface of all SS-treated plots throughout the experimental period (Choudhary & Kharche, 2015). This was also evidenced by the consistent emergence of same weed species in both unamended and amended soils testifying to the absence of any salinity-induced phytotoxicity caused by sludge addition (Wu et al., 2015). It is likely that the joint effect of rainfall and soil permeability could have resulted in the partial leaching of accumulated salts, which reduce the extent of soil salinization as well.

Soil instability index (Is) is another important physical parameter that reflects the relative state of aggregate water stability. This index has extensively been used for assessing changes in soil structural stability after organic amendments (Dimoyiannis et al., 1998; Tejeda & Gonzalez, 2007). Fig. 1 illustrates the variation of log10 (Is) with soil treatments after two annual successive SS applications. There was a significant dose-dependent amelioration of soil structure reflected by substantial decreases of instability index with respect to unamended control. Improvement rates were +13.3%, +28.8% and +59.4% for SS-40, SS-80 and SS-120, respectively. This was further highlighted by a significant correlation between Is and TOC content ($r = -0.89$) as indicated in Table 3. García-Orenes et al. (2005) reported that successive biosolids amendments significantly improved the aggregate stability percentage. Under comparable dryland conditions, Tejeda and Gonzalez (2007) observed also a structural stability improvement by 9% in soils treated for two years with SS at 25.6 t ha^{-1} $year^{-1}$. In general, enriching agricultural soils with organic matter affects soil mineralogy and particle-size distribution, resulting in soil cementing by flocculating particles to form stable aggregates (Spaccini et al., 2004; Tejeda & Gonzalez, 2007; Abdollahi et al., 2014; Shahbaz et al., 2017). As revealed before, the dose-dependent improvement of structural stability (Fig. 1) was also confirmed by the absence of signs of clay and/or organic matter dispersion in situ as well as by pH values consistently below the alkalinity range (<8.5) (Choudhary & Kharche, 2015).

In addition to organic matter effect, soil aggregation occurs also in presence of aluminium or iron oxides, colloidal silica or calcium carbonate (Braver et al., 1972, p. 498; Igwe et al., 2013). Several

Table 3
Pearson product-moment correlation coefficients (*r*) of soil parameters.

	Is	E_4/E_6	BN	BC	SMS	TOC	Ca^{2+}	Fe_2O_3	Al_2O_3
Is	-	*−0.99*	*−0.89*	*−0.99*	*−0.96*	*−0.89*	*−0.98*	*0.78*	*0.51*
E_4/E_6		-	*0.94*	*0.99*	*0.97*	*0.94*	*0.93*	*0.96*	*0.78*
BN			-	*0.92*	0.88	*0.99*	*0.87*	*0.85*	*0.67*
BC				-	*0.96*	*0.91*	*0.91*	*0.97*	*0.80*
SMS					–	*0.83*	*0.99*	*0.86*	*0.61*
TOC						–	*0.75*	*0.99*	*0.94*
Ca^{2+}							–	*0.79*	0.49
Fe_2O_3								–	*0.92*
Al_2O_3									–

Italic correlation coefficients are significant at $P \leq 0.05$. Is: Soil instability index; E_4/E_6: Welt ratio; BN: Biomass nitrogen; BC: Biomass carbon; SMS: Soil magnetic susceptibility; TOC: Total organic carbon.

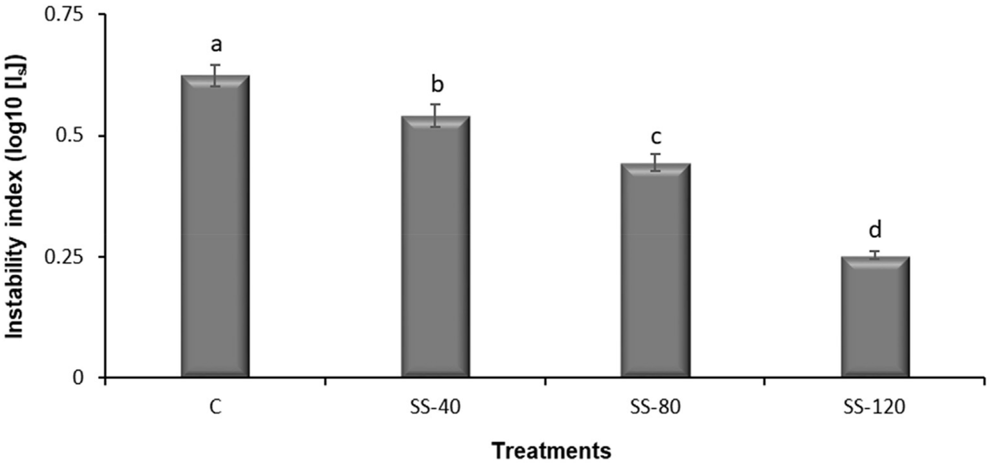

Fig. 1. Variation of soil instability index (Is) with soil treatments after two annual successive amendments with urban sewage sludge (SS). Numbers associated to treatment names represent sludge application rates in t ha^{-1} $year^{-1}$. C: unamended soil control. Means with the same lowercase letters are not statistically different at $P \leq 0.05$.

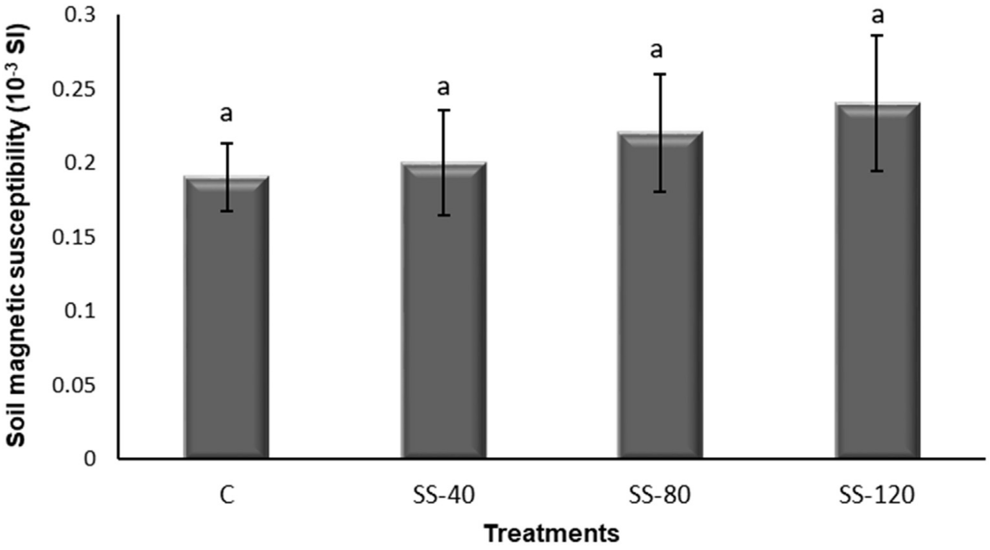

Fig. 2. Variation of soil magnetic susceptibility (SMS) with soil treatments after two annual successive amendments with urban sewage sludge (SS). Numbers associated to treatment names represent sludge application rates in t ha^{-1} $year^{-1}$. C: unamended soil control. Means with the same lowercase letters are not statistically different at $P \leq 0.05$.

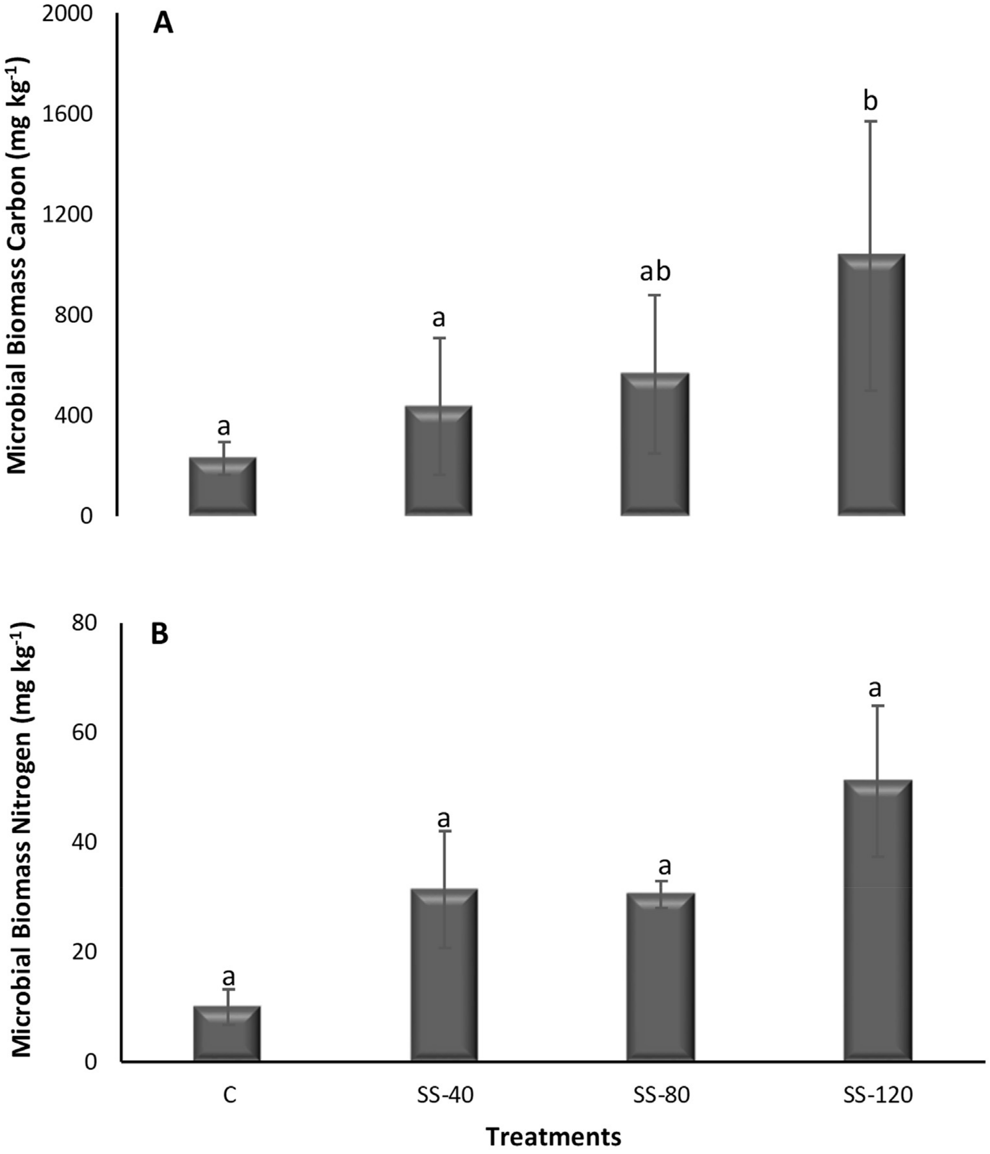

Fig. 3. Variation of microbial biomass carbon (A) and nitrogen (B) with soil treatments after two annual successive amendments with urban sewage sludge (SS). Numbers associated to treatment names represent sludge application rates in t ha^{-1} $year^{-1}$. C: unamended soil control. Means with the same lowercase letters are not statistically different at $P \leq 0.05$.

studies have highlighted the cation “bridging” effect of calcium between polycarboxylic molecules (humic acids) and clays, which results in additional soil aggregate stability (Muneer & Oades, 1989; Wuddivira & Camps-Roach, 2007). Interestingly, the current sludge had a substantial $CaCO_3$ content (11.8%) that caused a significant dose-dependent accumulation of calcium ions in amended soils, reaching 7.14 g kg^{-1} in SS-120 as compared to 6.1 g kg^{-1} in control soil (Table 2). The contribution of SS to Ca^{2+} accumulation in soil was also evidenced by significant correlation with TOC as illustrated in Table 3 ($r = 0.75$). On the other hand, an increase of soil magnetic susceptibility with SS rate was measured in situ (Fig. 2), showing strong correlations to TOC content and instability index (0.83 and −0.96, respectively) (Table 3). SMS reflects the presence of ferrimagnetic minerals in soil and is correlated to magnetite β-Fe_3O_4 and maghemite γ-Fe_2O_3 (Ramos et al., 2017; Yoshida et al., 2003). Consequently, the richness of SS in Fe_2O_3 (Table 1) was likely to further improve soil aggregation in a dose-dependent manner as well (Table 2). As compared to our findings (Tables 2 and 3), Sokolowska et al. (2016) observed also a linear relationship ($r = 0.76$) between SMS and Al_2O_3 content in soil after irrigation with wastewater. Goldberg (1989) explained the stabilizing role of iron and aluminium oxides in soil by decreasing critical coagulation concentration, clay dispersion and modulus of rupture; and by increasing micro-aggregation and hydraulic conductivity. These oxides precipitate on clay surfaces and play the role of stable coatings at high pH levels. The lowest SMS and structural stability in control treatment are indicators of degradation trends in absence of organic matter input under the current experimental conditions.

The microbiological assessment of agricultural soils is also important for quality monitoring (Wall et al., 2019). In this study, microbial biomass carbon and nitrogen increased as function of SS addition rates (Fig. 3). More precisely, BC (Fig. 3A) and BN (Fig. 3B) increased by almost five-fold in SS-120 treatment comparing to unamended control. This shows that excessive SS rates had no detrimental effects on the proliferation of biomass C and N (Fernandes et al., 2005). In general, the addition of biowastes provides exogenous microorganisms to soil and stimulates indigenous populations simultaneously, which increases aggregate formation and stabilization (Annabi et al., 2007). This contribution to structural stability is due to the production of microbial mucilage that act as soil binding agents (Caesar-Tonthat, 2002; Six et al., 2004; Tejeda & Gonzalez, 2007). On the other hand, 1–5% of total soil organic matter is represented by microbial biomass, which is a more sensitive indicator of changing soil conditions than direct analysis of organic carbon (Leita et al., 1999). Soil BC/TOC ratio, or microbial quotient, has widely been used as an indicator for future changes in organic matter due to alterations of soil conditions (Leita et al., 1999; Yang et al., 2010). This ratio is also used for comparison of soil quality across soils with different organic matter contents (Joergensen & Scheu, 1999). In other words, the microbial quotient is interpreted as substrate available and the portion of total soil carbon immobilized in microbial cells (Yang et al., 2010). In this study, TOC and BC were significantly correlated ($r = 0.91$) as shown in Table 3. Calculated BC/TOC ratios increased significantly with SS dose reaching 1.04%, 2.12%, 4.32% and 5.48% for control, SS-40, SS-80 and SS-120, respectively. This increase confirms the consistent dose-dependent improvement of soil quality with respect to unamended control under the described pedo-climatic conditions (Leita et al., 1999; Powlson, 1994; Zoghlami et al., 2016).

The quality of soil organic matter could be evaluated by determining the Welt ratio as well (Stevenson, 1994, p. 496), which is calculated to characterize soil HA and FA. Similar to BC/TOC, we observed a significant increase of E_4/E_6 ratio with sludge dose after two successive annual amendments (Fig. 4). As such, E_4/E_6 ratio passed from 1.45 in control to 3.48 in SS-120 implying significant correlation to TOC build up in soil as indicated in Table 3 ($r = 0.94$). Welt ratio is the parameter indicative of decomposition status and molecular size of soil organic matter. It mirrors the carbon fraction associated with humic acids in soil, which represent the major forms of stabilized carbon (Angelova et al., 2013; Zhao et al., 2013). According to Hevia et al. (2003), E_4/E_6 quotients lower than 5 indicate that humic rather than fulvic acids exist in the organic fraction of soils. This corresponds to ratios observed in all SS-amended soils (Fig. 4), which implies higher humus polymerization degree that favours organic matter adsorption onto the mineral matrix of the soil (Liu et al., 2015). This adsorption implicates a strong complexation between soil minerals and organic acid ligands especially those associated with aromatic structures

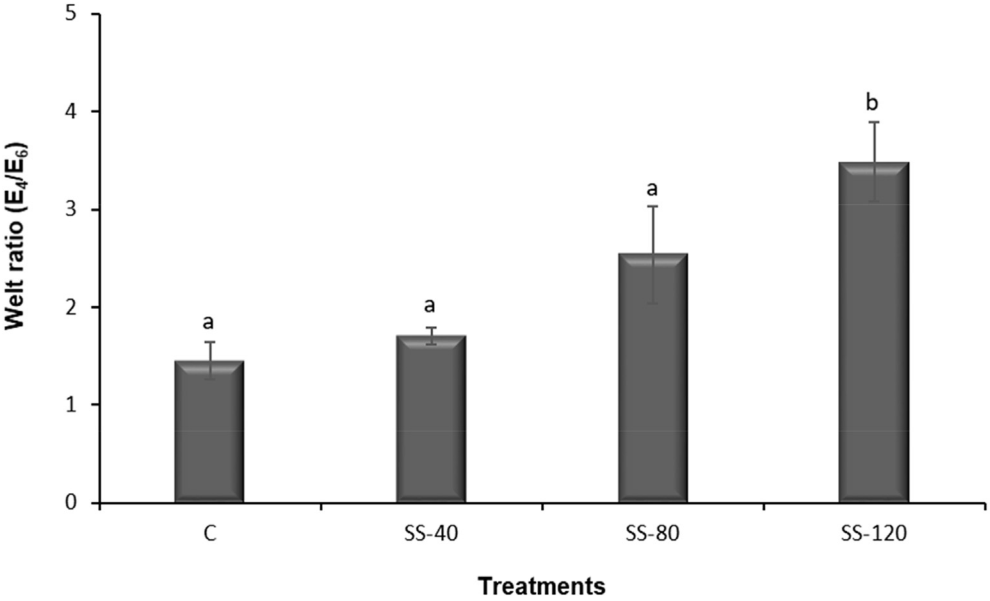

Fig. 4. Variation of the Welt ratio (E_4/E_6) with soil treatments after two annual successive amendments with urban sewage sludge (SS). Numbers associated to treatment names represent sludge application rates in t ha^{-1} $year^{-1}$. C: unamended soil control. Means with the same lowercase letters are not statistically different at $P \leq 0.05$.

(Verchot et al., 2011). In this study, the strong negative correlation between the Welt ratio and soil instability index ($r = -0.99$) evidences the beneficial effect of humic acids on soil structural stability through their contribution to the formation of clay-humic complexes, which prevents soil degradation (Wang and Xing, 2005). The lowest E_4/E_6 value calculated for unamended soil indicates that its small “aged” organic fraction has a higher degree of humification than the fresh one added with sludge.

4. Conclusions

The current field study investigated the quality of a semi-arid agricultural soil treated twice with increasing sludge amounts. Under the described pedo-climatic conditions, this practice resulted in a significant dose-dependent variation of all studied parameters. Overall, organic matter accumulation and its subsequent mineralization over two years improved soil structural stability in proportion to sludge dose. In this regard, soil magnetic susceptibility could be an interesting parameter in situ that correlates with soil quality. From a pure pedological point of view, excessive sludge rates of 120 t ha^{-1} $year^{-1}$ did improve soil structure and fertility without causing physical degradation. By conducting the field trials in absence of rhizosphere effect, we could highlight the intrinsic capacity of the agricultural soil in mitigating possible negative effects of sludge reuse.

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