Change and spatial variability of soil organic matter humification after long-term tillage and olive mill wastewater application in arid regions

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Change and spatial variability of soil organic matter humification after long-term tillage and olive mill wastewater application in arid regions

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Abstract. The changes of soil organic matter (SOM) humification induced by long-term combination of tillage and olive mill wastewater (OMW) application compared to natural and cultivated soil have been little investigated. This study aimed to compare effects of no cultivation with natural vegetation soil (NC), tillage (CT1) for 80 years and combination of tillage with OMW application (CT2) for 20 years on SOM humification degree. Fluorescence spectroscopy and UV-visible ratios (E4/E6 and C_{HA}/C_{FA}) were used to study soil humic acids (HAs). The SOM and humification distribution was determined for the whole field area using the Inverse Distance Weighting method. Results showed that SOM content, fluorescence emission area and E4/E6 and C_{HA}/C_{FA} ratios were higher in NC. Tillage reduced SOM amount, molecular size, aromatic condensation and humification degree as shown by the strong correlation between fluorescence area and C_{HA}/C_{FA} ratio in CT1 conversely to E4/E6. Contradictory results between fluorescence emission area and E4/E6 ratio found in NC and CT1 indicated that E4/E6 ratio was not a reliable indicator of SOM humification degree. The SOM amount, C_{HA}/C_{FA} ratio and emission fluorescence area increased conversely to E4/E6 ratio in CT2. This revealed a greatly humified organic matter and aromatic structure condensation with tillage and OMW application. Spatial distribution showed a progressive increase of SOM and C_{HA}/C_{FA} from north-west to south-east linked to the positive relationship between C_{HA}/C_{FA} ratio and SOM amount independent of soil management practices. Soil amended with OMW provided a favourable environment for the development of HAs which improved soil quality. The UV-visible ratio C_{HA}/C_{FA} with fluorescence emission area can be used as parameters to investigate SOM humification degree.

Additional keywords: aromatic structure, fluorescence spectroscopy, humic acids, organic wastes, UV-visible spectroscopy, SOM.

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Introduction

Soil organic matter (SOM) comprises all substances of biological origin, mainly plant and animal residues (Hernandez-Soriano *et al*. 2013). The SOM is considered an indicator of soil fertility due to its impact on soil chemical, physical and biological properties (Robertson *et al*. 2014). The SOM modifies porosity, increases aeration and water holding capacity, provides habitat for soil organisms that fuel nutrient cycling, and retains and provides nutrients critical to productivity (Brady and Weil 2007). The SOM content and quality change when soil is modified by different land uses such as tillage, application of amendments, natural vegetation and also by climatic conditions. In the Mediterranean regions, SOM content in agricultural soils is very low (<1%) especially in arid areas (Gargouri *et al*. 2013). This low amount is due to the effect of high temperatures, low rainfall and the intensified agricultural activities especially when water is available (Giongo *et al*. 2011). Indeed, higher temperature levels accelerate organic matter decomposition; this is likely to cause a serious agriculture problem in the future (Conant *et al*. 2011). Moreover, intensive tillage also decreases SOM content (ParrasAlcántara *et al*. 2015) and SOM compounds. Indeed, SOM humification or mineralisation is highly influenced by tillage. Several studies have shown that tillage enhances mineralisation against humification and reduces content of soil humic acids (HAs). Slepetiene and Slepetys (2005) noted that HA content increases in minimum tillage treatments compared to conventional tillage. However, Bayer *et al*. (2002) reported that the humification degree of HAs is lower in no tillage (NT) than conventional tillage treatment. Agricultural practices such as NT technologies help to restore soil fertility (Carr *et al*. 2013). According to Lal *et al*. (2007), NT systems are very effective in reducing erosion losses. More stable aggregates in the soil surface have been associated with

NT and this correspondingly results in high total porosity. A developed soil structure and high aggregate stability are important for enhancing soil fertility. However, NT systems are difficult to apply for improving soil fertility in arid areas with high water scarcity. In arid areas, water scarcity is the major limiting factor and long drought can be faced every year for 4–6 months. During this period any competition for water with the cultivated crop can be avoided by tillage and, due to lack of water, it is hard to maintain cover crops. Thus, a combination of tillage with amendment may be the best solution to enhance soil quality for crop production in agricultural land.

Olive growing in arid areas is a strategic socioeconomic sector. Almost 95% of olive trees are cultivated (IOC 2015) and more than 30 million m^3 of olive mill wastewater (OMW) are produced annually in the Mediterranean region (D'Annibale *et al*. 2004). The OMW contains high concentrations of phenolic compounds that are phytotoxic and difficult to biodegrade (Nikolopoulou and Kalogerakis 2007). However, olive oil producing regions (e.g. Tunisia, Morocco, Italy, Greece and Portugal) use OMW as soil biofertiliser especially because it contains 83–94% water, 4–16% organic compounds and 0.4–2.5% minerals (Ammar *et al*. 2005). Improvements in soil properties (chemical, physical and biological) have been observed following the use of OMW for both short and long term. El Hassani *et al*. (2010) reported that OMW spreading improves soil quality, which means recycling organic matter and enriching mineral elements that increase soil fertility. Chaari *et al*. (2015) found that long-term (9 years) application of raw OMW induces a significant increase in SOM, electrical conductivity, potassium, phosphorus and nitrogen. Other studies (Piotrowska *et al*. 2006; Rousidou *et al*. 2010) reported OMW spreading on soil provided a favourable environment for the development of soil microbial communities. Conversely, Mollaei *et al*. (2010) and Mekki *et al*. (2006) observed an inhibition of multiplication of soil microorganisms after OMW application due to the toxicity of phenolic compounds. Furthermore, Rusan *et al*. (2016) reported that short-term irrigation of soils with OMW can promote plant growth and production.

It is believed that humic substances are the main indices of soil fertility influencing crop productivity (Ufimtseva and Kalganov 2011). It is established that the HA content increases with natural vegetation (Traversa *et al*. 2011) as well as with addition of compost and manure (Ben Mbarek *et al*. 2019). During the humification process, organic matter in OMW is transformed to polymerised polyphenolic compounds, which include HA-like substances (Cox *et al*. 1997). Information concerning the impact of OMW on soil and the HA structures extracted from OMW are available. In contrast, there is a lack of knowledge on the impact of the long-term combination of tillage with OMW application on SOM humification degree. Soil microorganisms are able to produce with OMW compounds a mixture of aromatic structures (Niaounakis and Halvadakis 2004), which comprise humic compounds that improve soil fertility.

Current research assesses the impacts of combination of tillage with amendment as compared to plant residue input on SOC rates. Thus, studies in northern Japan limited their work to quantitative evolution of SOC with no information on qualitative aspects (Koga 2017). Other researchers examined SOM humification degree for long-term tillage compared to native forest and NT soils in tropical and subtropical regions with no focus on the effect of combination of organic waste with tillage. The SOM humification degree can be determined using UV-visible spectroscopy and fluorescence spectroscopy. Due to their sensitivity, non-destructivity and simplicity, these two spectroscopy methods have been used to provide qualitative and quantitative information on HAs. Indeed, UV-visible spectroscopy is often used to assess humification degree of organic matter in organic amendments (Chen *et al*. 1977; Ben Mbarek *et al*. 2019). Martins *et al*. (2016) used this technique to determine HA and fulvic acid (FA) contents in amended soil. Fluorescence spectroscopy is reliable for detecting changes in SOM humification due to cultural practices (Bayer *et al*. 2002; Milori *et al*. 2006). Fuentes *et al*. (2006) combined these two techniques to assess organic matter humification in soil and amendments but did not confirm their results by analytical methods. They concluded that, despite use of six humification indices, the results needed confirmation to be useful. In this work three spectroscopic humification indices with analytical methods are used to assess SOM humification. Indeed, the correlation between the UV-vis ratios (E4/E6 and C_{HA}/C_{FA}) and fluorescence area explains how different practice managements affect HA structure and SOM humification degree. This study aims to (1) investigate the change in SOM humification degree under long-term combination tillage with OMW application compared to uncultivated soil with natural vegetation and to tilled soil without amendment using UV-visible and fluorescence spectroscopy, (2) evaluate the accuracy of spectroscopic techniques to reveal aromatic structure diversity and the SOM humification degree and (3) map studied parameters using the interval distance weighting (IDW) method of the studied area in arid agro-ecosystems of south-eastern Tunisia.

Materials and methods

Site descriptions

The study area is located 60 km south-west of Sfax city (34°3′N, 10°20′E) in south-eastern Tunisia (Fig. 1). It is a part of organic

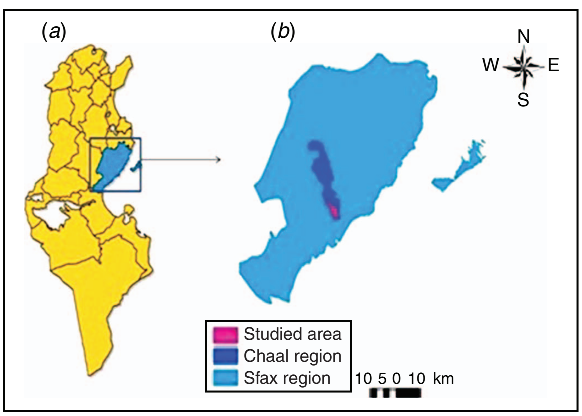

Fig. 1. Location of studied area: (*a*) Tunisia map and (*b*) Sfax region, Chaâl region and studied site.

farm called Chaâl. The Chaâl area is 18 000 ha and the majority of soil texture is sandy. The experimental field was 80 ha. The studied region has an arid Mediterranean climate with mean temperature 22°C and mean annual rainfall 156 mm during 2011–2017 as presented in Table 1 (climatic data provided by the Chaâl farm station in 2017).

Experimental design and treatment

The experimental field was a site covering three treatments corresponding to different long-term soil management practices. Each treatment was composed of three parcels, which were considered as replicates for each treatment. The first treatment (NC) was 20 ha of soil uncultivated for 80 years (since 1937). In NC treatment, three parcels were considered, each covering 2 ha and separated by at least 200 m. The NC treatment was colonised by native arid vegetation such as *Ziziphus lotus*, *Retama raetam* and *Stipagrostis pungens*. It was considered as a control soil without any farming practices (NT, no amendment, no olive tree and no plantation).

The second treatment (CT1) was 40 ha of cultivated soil, planted with olive trees with frequent soil tillage and without amendment for the last 80 years. Within CT1 three parcels were considered, each covering 6 ha and separated by at least 200 m.

The third treatment (CT2) was 20 ha of tilled soil with addition of 50 m^3 ha^{-1} of OMW for 20 years. Within CT2 three parcels were considered, each covering 2 ha and separated by at least 200 m. During the olive mill operation (early November to late December), OMW was spread from 1997 until 2017 yearly and homogenously on the soil covering the entire surface between trees. The OMW is an effluent characterised by acidic pH (4–5.5). More importantly, OMW presents high organic matter content (12.32%) and high concentrations of potassium, calcium and sodium (Gargouri *et al*. 2014).

The CT1 and CT2 treatments were planted with olive trees and tilled to depths varying within 5–25 cm using a tractor-driven cultivator. Planting was at the common density used in the region, corresponding to 17 trees/ha with a squared grid of 24 m. The only difference between the two treatments CT1 and CT2 was OMW amendment. These soils were tilled five times a year: twice before blooming during winter and early spring (for weed control and aeration of soil), one more in late spring (for weed control), one very superficial during summer to destroy capillarity channels and reduce evaporation and one reaching a depth of 20–25 cm during autumn to break compaction according to local agricultural management practices. In fact, in this arid region, farmers' practices to mitigate high temperatures and low rainfall aim to avoid any competition for water between the cultivated crop and natural vegetation by eliminating the latter and reducing evaporation through tillage.

Table 1. Annual rainfall of studied area during 2011–2017
Climatic data provided by the Chaâl farm station

Years	Annual rainfall (mm)
2011	194
2012	82.0
2013	112
2014	290
2015	164.5
2016	65.5
2017	184.0
Average	156.0

Soil sampling

Soil samples were taken using a soil auger from the upper layer corresponding to a depth of 0–20 cm. Fifteen soil samples were collected randomly from each treatment (Fig. 2). Each soil sample was composed of three samples from previously mixed three sampling points. The mean value was calculated on the basis of 15 soil samples collected from each plot. The characteristics of studied soils are presented in Table 2. Coordinates of soil samples were taken using a portable global positioning system (GPS). Soil samples were air-dried and passed through a 2-mm sieve before chemical analyses.

Soil analysis

The amount of organic matter was indirectly determined through carbon content determination using a multiplication factor of 1.72. Soil organic carbon content was determined by dichromate method according to Conyers *et al*. (2011).

Humic compounds were extracted from soil samples according to Rivero *et al*. (1998). Briefly, the soil was shaken overnight with a solution of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ with an extraction ratio 10 : 1. After centrifugation, the HAs were separated from the supernatant (i.e. FAs) by precipitation at pH 1.0. The decrease of pH was achieved by adding HCl to the solution. The precipitated HAs were separated by centrifugation, and then dissolved in NaOH and precipitated by adding HCl. After extraction of HAs and FAs from different samples, the ratio of C_{HA}/C_{FA} was calculated. The carbon content of FAs was obtained from the difference between the carbon content of humic extracts and the carbon content of HAs.

Spectroscopy analysis

UV-visible spectroscopy

The E4/E6 ratio of HAs was determined using methods cited by Plaza *et al*. (2002). Lyophilised HA samples (3.0 mg) were dissolved in 10 mL of $NaHCO_3$ solution. The pH of $NaHCO_3$ solution was adjusted to 8.00 with NaOH. The ratio of the absorbance at 465 and 665 nm (E4/E6) was measured by a PerkinElmer model Lambda 15 UV-visible spectrophotometer.

Fluorescence spectroscopy

Fluorescence experiments were realised on HA aqueous solutions (20 mg L^{-1}) in a fluorescence spectrometer (Fluoro Max-4, Horiba) equipped by a 150-W Xenon lamp using the following conditions: excitation wavelength at 470 nm and emission spectral region of 450–700 nm. Emission slits were set at 4-nm band width, with 5 nm for emission increment. Duplicate measurements were performed on solutions of HAs using quartz cells (1 cm) at room temperature. Fluorescence experimental parameters were based on literature (Milori *et al*.

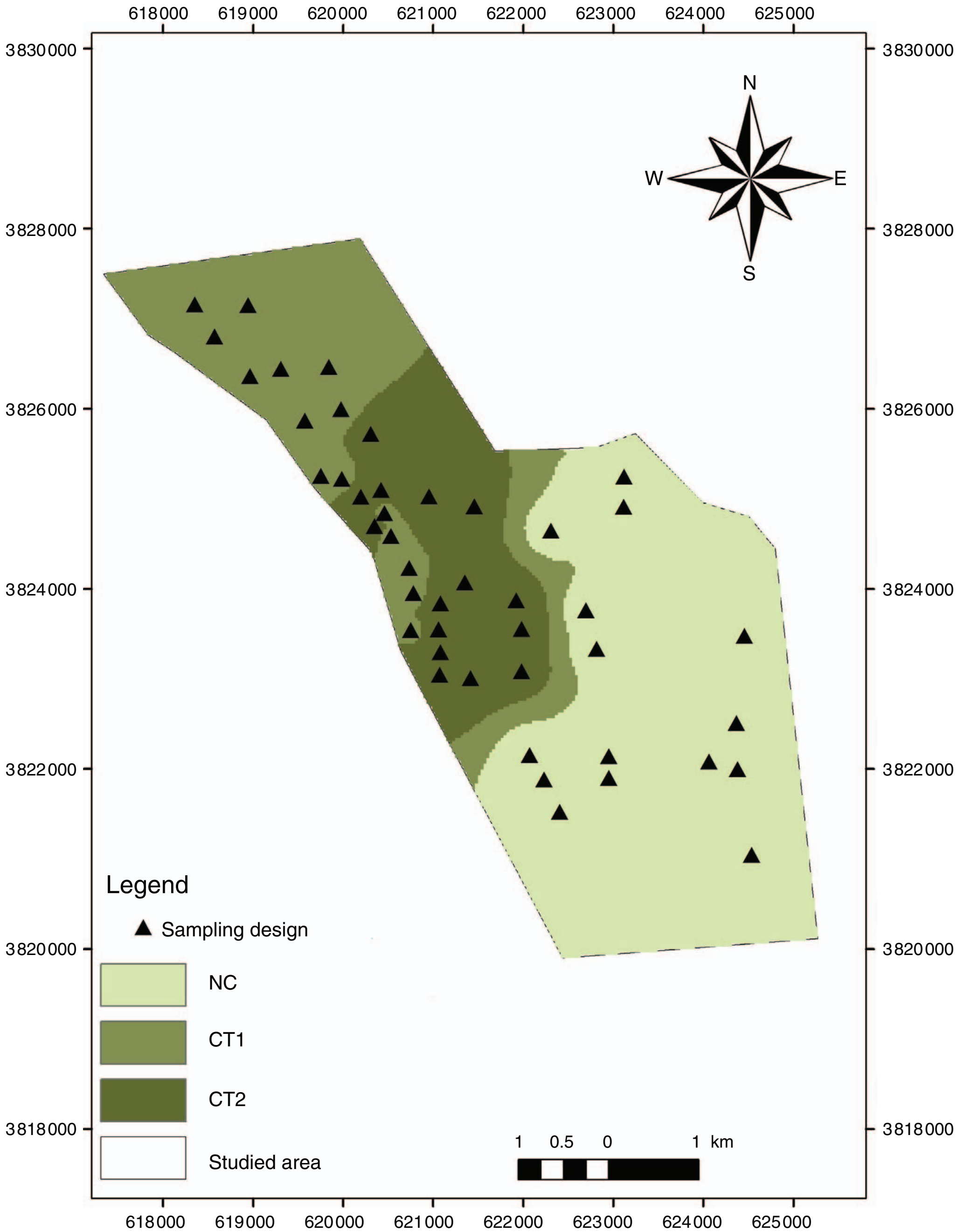

Fig. 2. Total surface of studied site, treatments and sampling design. CT2, soil tilled with 50 m^3 ha^{-1} of OMW for 20 years; CT1, soil tilled without amendment for 80 years; and NC, soil uncultivated for 80 years and with native vegetation.

2002, 2006; González Pérez *et al*. 2004) and tests achieved to optimise fluorescence parameters.

Statistical analysis

Data analysis

The data analyses were carried using SPSS 13.0 for Windows. The mean values of the treatments were compared using Duncan's multiple range tests at $P < 0.05$. All analyses were determined in triplicate.

Spatial prediction methods

Spatial distribution of soil parameters was achieved in the experimental field by IDW methods using ArcMaps 10.1.

The IDW is one of the most applied techniques in soil science for predicting spatial distribution of soil parameters. The weights assigned to the interpolating points are the inverse of its distance from the interpolation point. Consequently, the close points are given more weight than distant points and vice-versa. The known sample points are assumed to be independent of each other (Robinson and Metternicht 2006). The interpolation equation used can be written as follows:

$$Z(X_0) = \frac{\sum_{n}^{i=1} \frac{x_i}{h_{ij}^{\beta}}}{\sum_{n}^{i=1} \frac{x_i}{h_{ij}^{\beta}}}$$

where $Z(X_0)$ is the interpolated value, n is the total number of sample data values, x_i is the data value, h_{ij} shows the separation distance between interpolated value and the sample data value and β indicates the weighting power.

Results

SOM concentration

The SOM had the highest mean value (~2.60%) for treatment NC, followed by CT2 with ~1.95% and CT1 with 0.65%. Results showed significant variation in SOM among the NC, CT1 and CT2 treatments (Fig. 3*a*).

Table 2. Physical and chemical characteristics of studied soils: CT2, soil tilled with 50 m^3 ha^{-1} of OMW for 20 years; CT1, tilled soil without amendment for 80 years ago; and NC, uncultivated soil with native vegetation for 80 years (average values ± s.d.)

Significant differences are presented among soils in pH, electrical conductivity (EC), cation exchange capacity (CEC) and calcium content. CT2 showed lower pH, higher EC related to OMW characterised by acidity and higher EC

Soil properties	Soil treatment CT1	CT2	NC	Statistical analyses $P < 0.05$
Sand %	61.75	27.94	65.30	_
Clay%	0.18	0.38	0.25	_
Silt %	38.06	71.61	34.42	_
pH ± s.d.	8.39 ± 0.09	5.65 ± 0.09	9.56 ± 0.17	Significant
EC (cm/Sm) ± s.d.	0.57 ± 0.80	1.43 ± 0.07	0.44 ± 0.06	Significant
CEC ± s.d.	5.68 ± 0.90	4.29 ± 0.11	5.68 ± 0.09	Significant
Calcium % ± s.d.	0.34 ± 0.01	0.49 ± 0.03	0.51 ± 0.02	Significant
Potassium % ± s.d.	0.02 ± 0.05	0.06 ± 0.01	0.08 ± 0.03	Not significant
Sodium % ± s.d.	0.006 ± 0.02	0.007 ± 0.01	0.008 ± 0.01	Not significant

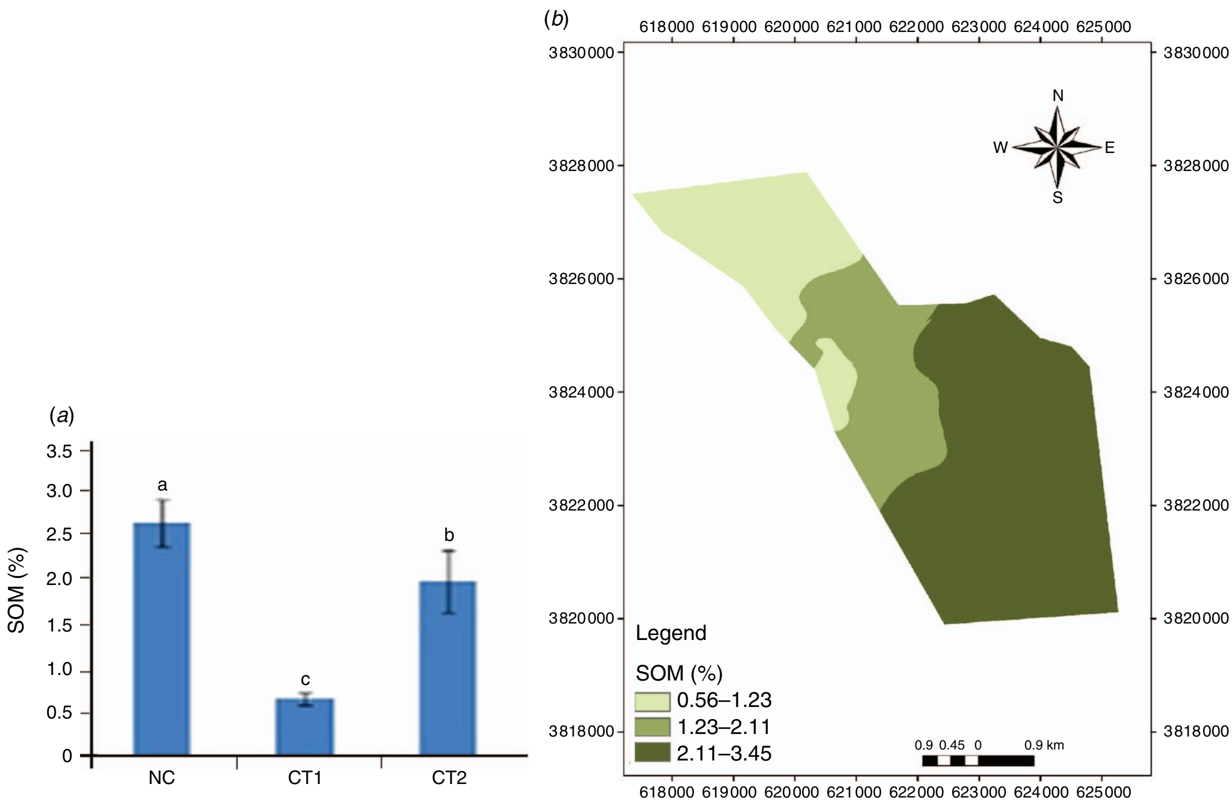

Fig. 3. Soil organic matter (SOM) amount with (*a*) soil management and (*b*) spatial distribution. Means with different letters indicate a significant difference at $P < 0.05$. CT2, soil tilled with 50 m^3 ha^{-1} of OMW for 20 years; CT1, soil tilled without amendment for 80 years; and NC, soil uncultivated for 80 years and with native vegetation.

The spatial distribution of SOM in the studied area showed a progressive increase of SOM content from north-west to south-east (Fig. 3*b*). A zone of SOM > 2.12% covered the south-eastern area, a second zone located in the centre of the studied area had SOM of range 1.23–2.12% and the extreme north-west had SOM < 1%.

Humification parameters

C_{HA}/C_{FA} ratio

The C_{HA}/C_{FA} ratio indicates humus quality because it expresses the degree of evolution of SOM humification process (Benites *et al*. 2003). The CT1 had the lowest C_{HA}/C_{FA} ratio of ~0.37, and the highest value was 2.19 for NC (Fig. 4*a*). The $C_{HA/FA}$ ratio was significantly higher in CT2 (1.62) compared to CT1. The spatial distribution of C_{HA}/C_{FA} ratio showed a significant decrease towards the south-east of the studied area (Fig. 4*b*). Near the north-western quarter of the studied area there was $C_{HA}/C_{FA} < 1$ especially in the western area, and the other three-quarters of the area had $C_{HA}/C_{FA} > 1$.

E4/E6 ratio

The E4/E6 ratio has been widely used to study HAs (Aranda *et al*. 2011). This ratio is considered to be inversely related to the degree of condensation and aromaticity of the HAs and to their humification degree (Senesi *et al*. 2003).

The E4/E6 ratio significantly differed among treatments (Fig. 5*a*). Indeed, NC soil exhibited the highest average value of 3.72 followed by CT1 (2.83) and CT2 (2.20). The spatial distribution of E4/E6 ratio showed a gradual decrease towards the centre of the area. In this area, five zones exhibited E4/E6 < 2.10 (Fig. 5*b*). The rest of the studied site had E4/E6 of range 2.49–3.88.

Fluorescence area

The fluorescence spectroscopy facilitates the examination of chemical structure of HAs and FAs (Rivero *et al*. 2004). The intensity of fluorescence emission can be related to condensation of aromatic groups, and can be used for revealing humification degree (Bayer *et al*. 2002; Milori *et al*. 2002). The HA analysis showed a band with a maximum intensity at 520 nm for all treatments (Fig. 6*a*). The HAs extracted from NC, CT1 and CT2 showed differences in band intensities. Thus, separation between all spectra was clear. The highest intensity was for NT, followed by CT2 and then CT1 (Fig. 6*a*). The area of a fluorescence spectra obtained by excitation is proportional to the humification degree of the sample and can be used as a humification index (González Pérez *et al*. 2004). Both NC and CT2 treatments had larger areas of fluorescence spectra than CT1 (Fig. 6*b*). The NC soils had a higher value of SOM and lower humification degree compared to CT2.

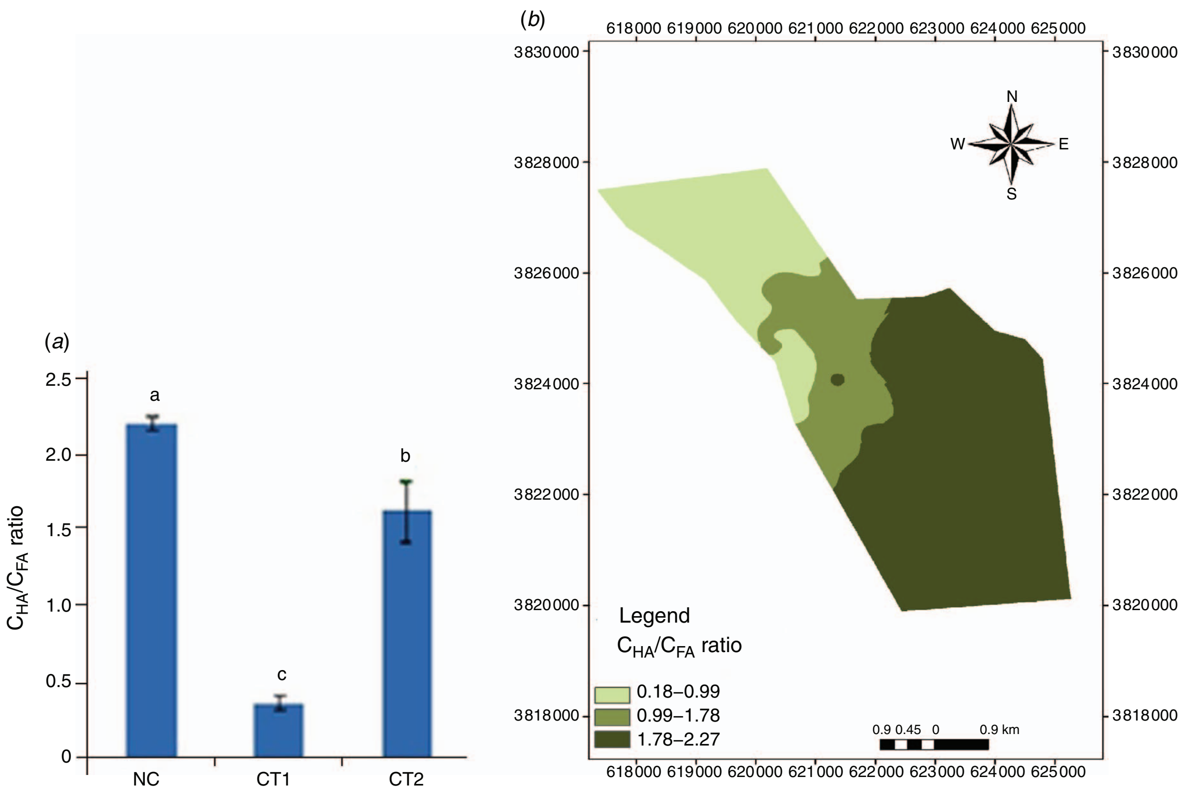

Fig. 4. (*a*) C_{HA}/C_{FA} ratio values and (*b*) mapping of C_{HA}/C_{FA} in studied soils. Means with different letters indicate a significant difference at $P < 0.05$. CT2, soil tilled with 50 m^3 ha^{-1} of OMW for 20 years; CT1, soil tilled without amendment for 80 years; and NC, soil uncultivated for 80 years and with native vegetation.

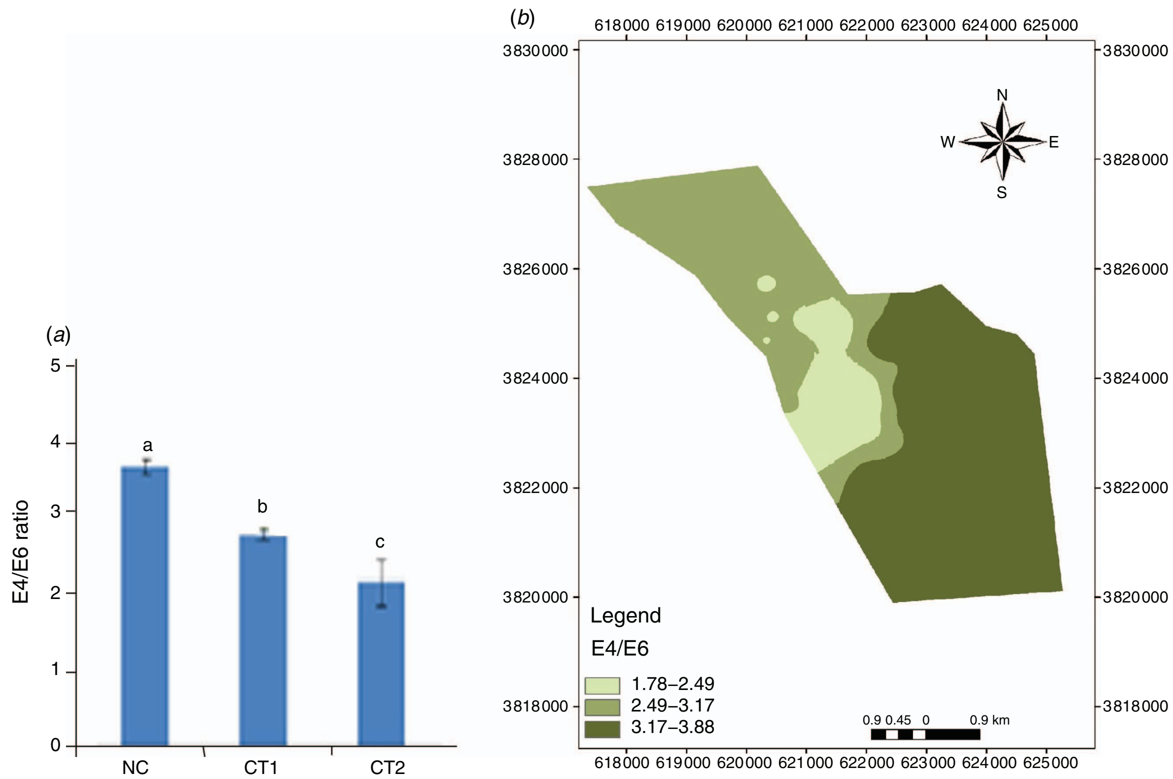

Fig. 5. (*a*) Variation of E4/E6 ratio and (*b*) its spatial distribution in the studied area. Means with different letters indicate a significant difference at $P < 0.05$. CT2, soil tilled with 50 m^3 ha^{-1} of OMW for 20 years; CT1, soil tilled without amendment for 80 years; and NC, soil uncultivated for 80 years and with native vegetation.

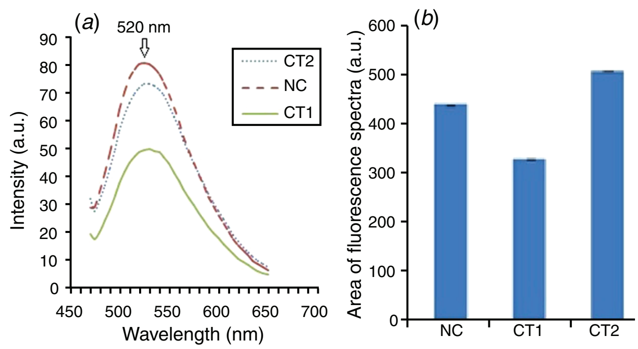

Fig. 6. (*a*) Fluorescence emission spectra of soil humic acids. (*b*) Area of fluorescence spectra of soil humic acids (area of emission spectra with maximum of intensity around 520 nm, excitation wavelength of 460 nm). CT2, soil tilled with 50 m^3 ha^{-1} of OMW for 20 years; CT1, soil tilled without amendment for 80 years; and NC, soil uncultivated for 80 years and with native vegetation.

Discussion

SOM variation in different soils

Evaluating the impact of management practices on SOM spatial distribution is important to maintain and improve soil quality. In fact, the spatial distribution of soil parameters is valuable to identify the gradient of soil degradation (Brevik *et al*. 2016). Many spatial interpolation methodologies have been used to predict the distribution of variables (Li and Heap 2008). Mueller *et al*. (2004) cited that kriging and IDW methods have been used widely to predict soil fertility. Tang *et al*. (2017) found that IDW and ordinary kriging methods generate similar results of soil organic carbon pool distribution for Moso bamboo forests.

The SOM was higher in the NC than in CT2 and CT1 treatments. Spatial distribution maps also indicated that the south-east of the studied area had the highest SOM content. This area corresponded to soil uncultivated for at least 80 years and covered by native vegetation, and confirmed that with NT, plant residues left on the soil surface enhance the SOM concentration in topsoil (Conceição *et al*. 2013). Zones with very low SOM corresponded to cultivated soils without amendment, which had been tilled for 80 years (CT1). Many studies reported that SOM in soils of the Chaâl area is very low (<1%) (Gargouri *et al*. 2014). This very low SOM in CT1 can be explained by the tillage effect, which reduces natural biomass production. When combined with aridity, biomass production is further reduced. Moreover, aridity and exposure of organic matter to oxidation by tillage accelerate the SOM degradation and mineralisation. Indeed, Busari *et al*. (2015) reported that higher mineralisation of organic carbon in tilled fields is due to soil structure deterioration following tillage. Bista *et al*. (2017) found that intensive tillage creates dry and loose soil particles and so boosts erosion. The cultivated areas were of sandy texture with SOM content of 1.1–2.2%, especially CT1 had a sandy texture in contrast to CT2 with silt loam texture. Hamarashid *et al*.

(2010) assessed the effects of soil texture of six textural classes (sandy loam, loamy sand, silty loam, silty clay loam, clay loam and loam) on chemical compositions and carbon mineralisation in soil. They found that carbon mineralisation in fine soil textures (clay loam, loam and silty clay loam) was significantly ($P \leq 0.01$) higher than in coarser soil textures (silty loam, loamy sand and sandy loam). They found no differences between sandy loam and silty loam textures. However, the authors indicated that capacity to preserve SOM is greater in silty than sandy soils. The CT2 was also treated with OMW for 20 years successively, and so the increase of SOM in CT2 can be related to the both the silt fraction and the OMW application. The CT2 had silt content of 71.61%, which could preserve organic carbon in soil against degradation (Hamarashid *et al*. 2010). In fact, González Pérez *et al*. (2007) assessed the SOM humification in latosols treated with sewage sludge and found higher carbon content in the silt fraction. Saab and Martin-Neto (2003) also reported that carbon in the silt fraction is more stable than in other fractions. According to these authors, this high stability is related to its linkage to the organic–mineral fraction. In addition, many studies have reported that OMW application increases the SOM in several soil types. Mahmoud *et al*. (2010) found an increase of SOM in the soil surface with long-term irrigation using OMW compared with a control soil. Other authors confirmed that OMW application induced an increase in SOM concentrations in the soil surface (Zenjari and Nejmeddine 2001; Gargouri *et al*. 2014). Our results showed that the combination of OMW addition and a high amount of silt induced higher SOM in CT2 (Fig. 3*b*).

Humification parameters under different treatments

The $C_{HA}/C_{FA} < 1$ found in CT1 indicated that the major part of soil organic carbon was formed by FAs. This can be explained by the organic matter supply derived from natural biomass and plant debris. The plant debris contains high amounts of labile organic matter leading to direct mineralisation and FA production, which is the first product of the humification process (Chaker *et al*. 2018). The lower C_{HA}/C_{FA} ratio indicates higher carbon mobility in the soil, with predominance of carbon in FAs (more soluble). This can be associated with tillage that was unfavourable to the formation of more stable HAs. In CT1 soils, $C_{HA}/C_{FA} < 1$ was due to the lower degree of humification, condensation and synthesis processes caused by intensive mineralisation of plant residues, and the lower content of exchangeable bases, which are unfavourable to biological activity in these soils (Canellas *et al*. 2002). The higher C_{HA}/C_{FA} ratio in the NC area indicated that native vegetation and NT system induced improvement of C_{HA}/C_{FA} in soil. The C_{HA}/C_{FA} was higher in CT2 soil compared to CT1 after OMW addition for 20 years. This increase is indicative of an increase in the carbon associated with the HA fraction (Rivero *et al*. 2004). Treatments CT2 and NC contributed to the SOM humification process. Indeed, these treatments had $C_{HA}/C_{FA} > 1.0$, exceeding that for CT1. These results indicated that the addition of OMW in CT2 and vegetation accumulated in NC produced high SOM quality, favouring physical and chemical properties beneficial to plant development (Fontana *et al*. 2006).

In general, a $C_{HA}/C_{FA} > 1.0$ is beneficial, showing that organic matter is stable and that permanent bonds with the mineral soil phase dominate over mobile formations, which easily migrate into deep layers (Kononova 1966).Thus, the combination of tillage with OMW application for 20 years in this arid region improved soil fertility by increasing SOM content from 0.65% (CT1) to 1.95% (CT2) and C_{HA}/C_{FA} ratio from 0.37 (CT1) to 1.62 (CT2).

The highest E4/E6 ratio was in the NC area, indicating that this treatment had high quantities of aliphatic groups and low quantities of aromatic groups (Chen *et al*. 1977). Vicente-Vicente *et al*. (2015) also found that a soil with plant cover had a higher E4/E6 ratio than soil of an olive grove with conventional tillage. The highest value in the NC treatment covered with native vegetation was mainly due to the high proportion of fresh SOM accumulated in the soil surface. This natural SOM is always available for soil microorganisms. The formation of SOM will be rich in aliphatic structures with lower aromatic carbon groups (Bayer *et al*. 2002). Higher values of E4/E6 ratio indicated the presence of larger organic molecules and higher quantities of aliphatic structures (Stevenson 1994). Soils under natural vegetation that have high organic inputs and high microbiological mass and their metabolites have more pronounced mineralisation and less degradation, and HAs that consist of aliphatic components (Aranda *et al*. 2011). Our results demonstrated positive relationships between E4/E6 ratio, C_{HA}/C_{FA} ratio and SOM content in NC. Indeed, a higher E4/E6 ratio was linked to the high SOM amount and C_{HA}/C_{FA} in the HAs due to the incorporation of fresh organic matter accumulated in NC compared to CT1. These results are in accordance with those obtained by Vicente-Vicente *et al*. (2015).

The E4/E6 ratio was lower in CT2 and CT1 than in NC soil. This confirmed the higher aromaticity and polycondensation degree, and the higher molecular weight of the HAs in these cultivated treatments (Pertusatti and Prado 2007). Mahieu *et al*. (1999) also showed that there were more aromatic structures determined in HAs extracted from cultivated soil than HAs extracted from uncultivated soils. Aranda *et al*. (2011) reported that in arable land under conventional agriculture there is a small input of organic matter that remains in the soil, more pronounced degradation and less mineralisation, so HAs consist of more stable, aromatic compounds with a higher condensation degree. Using OMW with tillage for 20 years induced a decrease of E4/E6 ratio in CT2 soils compared to CT1. This could be due to the addition of OMW in soils, which increased the SOM humification degree, aromatic structures and the molecular weight of HAs extracted from CT2 (Chaker *et al*. 2018). Moreover, this effluent is characterised by high amounts of aromatic compounds, which are responsible for the effluent's phytotoxic impact (Procida and Ceccon 2006). El Hajjouji *et al*. (2007) evaluated OMW characteristics using Fourier-transform infrared spectroscopy and ^{13}C-nuclear magnetic resonance (^{13}C NMR) analysis and showed a decrease in the density of aliphatic compounds, indicating that polymerisation of organic matter occurred during the storage period due to natural bioprocesses.

The highest fluorescence intensity in NC indicated that HAs extracted from NC zones were characterised by lower molecular size and lower aromatic condensation degree compared to HAs extracted from CT1 and CT2 treatments. Aoyama (2001) also found an inverse correlation between molecular size and aromatic condensation degree with the fluorescence intensity in HAs. The HAs extracted from NC treatment (that had higher accumulation of native vegetation) showed a weak humification degree, indicating a lower concentration of condensed aromatic carbon than CT2 (Bayer *et al*. 2002; Milori *et al*. 2002).

The emission fluorescence area was related to the condensation of aromatic groups, and may be used to reveal the SOM humification degree. The high fluorescence area in NC indicated that HAs extracted from NC zones were characterised by high molecular size, high aromatic condensation degree and high humified organic matter as shown in CT2. The emission fluorescence area increased in CT2, indicating that concentration of aromatic carbon and humification degrees increased after long-term spreading of OMW. Mekki *et al*. (2006) showed that long-term OMW caused negative changes in microbial soil properties in arid fields. The higher SOM humification in CT2 soils may indicate that OMW application increased SOM stability. These results were confirmed by the increase of C_{HA}/C_{FA} value after long-term OMW application.

The emission fluorescence area was lower in the NC compared to CT2 treatment. This is due to accumulation of plant residues at the soil surface exceeding the capacity of microorganisms to metabolise them, inducing less aromatic and less humified humic compounds (González Pérez *et al*. 2007). The higher SOM and the lower humification degree presented in NC compared to cultivated soils are consistent with other studies (Milori *et al*. 2006). Bayer *et al*. (2002) showed that cultivated soil had a higher degree of humification and lower carbon amounts compared to the same soil under a NT system. They reported that the humification was characterised by semiquinone free-radicals, which appeared in the samples. This is related to the presence of aromatic carbon or carbon in a more stable state of decomposition. This result was attributed to the increase and accumulation of vegetal residue on the NT soil surface. Thus, in the NC treatment we found that E4/E6 increased in line with the fluorescence emission area. These results indicated that molecular weight of extracted HAs increased with the SOM humification degree. Our results were similar to those of Fuentes *et al*. (2006), who found that E4/E6 ratios increased with SOM humification degree in the case of organic extracts from composted materials. These results indicate that E4/E6 was not a reliable indicator of SOM humification degree.

The low fluorescence emission area in CT1 indicated a low SOM humification degree, low molecular size and low aromatic structure compared to CT2 and NC. Martin *et al*. (1998) observed that intensive cultivation caused a decline of the condensation level of aromatic rings in humic substances of arable land and decreased the molecular weight of humic substances. This result was confirmed by the C_{HA}/C_{FA} ratio results. The HAs extracted from CT1 had $C_{HA}/C_{FA} < 1$, indicating that HAs had low aromatic structure, low molecular size and low SOM humification degree. In contrast, we found high values of E4/E6 ratio in CT1, which indicated that HAs extracted from CT1 had high molecular size with low aromatic structures. Thus the E4/E6 increased with the decrease of humification degree. We conclude that the high E4/E6 ratio with low fluorescence area was associated with changes in the molecular weight of extracted HAs converse to the humification degree (Fuentes *et al*. 2006). In fact, Fuentes *et al*. (2006) observed a decrease of E4/E6 with the increase of humification degree in soil HAs.

The contradictory results found in CT1 and NC between E4/E6 ratio and fluorescence area indicated that there was no clear relationship between humification degree and E4/E6 values. Moreover, Chen *et al*. (1977) suggested that E4/E6 was inversely correlated with molecular weight of HAs, but found no clear correlation with molecular properties directly related to humification. Our results suggest that E4/E6 was not a reliable indicator to assess SOM humification degree. The C_{HA}/C_{FA} was positively correlated with fluorescence emission area. Thus UV-visible ratio C_{HA}/C_{FA} with fluorescence area can be used as reliable indicators of SOM humification. In this context, complementary studies involving other analytical techniques such as ^{13}C NMR would be of great interest to better clarify the relative importance of conjugated systems of aromatic or aliphatic groups in the humic properties of organic materials.

Conclusion

Our study supports the conclusion that conventional tillage and OMW application for more than 20 years led to changes in SOM humification degree compared to soil uncultivated for the last 80 years.

The spatial distribution of maps showed a progressive increase of SOM and C_{HA}/C_{FA} from north-west to south-east linked to the positive relationship between C_{HA}/C_{FA} ratio and SOM amount independent of soil management practices. In fact, higher amounts of SOM as well as a higher C_{HA}/C_{FA} values were found in both uncultivated soil (NC) and cultivated soil with addition of OMW (CT2) than cultivated soil without amendment (CT1). High SOM humification degree was found in CT2, determined by fluorescence emission area and confirmed by C_{HA}/C_{FA} and E4/E6 ratios. Long-term tillage (CT1) reduced aromatic condensation and SOM humification degree of HAs, as shown by the strong correlation between fluorescence emission area and C_{HA}/C_{FA} ratio conversely to E4/E6 ratio. Long-term NT and native vegetation maintained significantly greater levels of SOM, C_{HA}/C_{FA} and humification degree. However, these findings were opposite to indications using the E4/E6 ratio. Contradictory results found in CT1 and NC between E4/E6 ratio and fluorescence area indicate that E4/E6 was not a reliable indicator of humification degree.

A decreasing gradient of the C_{HA}/C_{FA} ratio towards the middle of the studied area confirmed that HAs had the following aromatic condensation and humification degree order CT1 < NC < CT2, as confirmed by fluorescence emission area. The C_{AH}/C_{AF} ratio and fluorescence spectroscopy were reliable complementary indicators of SOM humification degree.

High silt content and OMW application for more than 20 years improved fertility levels by high content of SOM and also enhanced soil quality by a marked improvement of aromatic structures as confirmed by low E4/E6 ratio and high humification index. Combination of tillage with OMW may represent an alternative solution for promoting organic matter stabilisation in soils, enhancing sustainability of agroecosystems and reducing the possible negative environmental problems in arid climates.

Conflicts of interest

The authors declare no conflicts of interest.

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