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Sewage Sludge Application in Mediterranean Agricultural soils: Effects of Dose on the Soil Carbon Cycle

González-Ubierna, S.[*]**, Jorge-Mardomingo, I., Cruz, M.T., Valverde, I. and Casermeiro, M.A.**

Departamento de Edafología, Facultad de Farmacia, Universidad Complutense de Madrid, Pza.
Ramón y Cajal s/n (Ciudad Universitaria), 28040 Madrid, Spain

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ABSTRACT: This work investigates the effect of the application rate and type of sludge throughout the soil carbon cycle in a semiarid Mediterranean agro-ecosystem. We study the two-year evolution of the various pools of soil organic and inorganic carbon and their influence on soil respiration. We applied three rates (40, 80 and 160 Mg/ha) of two types of sludge –aerobically and anaerobically digested sewage sludge– in a calcareous Mediterranean soil. The study area is located in the southeast of Madrid (Spain) and is characterised by a Mediterranean climate with a marked seasonal and daily contrast. We analysed soil organic carbon, CO_2 emissions, organic carbon fractions, soluble carbon, and inorganic carbon forms. Measurements were madeat three times over two years, and bimonthly for organic carbon and CO_2.The results show that sludge type and rateof applicationexerta significant influence throughout the soil carbon cycle. Aerobic sludge has a greater effect over the short-term. Anaerobic sludge treatment appears to have less effect on the cycle at the beginning of the amendment, but is prolonged over time, as the differences with untreated soil persist even after two years. The application of organic amendments in calcareous Mediterranean soils also modifies the inorganic carbon pools and greatly increases the soil soluble hydrogen carbonates. All of these results are reflected in the rates of soil CO_2 emissions, with the highest values recorded in soils amended with aerobic sludge. Our data points to the advisability of a review of the European Union's recommendations regarding sludge and agriculture. We propose includinga sludge stabilization process and recommended application ratesaccording to the effects on soil biogeochemical cycles.

Key words: Soil Organic Carbon,CO_2 emissions, Sludge application,Fertilization, Agriculture

INTRODUCTION

The management oforganic waste producedin urban areas isa priority issuein the environmental policyof the European Union (EU), according toDirective2008/98/EC (CEC, 2008).The EU generatesapproximately 14milliontons of organic waste per year (d.w.), which requires the development and improvement ofenvironmentally friendly mechanisms for itsmanagement (Sheppard *et al.*, 2005). The EuropeanCommission considers that the application of organic waste(sewage sludge and biodegradableorganic waste) in agriculture to be theleast expensive solution(Hogg *et al.*, 2002), and this is the main outputatthe community level (Albiach *et al.,* 2001). However, the useof these amendmentsalso hasnegative impacts.In view of this fact, the effects of the heavymetal contentof these residues have been extensively studied (Cai *et al.,* 2007; Haynes *et al.,* 2009). The presence of organic contaminants has also been studied (Stevens *et al.*, 2003, Sánchez-Brunete*etal.*, 2007), and more recent works have focused onsoilbiogeochemicalcycles (i.e. Hemmat *et al.,* 2010 and González-Ubierna *et al.,* 2012 on calcareous soils). In current European legislation (Directives 86/278/EEC (CEC, 1986) and 91/692/EEC (CEC, 1991)), the maximum regulatory criteria for sludge application in soilsis based solely on its heavy metal content. One of the latest EC Working Documents on Sludge (CEC 2000), involving a revision of Directive 86/278/EEC (CEC, 1986), proposes limit values for a range of classes of organic contaminants in sludge. Unfortunately, the implications of sludge in biogeochemical cycles have not yet been addressed in EU policies. Thus, there may be cases where the maximum rate for carbon forms produces a negative effect on groundwater or the atmosphere, while other rates may fail to enhance soil fertility. Some EU countries (Denmark, Netherlands and Sweden) have developed regulations that take into account the amount of nutrients in sludge in order to establish the maximum rate (Aubain, 2002).

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*Corresponding author E-mail:sergonza@farm.ucm.es

Furthermore, European regulations have neglected to include sludge stabilisation processes when establishing recommendations for its disposal in soils.The importance of soil carbon on the global carbon cycle has already been noted (Giardina and Ryan, 2000; Lal, 2004; Almagro *et al.*, 2009). According to Lal (2004), the adoption of recommended management practices on agricultural soils can reduce the rate of enrichment of atmospheric CO_2. (Conant, Dalla-Betta *et al.,* 2004)(Maestre and Cortina 2003; Mermut 2003). Some authors have indicated that in Mediterranean soils, concentrations of organic matter tend to increase after the application of urban sludge, particularly in the humic fractions, which are the most persistent and difficult to degrade (Albiach *et al.*, 2001;Zinati *et al.*, 2001; Heras *et al.*, 2005). However, severalauthors (Quemadaand Menacho,2001; Torrietal.,2003) suggest thatmost ofthe organic carbonis releasedin the form of$CO_2$to the environment (mineralisation processes). Although the effects of different organic amendments (composted sewage sludge, thermally dried sludge and slurries) on soil properties and carbon mineralisation have been the object of numerous studies (Albiach *et al.*, 2001; Haynes *et al.*, 2009; Franco-Otero *et al.*, 2012); there is still little knowledge of the main drivers and controls for SOM-mineralisation (Bradford *et al.*, 2008). The relations among the different pools of soil carbon and CO_2 fluxes and the relevance of application rates and sludge type in these processes have been less widely studied, especially in Mediterranean environments.

The main aim of this work is to investigate the effect of the application rate of two types of sewage sludge (aerobically- and anaerobically-digested) throughout the evolution of soil carbon forms and soil properties in a semiarid Mediterranean agro-ecosystem. We study the soil carbon evolution through analyses of soil organic carbon, CO_2 emissions, organic carbon fractions (soluble, labile and recalcitrant pools),and inorganic carbon.

MATERIALS & METHODS

The study area is an experimental station located in the centre of the Iberian Peninsula, near the city of Arganda del Rey, in the southeast of the Madrid Region in Spain (UTM X: 457673.84, UTM Y: 4462824.553). Geomorphologically, the area lies on the former alluvial terrace on the left bank of the Jarama river basin, on quaternary calcareous sediments with high carbonate contents.The soil was analysed from a range of profiles on the experimental plot to obtain its correct characterisation (Table 1). The land on which the plot is located consists of quaternary sediments from the Jaramariver, which are basically sands and silts. These sediments are of alluvial origin, giving rise to a CalcaricFluvisol, which today has characteristics typical of an Anthrosol(FAO, 2006). This soil exhibits a marked human influence due to its use for agriculture. Morphologically, the followingelements can be differentiated: an Ap horizon (0-40 cm) with properties similar to an Anthragric horizon with an organic carbon content close to 1%, a moderately basic pH (pH =8), low surface stoniness and high permeability; and a subsurface horizon (40-80 cm) with the characteristics of agricultural land, showing subsurface compaction due to the intensive use of farm machinery. Morphologically, textural changes can also be observed in this latter horizon due to the accumulation of clays. This horizon could be categorised as Anthraquic, as it presents a significant increase in apparent density, which translates into a decrease in the effective permeability and a lower carbon content (see Table 1) (Casermeiro *et al.*, 2007).

Table 1. Specific characteristics of the Ap horizon, separated by two depths

Variable	Unit	Value
Texture	Fine Sand (%)	7.78
	Silt (%)	41.28
	Sand (%)	23.61
	Clay (%)	27.34
	Class	Coarse clay
Porosity	%	40.69
TOC	g/kg	13.08
Carbonates	%	8.8
CEC	meq NH_4^+ 100 g^{-1}	15.32
Na	Cmol/ kg	0.54
K	Cmol/ kg	1.47
Ca	Cmol/$_+$ kg	12.36
Mg	Cmol/ kg	1.03
pH	-	8.30
$EC_{1:5}$	dS/m	0.19

TOC: Total Organic Carbon. EC: Electrical Conductivity. CEC: Cation Exchange Capacity

The site is typical of a Mediterranean pluviseasonal-oceanic bioclimate, and is located within a dry meso-Mediterranean belt (www.globalbioclimatics.org). Theclimateischaracterised bydistinctseasonal anddailycontrasts. The average annual rainfall is 430mm, with a markedminimumin summer (50 mm). The average annual temperature is 19ºC, with maximums in summer that oftenexceed 35°C.These conditions confer singularity on the study, as the Mediterranean climateimposes a doublehardshipon biological systems: limited water in summer and unpredictable rainfall(Valladares, 2004).We selected two types of sewage sludge representative of urban areas: aerobic and anaerobic digested sludge. The sludge comes from the Canal de Isabel II water treatment plants in the Madrid Region: the aerobically-treated sludge from the Campo Real plant; and the anaerobically-treated sludge from the Guadarrama plant. After its generation, the aerobic sludge (AE) underwent only an air-drying process; however, the anaerobic sludge (AN) was treated in digesters without the addition of oxygen for its chemical stabilisation. The main chemical properties of the amendments applied are shown in Table 2. In terms of metal content, the sludge complies with the national and European legislation for agricultural use (CEC, 1986; RD, 1990).

Table 2. Specific characteristics of the organic amendments

Variable	Anaerobic Sludge	Aerobic Sludge
Dry weight (%)	16.50	14.20
TOC (g/kg)	76.30	74.50
N (g/kg)	6.30	4.20
C/N ratio	7.04	10.31
P (g/kg)	12.0	17.0
pH	7.50	8.20
$EC_{1:5}$ (dS/m)	14.86	14.35
CO_3^{2-} (%)	4.13	1.32
Ca (g/kg)	17.0	35.0
K (g/kg)	2.6	5.4
Mg (g/kg)	2.5	3.0
Fe (mg/kg)	6500	4400

TOC: Total Organic Carbon. EC: Electrical Conductivity. All data referred to dry weight (d.w.)

The property used as the site of the experimental plot had lain fallow for ten years, after which it was ploughed for the present experiment. Three randomised blocks of soil plots (10x15 m each) were designed. The plot treatments included an unamended control (CONT) and two types of organic amendments: AE and AN. The concentrations established were 40, 80 and 160 Mg/ha (d.w.), with a random distribution of eight plots per block (three plots with AE application, three plots with AN application and two blanks without sludge application as a control). Three replicates were thus obtained for each rate and type of sludge. Each plot measured 2.5x5 m^2. The blocks were separated by a distance of 10 m to avoid any possible influence or contamination between one block and another.

The amendments were applied and mixed with the topsoil using a rototiller to a depth of 20 cm. No maintenance work, watering or cutting of the vegetation was performed on the plots after the organic application. Before the CO_2 measurements, the vegetation was only removed in respiration chambers to eliminate the plant respiration effect.Thecollection andprocessingof samples wasperformedaccording toUSDAcriteria (Schoeneberger *et al.*,2002) in the first 20 cm soil depth. The organic amendments were applied in the summer of 2007, and the first sampling was taken 20 days after mixing the soil with the sludge. Data were collected at three times over two years, and bimonthly for organic carbon and CO_2.Climate datawere obtained from the Argandastation(Cod:3182Y)of the National Meteorological Agency(AEMET), which is located at the experimentalfarm “La Isla”. These data refer to hourly temperature andmoisture.Soil analyses were performed on the fine earth fraction. All of the variables were analysedthree times over two years: twenty days after the application, and one and two years after. CO_2 and organic carbon were measured bimonthly.Electrical conductivity (EC) was determined in a 1/5 soil/water suspension using a Crison Micro CM 2200 conductivity meter (ISRIC, 2002). The pH was determined in a 1/2.5 soil/water suspension using a Crison GLP 21 pH meter (ISRIC, 2002). The calcium carbonate equivalent and soluble hydrogen carbonate (SHC) were estimated according to the acid neutralisation method (FAO, 2006). Soil organic carbon (SOC) was determined using the Walkley-Black methodology through oxidation with potassium dichromate and subsequent titration (FAO, 2006). Dissolved organic carbon (DOC) was extracted in a 1/5 soil/water ratio (m/v) after one hour of agitation, and analysed using a micro NC Analytik Jena autoanalyser. To study the organic components, the carbon associated with labile fractions (hydrolysable organic matter) was separated from more stable carbon forms (recalcitrant organic matter) and was quantified by means of acid hydrolysis using the methodology proposed by Rovira and Ramón-Vallejo (2007). The labile fraction (LP) basically corresponds to sugars, amino acids and fatty acids with low molecular weight, and the recalcitrant fraction (R) contains compounds with a high molecular weight. The recalcitrance index, proposed by the same authors, was also calculated. This index is a ratio between recalcitrant organic carbon and total organic carbon. Soil respiration (Rs) data were measured in situ (Davidson *et al.*, 2002), using an infrared gas analyser, model Li-COR 8100, with a 20-cm-diameter chamber. Three PVC cylinders, 20 cm in diameter, were randomly installed in each plot for sampling. The cylinders were installed to a depth of 5cm. in order to exclude root ingrowth from the side.

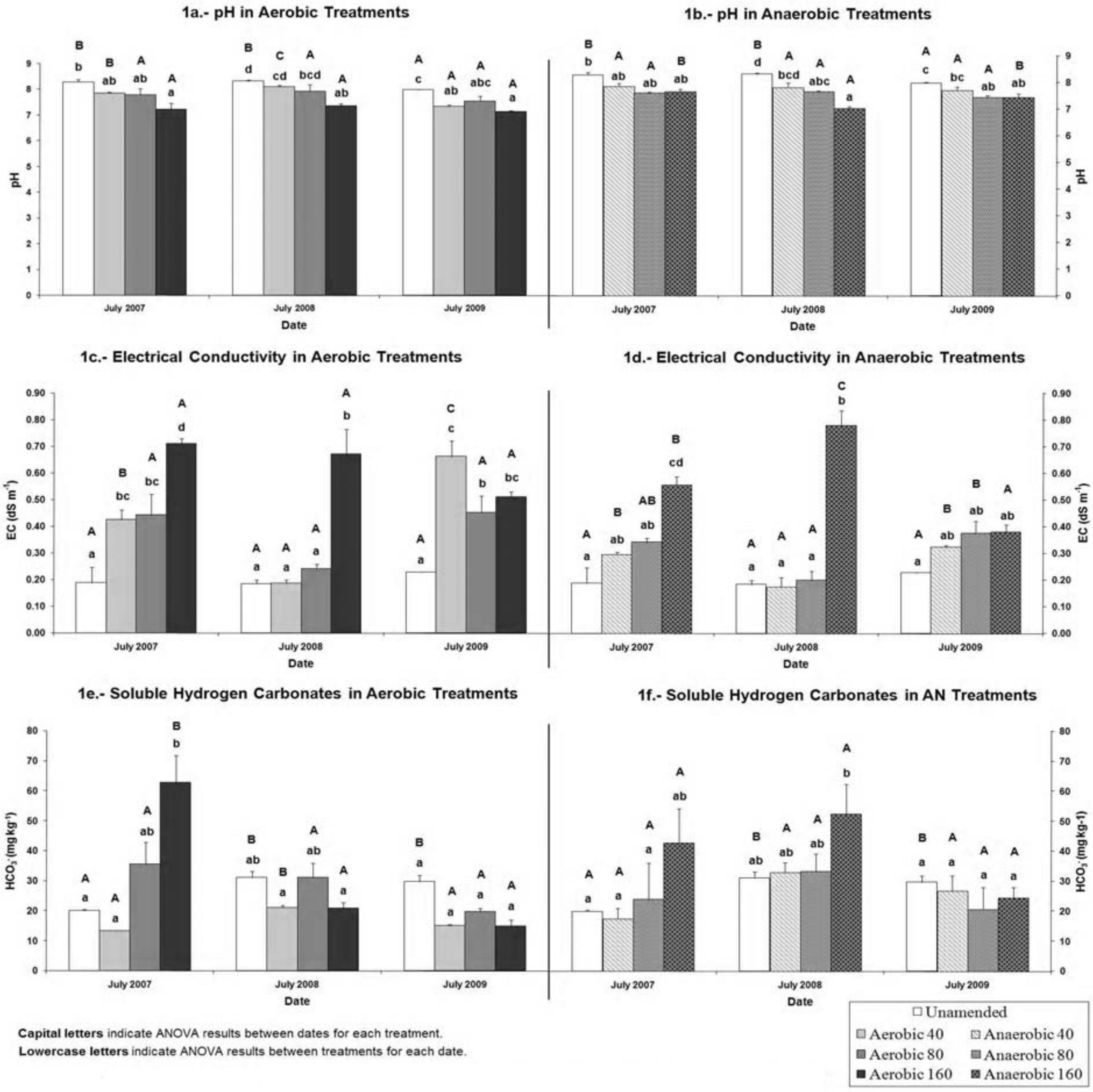
The statistical treatment of the results was performed by analysis of variance (ANOVA) using the F distribution method of Fisher-Snedecor with a confidence level of over 95% ($p < 0.05$) by SPSS v.17 for the Microsoft Windows operating system. To study therelationship between $CO_2$and variousforms of carbon, a multiple regression analysis was conducted to examine the variables that best explain the CO_2 variations and to determine the extent to whichthey are related.

RESULTS & DISCUSSION

As expected, the application of the sludgecauseda decreasein soil pH (Figs 1a and 1b), andanincrease in theEC (Figs 1c and 1d). These data were related to the sludge type and application rate and were similar to those proposed by other authors (Bastida *et al.*, 2007; Hemmat *et al.*, 2010; Morugán-Coronado *et al.*, 2011). AE treatments had a marked effect at the beginning of the experiment, with a 1-unit decrease in pH values,and an increase in EC of 0.5 dS/cm at the 160 rate; these differences continued after two years. The greatest differences between the AN-treated soils andthe CONT soil were observed after one year (a decrease of 1.3 units in pH values and an EC increase of 0.6 dS/m at the 160 rate). After two years, the AN-treated soils reached EC values close to the CONT soil, as reported by Antolin et al. (2005). AE addition affected soil chemistry to a greater degree than AN sludge.Although there was a clear decrease in pH values after the applicationof the amendments, no significant changes were observed in the calcium carbonate content (data not shown),since the soils had a high carbonate content, andthere were also carbonatesin the sludge (Table 2). This excess of calcium carbonate partially buffered the decrease inpH.However, there was anotable effecton the values ofsolublehydrogen carbonate (SHC) (Figs 1e and 1f), whose effects were clearly related to application rate and type of sludge.Thevalues of SHC were significantly influenced by pH values and the equilibrium of calcite weathering (Serrano-Ortiz *et al.*, 2010). The AE-treated soils showed the highest values at the first sampling. After one year, the soils recovered to the CONT soil values, with the exception of the AN 160 rate. The CONT soil increased its SHC content over time. In the treated soil, the main pattern was the decrease in SHC. After two years, allamendedsoilsreducedtheirvaluestobelowthe CONT soilcontents, and no significant differences were found between treatments.

A statistically significant increase was observed inSOCin thesoil after the application of the amendments (Figs 2a and 2b). These data were closely related to application rate and sludge type (Albiach *et al.*, 2001). We also found differences in the pattern of SOC evolution over time:AEinduced a moderate increase in SOC content at the beginning of the experiment, and after two years, its valueswerereduced and showed no significant differenceswiththe CONT soil. These results have been previously reported under a Mediterranean climate by Fernandez et al. (2007a). TheAN-treated soils underwent a greater increase in SOC than the AE-treated soils. This pattern was rate-dependent and showed a maximum for the 160 Mg/ha rate, one year after application. After two years, only the high rate continued to show a statistically significant difference with the CONT soil. Dissolved organiccarbon (DOC) (Figs 2c and 2d) followed the same pattern as total organic carbon. The applicationof the amendments generated asignificant increase in both types of treatments and was rate dependent, as previously observed by Franco-Otero etal. (2012). At the beginning of the experiment, the increase in the content of DOC was only significant in the AE-treated soils. These data were similar to those obtained by Pascual et al. (1998), perhaps due to the greaterpresence of carbohydrates, as noted by Ros et al. (2003). However, after one year we found nosignificant differences between the AE and CONT soil, while the AN-treated soil showed a significant increase compared with the initial values. Inboth cases, their contents reached the CONT soil values two yearsafter application. These decreases in DOC values could be explained by consumption and further mineralisation by the soil microbiota (Ros *et al.*, 2003).

After the applicationof the amendments,we observed an increase inall the carbon fractions analyzed (Fig.3), in correlationwith the rateand type ofsludgeapplied. The R form was the main fraction in CONT soil and in both types of sludge-amended soils. The co-evolution over time of the different carbon fractions in the AN-treated soils did not follow a clear pattern. After one year the R and LP contents increased, but after two years significant differences remained only between CONT soil and soil amended with a higher rate of AN. In the AE-treated soils, thetrend was towards the CONT soil values, since nosignificantdifferences in the R andLP forms were foundwith the CONT soil two years after the sludge application. The recalcitrance index (Rovira and Ramón-Vallejo, 2007) allows us to analyse the bioavailability of carbon pools. As this ratio rises, the importance of the R form increases. As expected, after the application, the lower sludge rate induced an increase in the recalcitrance index due to a faster consumption of the labile carbon forms. This effect is still unclear, but can be explained by the sludge being more easily decomposed when it is applied at low rates (Sommers *et al.*, 1979). The effect of the different rates on the recalcitrance index was more pronounced in the


1a.- pH in Aerobic Treatments

1b.- pH in Anaerobic Treatments

1c.- Electrical Conductivity in Aerobic Treatments

1d.- Electrical Conductivity in Anaerobic Treatments

1e.- Soluble Hydrogen Carbonates in Aerobic Treatments

1f.- Soluble Hydrogen Carbonates in AN Treatments

Capital letters indicate ANOVA results between dates for each treatment.
Lowercase letters indicate ANOVA results between treatments for each date.

fig.1. chemical properties and soluble hydrogen carbontes

AE- than in the AN-amended soils, due to their higher LP contents. This result may be due to the abovementioned higher mineralisation process at lower rates,causedbythegreater presence of easily biodegradable compounds in AE (Fernández *et al.*, 2007b). The differences in the consumption rate of the various carbon forms could be due to the fact that the consumption of R isgreater at high temperatures than at low temperatures (Bol *et al.*, 2003).

We found a clear seasonal pattern in the Rs rate, proving that the evolution ofRs ratesis drivenby temperature andmoisture (Maag and Vinther, 1999; Conant *et al.*, 2000 and 2004; Chen *et al.*, 2010), with significantly higher mineralisation values inspring, and aminimum in winter (Fig. 4). The application of organic amendments to soil promoted an increase in Rs immediately after the addition, and wascorrelated with application rate (Quemada and Menacho, 2001) and sludge type (Flavel *et al.*, 2005; Paramasivam *et al.*, 2008; Franco-Otero *et al.*, 2012). The stimulating effects of sewage sludge application on CO_2 fluxes have been previously reported in laboratory incubations (Raj and Antil, 2011) and in the field (Álvarez and Lidén, 2008). Throughout the study, the AE-amended soils had higher CO_2emission rates.Wefound no clearlyhigherflush in Rsafterthe amendment, as we expected following Kuzyakov et al. (2000 and 2010). This could be attributed to the fact that in the early

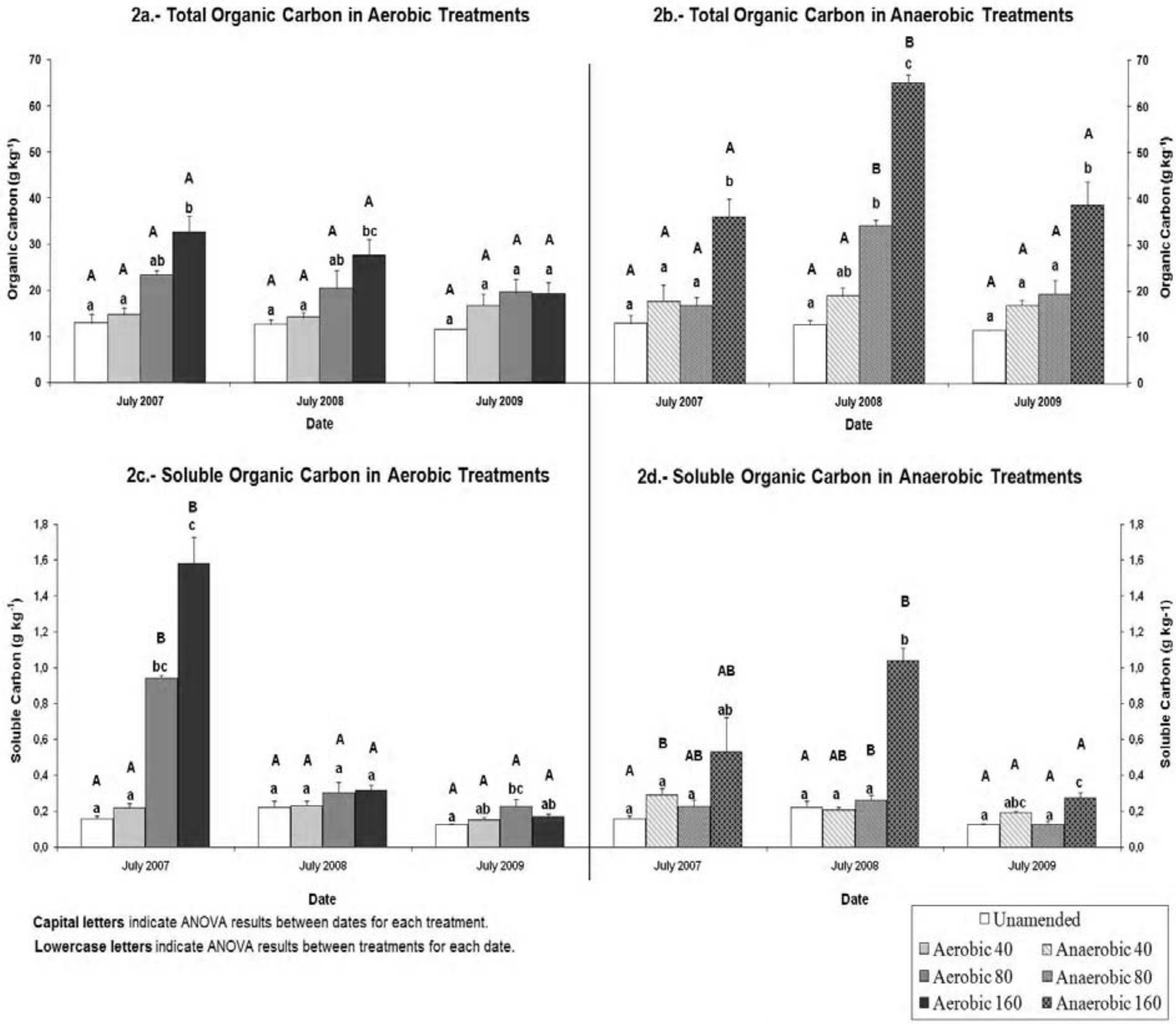
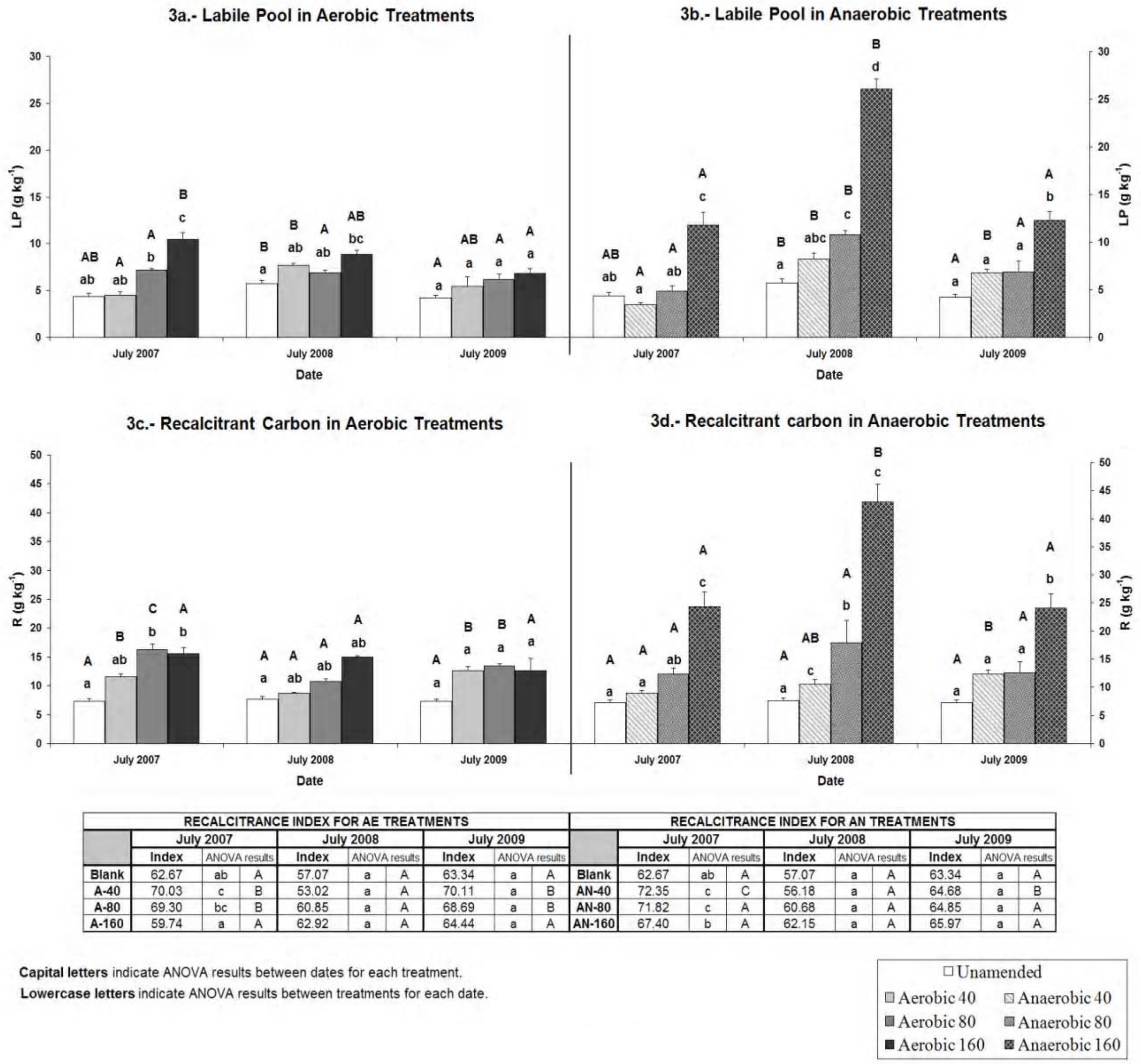


fig. 2. Total and soluble organic carbon

sampling stage (June to October) the weather was extremely dry (126 mm precipitation and 21ºC), which greatly reduced the mineralisation processes. Aftera rainy period (spring 2008, with 90 mm precipitation in May) and a moderate increase in the average temperatures (2ºC from April to May), the Rs rose dramatically in all treatments. Although this process was not tested using theisotope approach, these data may be explained by a priming effect, according to Kuzyakov et al. (2000). We found a clear rate response effect that was higher in the AE-treated plots. The increase inRs was related to the use of the rapidly available SOM fractions (Van Veen *et al.*, 1985; Flavel *et al.*, 2005). The effect of the type of stabilisation of an organic waste on the retention of organic C in soil after the amendment has been observed previously (Dere and Stenhouwer, 2011), pointing tothe possible advisability of using preferentiallyAN sludge in order to reduce CO_2 emissions. In the second year of the experiment, in the summerand wintersampling, the differences in rates and type of sludge Rspractically disappeared.At the endof the study (summer 2009), the sludge-treated soils showed values that were significantly lower thanfor the CONTsoil.

To verify the importance of climate in soil emissions after sludge application, we performed multiple regression analyses between CO_2 emissions and environmental conditions (temperatureand moisture). We also addedSOC as a factortostudy the behaviourof thesoil under the effect ofthe amendments. The results showed that temperatureis themainfactor in the CONT soil (41.4%), and,in combination with moisture, accounted for 57.4% of the changes in the CO_2 emissions.In the AE-treated soils,moisturewas thedetermining factor (22.3%), with temperature, explaining25.9%of the variation in theRs rates. Finally in the AN-amended soils, moisture wasagainthe main factor (29.2%);although in this case, temperature and theamount of SOCappeared to be an



RECALCITRANCE INDEX FOR AE TREATMENTS									
	July 2007			July 2008			July 2009		
	Index	ANOVA results		Index	ANOVA results		Index	ANOVA results	
Blank	62.67	ab	A	57.07	a	A	63.34	a	A
A-40	70.03	c	B	53.02	a	A	70.11	a	B
A-80	69.30	bc	B	60.85	a	A	68.69	a	B
A-160	59.74	a	A	62.92	a	A	64.44	a	A

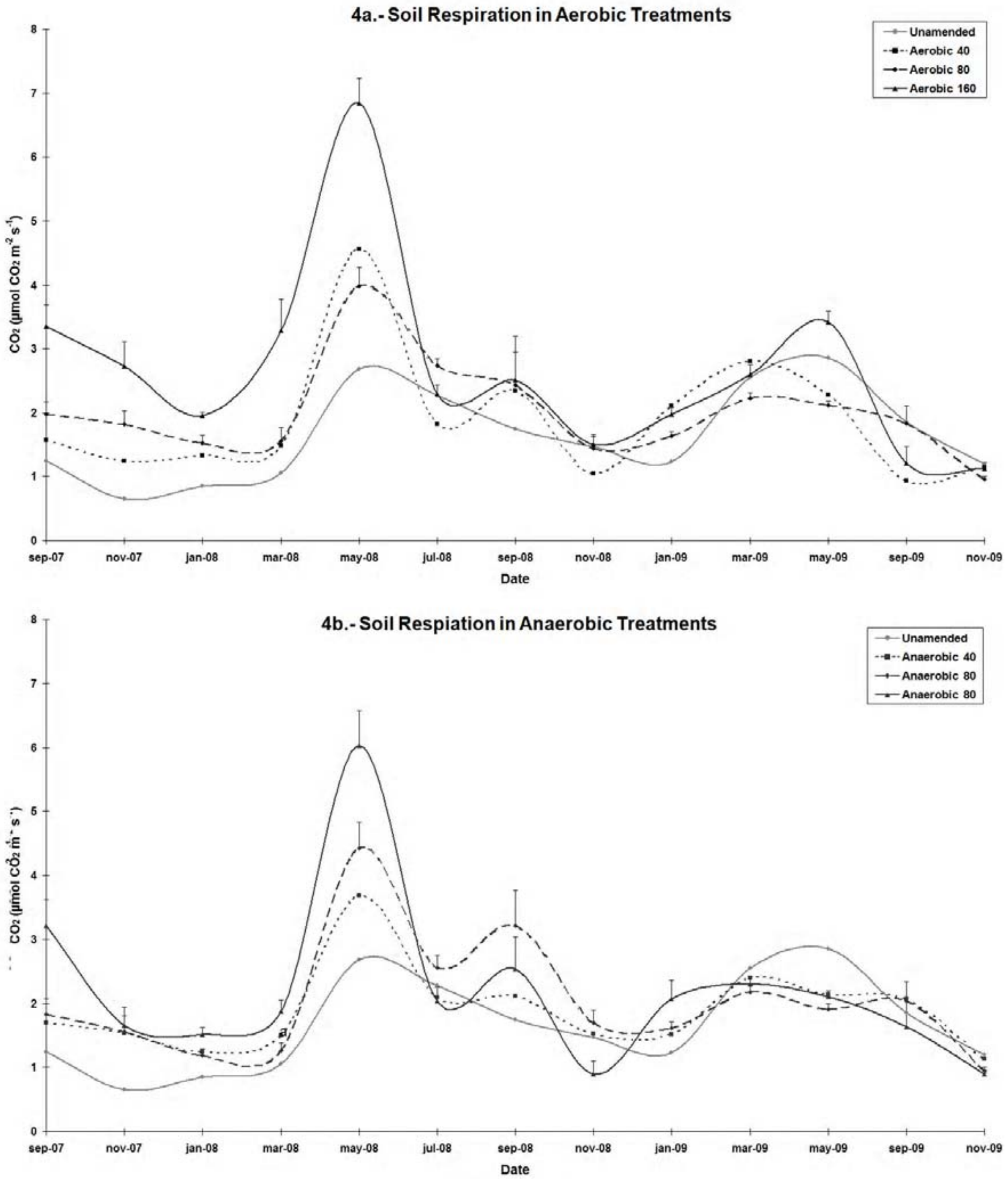
RECALCITRANCE INDEX FOR AN TREATMENTS									
	July 2007			July 2008			July 2009		
	Index	ANOVA results		Index	ANOVA results		Index	ANOVA results	
Blank	62.67	ab	A	57.07	a	A	63.34	a	A
AN-40	72.35	c	C	56.18	a	A	64.68	a	B
AN-80	71.82	c	A	60.68	a	A	64.85	a	A
AN-160	67.40	b	A	62.15	a	A	65.97	a	A

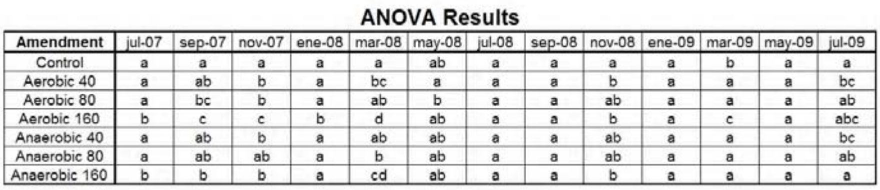
Capital letters indicate ANOVA results between dates for each treatment.
Lowercase letters indicate ANOVA results between treatments for each date.

fig. 3. organic carbon fractions

explanatory variable. These three variablesexplained33.9%of the variation inCO_2emissions. The application of sludge improves the significance of soil moisture on Rs, according to the results of Song and Lee (2010).The analysis ofthe separateeffects of the different rates showed that in the AE-treated soils,thepercentage explainedby the variablesdeclinedas the rateincreased (46.1to 40Mg/ha,23.2% for80 Mg/ha, and 21.4%to 160Mg/ha). Meanwhile, moisture was alwaysthe sole explanatory variable,except atthe lowest rate (40Mg/ha),where temperaturealso hadan impact. The AN-treated soils showed a similarpattern, with a reduced importance of climatic factors on Rsas the rate of application increased; this effect was lower than for AE sludge-amended soils. (46.2to 40Mg/ha,40.7%to80 Mg/ha, and 34.2%to 160Mg/ha).

The resultsindicate that the additionof sludgehada determining effect onRs, by introducingvariablesthat reducedthe influenceof environmental factorsin its variations.This result wasreflected inthe degree to which these variablesexplained changes inRs rates,with a reduction inthe explanationthat was inversely proportionalto the rateof sludgeapplied.Moreover, the improvementin the quality ofsoil organicmattershows that of all theenvironmental factors, the influence ofmoisture onCO_2 emissionswas enhancedin theamended soils. In addition, SOC was not observed to be a relevant factor in CO_2 emissions in the CONTsoil orin AE-treated soils,and onlycontributed1.7%of the explanation





ANOVA Results

Amendment	jul-07	sep-07	nov-07	ene-08	mar-08	may-08	jul-08	sep-08	nov-08	ene-09	mar-09	may-09	jul-09
Control	a	a	a	a	a	ab	a	a	a	a	b	a	a
Aerobic 40	a	ab	b	a	bc	a	a	a	b	a	a	a	bc
Aerobic 80	a	bc	b	a	ab	b	a	a	ab	a	a	a	ab
Aerobic 160	b	c	c	b	d	ab	a	a	b	a	c	a	abc
Anaerobic 40	a	ab	b	a	ab	ab	a	a	ab	a	a	a	bc
Anaerobic 80	a	ab	ab	a	b	ab	a	a	ab	a	a	a	ab
Anaerobic 160	b	b	b	a	cd	ab	a	a	b	a	a	a	a

fig. 4. Soil Respiration

insoils treated withAN.That is, thedecline inthe importanceof environmental factorson soil CO_2 variationswas notexplained by the SOC values. This could be due to the more minor variations in SOC than in moisture and temperature throughout the time of the experiment. It is also possiblethat onlycertain fractionsof organic carbonexplained thesevariations (Dumale *et al.*, 2011; González-Ubierna *et al.*, 2012).In summary, the results suggest that the increase in CO_2 emission is due to the consumption of the labile fraction of the sludge carbon while the original SOC remains stable.

The early sampling results showed that theeffect ofthe amendments on soilcarbon depends on the typeof sludge and the application rate. In theAE-amended soils, an increase was observed inmineralisation processes, with a clear rate effect. This result was reflected inasignificantincrease in Rs rates, whichproducedadrop in pH values andanincrease in SHC content. In the soilstreated withAN, the mineralisation processeswere less intense, and the rate effect was moreunclear.These differences may be explained bythe varying compositionsof the sludge, with AE presenting a higher proportion ofSOCand LPforms (Fernández *etal.*, 2009).

The analysis performed one year after the sludge applicationshowed adifferent effectthan expected based on the results of a previous work(González-Ubierna*et al.*,2012).There was an increasein TOC insoils treated withAN,while the values intheAE-amended soils were maintained, which can be explained bythe contribution of TOC fromvegetation thatgrew spontaneouslyonthe plots and was not harvested.The unusualrainfallinlate springandearly summerof 2008caused anexplosive growthof vegetation.Some authors(Dube *et al.*, 2012;Lopez *et al.*, 2012)estimateda contribution ofSOCfromannualvegetationof between 10and 20g/kg.The samplesanalysed two yearsafterthe sludge application evidenced a trendtowards recovery ofthe CONT soil values.Onlysoils treated withhigher ratesof ANcontinued to showsignificant differences from theCONTsoils in all carbonforms measured.In the AE-treated soils, onlydifferences insoil chemical properties(pHand EC) were observed.The differences foundbetween treatments revealed that theAN-amended soils hadmore complex carbonforms, which made a greater contribution to themaintenance ofsoil carbon, resulting in lower respiration rates.

CONCLUSION

- The application of both types of sludge showed a patentimpact on soil carbon evolution, with the greatest effects observed in soils treated with the highest rates.
- The type of sludge applied also influences soil carbon evolution, to a greater extent in the case of anaerobic sludge. The application of aerobic digested sludge had a greater influence at the early stages, but its effect decreased throughout the first year. This could be related to its high rate of readily mineralized carbon forms. Anaerobic sludge provided more polymerised forms. Although it appeared to have less effecton soil carbon at the beginning of the amendment, this impact became more extensive over time.
- The rates of soil CO_2 emissions were related to the sludge type and application rates, with the highest values observed in high doses of AE sludged-soils.
- The increase in CO_2 emissionsmay be related mainly to the consumption of the sludge labile carbon fraction; while the SOC content remained stable.
- Further study is requiredinto soil respiration after the application of organic amendments in order to reach a decision as tothe most effectivetype of amendments to configure the soil as a sink for carbon sequestration. These additional studies should be taken into account as part of the decision-making process within the sphere of agricultural policy.

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REFERENCES

Albiach, R., Canet, R., Pomares, F. andIngelmo, F. (2001). Organic matter components, aggregate stability and biological activity in a horticultural soil fertilized with different rates of two sewage sludges during ten years. Bioresour. Technol., **77(2)**, 109-114.

Almagro, M., López, J., Querejeta, J.I. and Martínez-Mena, M. (2009). Temperature dependence of soil CO_2 efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem.Soil Biol. Biochem., **41**, 594–605.

Alvarez, R. andLidén, G. (2008).Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste.Renew. Energy, **33(4)**, 726-734.

Antolín, M.C., Pascual, I., García, C., Polo, A. and Sánchez-Díaz, M. (2005). Growth, yield and solute content of barley in soils treated with sewage sludge under semiarid Mediterranean conditions. Field Crops Res., **94(2-3)**, 224-237.

Aubain, P., Gazzo, A., Le Moux, J., Mugnier, E., Brunet, H. andLandrea, B. (2002). Disposal and recycling routes for sewage sludge. Synthesis Report. Report for the EU Commission.

Bastida, F., Moreno, J. L., García, C. and Hernández, T. (2007). Addition of Urban Waste to Semiarid Degraded Soil: Long-term Effect. Pedosphere, **17(5)**, 557-567.

Bol, R., Kandeler, E., Amelung, W., Glaser, B., Marx, M.C., Preedy, N. and Lorenz, K. (2003). Short-term effects of dairy slurry amendment on carbon sequestration and enzyme activities in a temperate grassland. Soil Biol. Biochem., **35(11)**, 1411-1421.

Bradford, M. A., Fierer, N. and Reynolds, J.F. (2008). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Functional Ecology,**22**, 964-974.

Cai,Q.Y., Mo, C.H., Wu, Q.T., Zeng, Q. Y. andKatsoyiannis, A. (2007). Concentration and speciation of heavy metals in six different sewage sludge-composts.J. Hazard Mater., **147(3)**, 1063–1072.

Casermeiro, M. A., Castaño, S., de la Cruz, M. T., García-Montero, L. G., Hernando, M. I. and Navarro-García, F. (2007). Metodología para estudiar el impacto sobre el cambio climático de la aplicación de lodos de depuradora al suelo.

Efectos en el secuestro de carbono. in: Casermeiro, M.A., Espluga, A.P., Desdentado, L.D., Díaz, M., García-Montero, L.G., Sobrini, I. and Andrés, M. (Eds.), Evaluación de Impacto Ambiental en España: nuevas perspectivas. Asociación Española de EIA (pp. 29-34). Madrid.

CEC, (1986).Council of the European Communities.Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/ 278/EEC). Off. J. Eur. Communities L.,**181**, 6-12.

CEC, (1991).Council of the European Communities. Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/ EEC). Off. J. Eur. Communities L., **375**, 1-8.

CEC, (2000).Working Document on Sludge. Third Draft, Brussels 27 April 2000, DG. Environment, 18 pp.

CEC, (2008).Council of the European Communities.Council Directive of 19 November 2008 on waste and repealing certain Directives (2008/98/EC). Off. J. Eur. Communities L, **312**, 3-29.

Chen, S., Huang, Y., Zou,J., Shen, Q., Hu, Z., Qin, Y., Chen, H. and Pan, G. (2010).Modelinginterannual variability of global soil respiration from climate and soil properties.Agric. and For.Meteorol.,**150(4)**, 590-605.

Conant, R.T., Klopatek, J.M. and Klopatek, C.C. (2000). Environmental factors controlling soil respiration in three semiarid ecosystems. Soil Sci. Soc. of Am. J.,**64(1)**, 383-390.

Conant, R.T., Dalla-Betta, P., Klopatek, C.C. andKlopatek, J.M. (2004).Controls on soil respiration in semiarid soils. Soil Biol. Biochem., **36(6)**, 945-951.

Davidson, E. A., Savage, K., Verchot, L. V. and Navarro, R. (2002). Minimizing artifacts and biases in chamber-based measurements of soil respiration. Agri. and For.Meteorol.,**113**, 21-37.

Dere, A.L. andStehouwer, R.C. (2011). Labile and Stable Nitrogen and Carbon in Mine Soil Reclaimed with Manure-Based Amendments *Soil Sci. Soc. of Am. J.,* **75(3)**, 890-897.

Dube, E., Chiduza, C. andMuchaonyerwa, P. (2012). Conservation agriculture effects on soil organic matter on a HaplicCambisol after four years of maize–oat and maize–grazing vetch rotations in South Africa. Soil and Tillage Res.,**123**, 21-28.

Dumale, W.A. Jr, Miyazaki, T., Nishimura, T. and Seki, K. (2011). Short-Term Dynamics of the Active and Passive Soil Organic Carbon Pools in a Volcanic Soil Treated With Fresh Organic Matter. E-Int. Sci. Res. J., **3(2)**, 128-144.

FAO, (2006).World reference base for soil resources (2006).A framework for international classification, correlation and communication.FAO, Roma, 145 pp.

Fernández, J. M., Hernández, D., Plaza, C. and Polo, A. (2007a). Organic matter in degraded agricultural soils amended with composted and thermally-dried sewage sludges. Sci Total Environ., **378(1-2)**, 75-80.

Fernández, J. M., Plaza, C., Hernández, D. and Polo, A. (2007b). Carbon mineralization in an arid soil amended with termally-dried and composted sewage sludge. Geoderma, **137**, 497-503.

Fernández, J. M., Plaza, C., García-Gil, J. C. and Polo, A. (2009). Biochemical properties and barley yield in a semiarid Mediterranean soil amended with two kinds of sewage sludge. App. Soil Ecol., **42(1)**, 18-24.

Flavel, T.C., Murphy, D.V., Lalor, B.M. andFillery, I.R.P. (2005). Gross N mineralization rates after application of composted grape marc to soil. Soil Biol. Biochem., **37(7)**, 1397-1400.

Franco-Otero, V. C., Soler-Rovira, P., Hernández, D., López-De-Sá, E. and Plaza, C. (2012). Short-term effects of organic municipal wastes on wheat yield, microbial biomass, microbial activity, and chemical properties of soil. Biol. and Fertil.of Soils, **48(2)**, 205-216.

Giardina, C.P. and Ryan, M.G. (2000).Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature.Nature, **404**, 858-861.

González-Ubierna, S., Jorge-Mardomingo, I., Carrero-González, B., de la Cruz, M. T. and Casermeiro, M. A. (2012). Soil organic matter evolution after the application of high rates of organic amendments in a Mediterranean calcareous soil. J Soils Sediments.,**12(8)**, 1257-1268.

Haynes, R.J., Murtaza, G. and Naidu, R. (2009). Inorganic and Organic Constituents and Contaminants of Biosolids: Implications for Land Application. Advances in Agronomy, **104**, 165-267.

Hemmat, A., Aghilinategh, N., Rezainejad, Y. andSadeghi, M. (2010). Long-term impacts of municipal solid waste compost, sewage sludge and farmyard manure application on organic carbon, bulk density and consistency limits of a calcareous soil in central Iran. Soil and Tillage Res.,**108(1-2)**, 43-50.

Heras, J., Manas, P. and Labrador, J. (2005). Effects of several applications of digested sewage sludge on soil and plants. J. Environ. Sci. Health A Tox.Hazard. Subst. Environ. Eng., **40**, 437-451.

Hogg, D., Favoino, E., Nielsen, N., Thompson, J., Wood, K., Penschke, A., Economides, D. andPapageorgiou S. (2002).Economic Analysis of Options for Managing Biodegradable Municipal Waste.Final Report.

ISRIC, (2002).Procedures for soil analysis. International Soil Reference And Information Center (ISRIC). L.P. van Reeuwijk (Ed.).

Kuzyakov, Y., Friedel, J.K. andStahr, K. (2000). Review of mechanisms and quantification of priming effects. Soil Biol. Biochem.,**32(11-12)**, 1485-1498.

Kuzyakov, Y. (2010).Priming effects: Interactions between living and dead organic matter. Soil Biol. Biochem.,**42(9)**, 1363-1371.

Lal, R. (2004). Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science, **304**, 1623-1627.

López, M.V., Blanco-Moure, N., Limón, M.A. andGracia, R. (2012).No tillage in rainfed Aragón (NE Spain): Effect on organic carbon in the soil surface horizon. Soil and Tillage Res.,**118**, 61-65.

Maag, M. andVinther, F.P. (1999). Effect of temperature and water on gaseous emissions from soils treated with animal slurry. Soil Sci. Soc. of Am. J., **63(4)**, 858-865.

Morugán-Coronado, A., García-Orenes, F., Mataix-Solera, J., Arcenegui, V. andMataix-Beneyto, J. (2011).Short-term effects of treated wastewater irrigation on Mediterranean calcareous soil. Soil and Tillage Res., **112(1)**, 18-26.

Paramasivam, S., Fortenberry, G.Z., Julius, A., Sajwan, K.S.andAlva, A.K.J. (2008). Evaluation of emission of greenhouse gases from soils amended with sewage sludge. J. of Environ. Sci. and Health.Part A, Toxic/hazardous substances & Environmental Engineering., **43(2)**, 178-185.

Pascual, J.A., Hernandez, T., García, C. andGarcía, A. (1998).Changes in the organic matter mineralization rates of an arid soil after amendment with organic wastes. Arid and Soil Res. and Rehabilit.,**12(1)**, 63-72.

Quemada, M. andMenacho, E. (2001).Soil respiration 1 year after sewage sludge application.Short communication. Biology and Fertility of soils.,**33**, 344-346.

Raj, D. andAntil, R.S. (2011). Evaluation of maturity and stability parameters of composts prepared from agro-industrial wastes. Bioresour. Technol., **102(3)**, 2868-2873.

RD, (1990). Real Decreto 1310/1990, de 29 de octubre, por el que se regula la utilización de los lodos de depuración en el sector agrario. BOE número 262 de 1/11/1990, 32339-32340.

Ros, M., Hernandez, M. T. and García, C. (2003). Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biol. Biochem., **35(3)**, 463-469.

Rovira, P. and Ramón-Vallejo, V. (2007).Labile, recalcitrant, and inert organic matter in Mediterranean forest soils. Soil Biol. Biochem., **39(1)**, 202-215.

Sánchez-Brunete, C., Miguel, E. andTadeo, J.L. (2007). Analysis of 27 polycyclic aromatic hydrocarbons by matrix solid-phase dispersion and isotope dilution gas chromatography-mass spectrometry in sewage sludge from the Spanish area of Madrid. J. Chromatogr. A., **1148(2)**, 219-227.

Serrano-Ortiz, P., Roland, M., Sanchez-Moral, S., Janssens, I.A., Domingo, F., Goddéris, Y. and Kowalski, A.S. (2010). Hidden, abiotic CO_2 flows and gaseous reservoirs in the terrestrial carbon cycle: Review and perspectives. Agric. and For.Manag.,**150**, 321-329.

Schoeneberger, P.J., Wysocki, D.A., Benham, E.C. andBroderson, W.D. (2002).Field book for describing and sampling soils, Version 2.0.Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE.

Sheppard, S.K., Gray, N., Head, I.M. and Lloyd, D. (2005). The impact of sludge amendment on gas dynamics in an upland soil: monitored by membrane inlet mass spectrometry. Bioresource Technology, **96**, 1103-1115.

Sommers, L.E., Nelson, D.W. andSilviera, D.J. (1979). Transformations of carbon, nitrogen and metals in soils treated with waste materials. J. Environ. Qual. **8**, 287-294.

Song, U. and Lee, E.J. (2010).Environmental and economical assessment of sewage sludge compost application on soil and plants in a landfill. Resources, Conservation and Recycling, **54**, 1109-1116.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T. and Jones, K.C. (2003). PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in U.K. sewage sludge: Survey results and implications. Environ Sci Technol., **37**, 462-467.

Torri, S., Alvarez, R. andLavado, R.L. (2003).Mineralization of Carbon from Sewage Sludge in Three Soils of the Argentine Pampas.Communications in Soil Science and Plant Analysis, **34**, 2035-2043.

Valladares, F. (2004). Ecología del bosque mediterráneo en un mundo cambiante. pp: 163-190. Ministerio de Medio Ambiente, EGRAF, S. A., Madrid. ISBN: 84-8014-552-8.

Van Veen, J. A., Ladd, J. N. and Amato, M. (1985). Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [14C(U)] glucose and [15N](NH4)2So4 under different moisture regimes. Soil Biol. Biochem.,**17(6)**, 747-756.

Zinati, G.M., Li, Y.C. and Bryan, H.H. (2001). Utilization of compost increases organic carbon and its humin, humic and fulvic acid fractions in calcareous Soil. Compost Science & Utilization, **9**, 156-162.