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Organic matter mineralisation in contrasting agricultural soils amended with sewage sludge

Mineralización de la materia orgánica en diferentes suelos agrícolas enmendados con lodos de depuradora
Mineralização damatéria orgânica em diferentes solos agrícolas fertilizados com lamas de esgoto

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ABSTRACT

The mineralisation of organic matter (OM) when sewage sludge was used as amendment in 70 contrasting agricultural soils from Spain was analysed. Soils received a single dose of sewage sludge (equivalent to 50t dry weight ha^{-1}) and the O_2 consumption was continuously monitored for 30 days using a multiple sensor respirometer in a laboratory experiment. The cumulative O_2 consumption and rates after 8 and 30 days of incubation ($O_{2\ cum}$ 8d, 30d and $O_{2\ rate}$ 8d, 30d), the respiratory quotient (RQ), the maximum O2 rates over the incubation period ($O_{2\ max}$) and time from the beginning of the incubation when $O_{2\ max}$ occurred (T_{max}), were determined in both amended and non-amended soils. Sewage sludge application resulted in increased values for $O_{2\ max}$, $O_{2\ rate}$ 8d, and $O_{2\ cum}$ 30d. Differences were minor for T_{max}, RQ 8d and $O_{2\ rate}$ 30d. A considerable amount of the initial OM applied was mineralised during the first 8 days. Organic matter decomposition (as expressed by $O_{2\ cum}$ 30d) was favoured in soils with high values of pH, carbonates, soil organic carbon and low values of amorphous Mn. Soils with these characteristics may potentially lose soil C after sewage sludge application.

RESUMEN

Se estudió la mineralización de la material orgánica de 70 suelos agrícolas españoles tras la aplicación de lodos de depuradora como enmienda orgánica. Los suelos recibieron una dosis única de enmienda (equivalente a 50t en peso seco ha^{-1}). Se determinó el consumo de O_2 de forma continua durante 30 días usando un respirómetro múltiple. El consumo acumulado de O_2 y las tasas de respiración después de 8 y 30 días de incubación ($O_{2\ cum}$ 8d, 30d y $O_{2\ rate}$ 8d, 30d), el cociente de respiración (RQ), la tasa máxima de O_2 durante el periodo de incubación (O2 max) y el tiempo desde el comienzo de la incubación cuando se produjo $O_{2\ max}$ (T_{max}), fueron determinados para las muestras de suelo enmendadas y no enmendadas. La adición de lodo de depuradora produjo un incremento en los valores obtenidos para $O_{2\ max}$, $O_{2\ rate}$ 8d, y $O_{2\ cum}$ 30d. Las diferencias fueron menos importantes para los resultados obtenidos de T_{max}, RQ 8d y O2 rate 30d. Se observó una mineralización importante de la materia orgánica añadida durante los primeros 8 días. La descomposición de la materia orgánica (expresada como $O_{2\ cum}$ 30d) se vio favorecida en aquellos suelos que mostraron los valores más altos de pH, carbonatos y carbono orgánico, y valores bajos de compuestos amorfos de Mn. Los suelos que presenten estas características son potencialmente susceptibles de perder rápidamente C por mineralización del lodo de depuradora aplicado como enmienda orgánica.

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RESUMO

A mineralização da matéria orgânica de 70 solos agrícolas espanhóis foi estudada após a aplicação de lamas de esgoto como corretivo orgânico. Os solos receberam uma dose única de corretivo (equivalente a 50t peso seco ha^{-1}). O consumo de O_2 foi determinado ao longo de 30 dias de forma contínua usando um respirómetro múltiplo. O consumo acumulado de O_2 e as taxas respiração após 8 e 30 dias de incubação ($O_{2\ cum}$ 8d, 30d e $O_{2\ rate}$ 8d, 30d), o quociente respiratório (RQ), a taxa máxima de O_2 durante o período de incubação (O2 max) e o tempo desde o início da incubação até quando ocorreu $O_{2\ max}$ (T_{max}) foram determinados para as amostras de solo com e sem adição de corretivo. A adição de lamas de esgoto conduziu a um aumento dos valores obtidos para o $O_{2\ max}$, $O_{2\ rate}$ 8d, e $O_{2\ cum}$ 30d. As diferenças foram menos evidentes para os resultados de T_{max}, RQ 8d e $O_{2\ rate}$ 30d. Observou-se uma mineralização significativa do material biológico adicionado durante os primeiros 8 dias. A decomposição da matéria orgânica (expressa como $O_{2\ cum}$ 30d) foi favorecida nos solos que apresentavam os valores mais elevados de pH, carbonatos e carbono orgânico, e valores baixos em compostos amorfos de Mn. Os solos que apresentam essas características são potencialmente mais susceptíveis de perder rapidamente C por mineralização de lamas de esgoto aplicadas como corretivo orgânico.

KEY WORDS
C sequestration, soil carbon, biosolids, soil respiration

PALABRAS CLAVE
Secuestro de carbono, carbono del suelo, biosólidos, respiración edáfica

PALAVRAS-CHAVE
Sequestro de carbono, carbono do solo, biosólidos, respiração do solo

1. Introduction

The production of sewage sludge in Europe has increased dramatically in the last decades. With the increment of economic restraints and environmental concerns about landfilling and incineration (Liang et al. 2011), there has been increased interest in the land application of these wastes (O'Connor et al. 2005). Land application enhances the recycling of nutrients and organic matter to soil and can contribute to the reduction of the atmospheric concentration of CO_2, increasing the levels of soil organic carbon (SOC) and associated fertility (Ajwa and Tabatabai 1994; Bernal et al. 1998; Tian et al. 2009). The conversion of natural to agricultural ecosystems has resulted in the depletion of SOC, as much as 60% in soils of temperate regions and 75% or more in the tropics. The land application of sewage sludge represents an opportunity for carbon (C) sequestration (Lal 2004; Lal 2008). This practice is of particular relevance in soils from arid and semi-arid regions, where soil erosion and decline of organic matter are major threats.

However, recycling sewage sludge onto agricultural land poses risks for humans and the environment due to the presence of high levels of nutrients, labile organic matter and pathogens as well as organic pollutants and toxic metals (McBride 2003; Basta et al. 2005; Cesar et al. 2012). The intense microbial activity is enhanced by the land application of waste materials with high levels of labile organic matter (Saviozzi et al. 2002; Belyaeva and Haynes 2012), which may promote the mineralisation of the native SOC (Bernal et al. 1998) known as the priming effect (Dalenberg and Jager 1989).

The decomposition of exogenous organic matter (EOM) in soil following the application of sewage sludge is thought to play a crucial role in the balance between the potential positive or negative effects derived from these applications. In particular, this process drives C, N and other important elements cycles in agroecosystems (Curtin et al. 1998; Spargo et al. 2011) and conditions potential adverse effects and the contribution to soil C storage of sewage sludge applications (Navarro-Pedreño et al. 1996; Kaur et al. 2008).

The mineralisation of EOM following the land application of organic wastes is dependent on a myriad of biotic (Blagodatskaya and Kuzyakov 2008; Blagodatsky et al. 2010) and abiotic factors including EOM composition (Levi-Menzi et al. 1990; Bernal et al. 1998), environmental conditions (Hsieh et al. 1981) and soil properties (Pedra et al. 2007; Huang and Chen 2009), which affect the microbial activity that drives this process (Blagodatskaya and Kuzyakov 2008). Studies evaluating the mineralisation of EOM following sewage sludge application in a range of soils are sparse and the soil mechanisms responsible for this process still remain unclear (Bradford et al. 2008). In particular, there is a considerable controversy about the effects of sewage sludge application on the SOC pool (Jones et al. 2006; Soriano-Disla et al. 2010; Tian et al. 2013).

Studies evaluating the mineralisation of EOM in soil should involve the combined information about short- (hours to days) and long-term (weeks to months) respiratory parameters since they reveal different mechanisms of the decomposition of EOM (Smith et al. 1985; Adani et al. 2004; Blagodatskaya and Kuzyakov 2008; Ponsá et al. 2010).

The objective of this study was to assess the mineralisation of OM when sewage sludge was applied in 70 contrasting agricultural soils and the influence of the soil factors responsible for short-term mineralisation.

2. Material and methods

Seventy agricultural soils from different parts of Spain were chosen. The soils were sampled from the ploughed layer (0-30 cm) and then air-dried, homogenised and sieved (< 2 mm). A synthesis of the selected properties of the soils is shown in **Table 1** (more detailed information in Supplementary Material 1).

Selected soil properties were analysed as follows: pH (1:2.5 wv^{-1}, distilled water), electrical conductivity (1:5 wv^{-1}, distilled water), equivalent calcium carbonate (CO_3^{2-}) determined using the Bernard calcimeter (Hulseman 1966), soil organic carbon (SOC) determined by potassium

Table 1. Selected properties of the soils (n=70)

Properties[a]	Mean±SD[b]	Range	25_{th}perc	Median	75_{th}perc
pH	7.9±1.1	5.0-9.1	7.0	8.4	8.6
EC (μs cm^{-1})	218±411	18-2370	83	120	186
CO_3^{2-} (%)	22.9±22.8	<0.1-78.7	<0.1	17.6	42.1
SOC (g kg^{-1})	7.4±4.3	2.1-20.0	4.1	6.4	9.3
SOC/Nk	10.0±1.8	5.0-14.1	8.7	10.0	11.3
Clay (%)	19±10	1-53	12	17	22
Sand (%)	56±19	17-90	41	59	73
AmFe (mg kg^{-1})	313±223	71-1024	172	246	369
AmMn (mg kg^{-1})	96±122	7-810	25	49	132

[a]EC, electrical conductivity; CO_3^{2-}, equivalent calcium carbonate; SOC, soil organic carbon; N_k, Kjeldahl N; AmFe, amorphous Fe oxides; AmMn, amorphous Mn oxides.
[b]SD, standard deviation.

dichromate oxidation (Nelson and Sommers 1982), Kjeldahl nitrogen (Nk) to measure the ratio SOC/Nk (Bremner and Mulvaney 1982), texture determined by the Bouyoucos method (Gee and Bauder 1986), amorphous iron (Fe) and manganese (Mn) extracted by an ammonium oxalate/oxalic acid extraction (Houba et al. 1989) and determined by atomic absorption spectrophotometry (Unicam 969, UK).

Sewage sludge was from a municipal wastewater treatment plant in Alicante (SE Spain). Wastewater comprised mainly domestic sewage. Sludge collected during the water treatment process was digested aerobically. The digested sludge was transferred to gravity thickening tanks and finally dewatered by belt filters. The characteristics of the sludge are shown in **Table 2**. Total metals and phosphorous were determined after microwave acid extraction using HNO_3/H_2O_2 at a ratio of 4:1 (v/v) (Moral et al. 1996). Concentrations of heavy metals in the sludge were far below of the limit values set by the European Directive 86/278/EEC.

Table 2. Selected properties of the sewage sludge

EC[a] (ms cm^{-1})	N_k[b] (g kg^{-1})	OC[c] (g kg^{-1})	Total P (g kg^{-1})	Total Cd (mg kg^{-1})	Total Cr (mg kg^{-1})	Total Cu (mg kg^{-1})	Total Ni (mg kg^{-1})	Total Pb (mg kg^{-1})	Total Zn (mg kg^{-1})
4.9	58	234	22	0.84	35.6	207	14.3	39.0	646

[a]EC, electrical conductivity.
[b]N_k, Kjeldahl nitrogen.
[c]OC, organic carbon.

Microbial respiration was evaluated using a multiple sensor respirometer (Micro-Oxymax, Columbus Inc, EEUU) that analysed the O_2 concentration at time intervals using paramagnetic sensors. Fifteen grams of each soil were mixed with sewage sludge at a single dose equivalent to 50 t dry weight ha^{-1} (0.016 g sewage sludge g soil^{-1}) in tightly closed 250 ml vessels, and maintained at 60% water-holding capacity (determined according to Forster 1995) at 25 °C for 30 days. Respiration was also measured for control soils by maintaining 30 g of each soil at the same conditions for 30 days.

The cumulative O_2 consumption and rates after 8 and 30 days ($O_{2\ cum}$ 8d, 30d and $O_{2\ rate}$ 8d, 30d, respectively), the maximum rates during the incubation time ($O_{2\ max}$) and time from the beginning of the incubation experiment (T_{max}) when $O_{2\ max}$ is reached, were the parameters selected. For non-amended (na) soils, $O_{2\ max\_na}$, T_{max_na}, $O_{2\ cum\_na}$ 8d, 30d and $O_{2\ rate\_na}$ 8d, 30d were calculated.

Simultaneous analysis of CO_2 release using an infrared sensor was done. In order to know the percentage of organic C release from the mineralisation of the added EOM, the cumulative CO_2 release after 30 d for both amended and non-amended soils was calculated: $CO_{2\ cum}$ 30d and $CO_{2\ cum\_na}$ 30d. In addition, the cumulative CO_2 release after 8 d was also determined for both amended and non-amended soils for the calculation of the respiratory quotient (RQ), expressed as the ratio of mol CO_2 evolution over mol O_2 consumption (Dilly 2003).

For the determination of the percentage of organic C from EOM mineralised after 30d (% EOM 30d), the $CO_{2\ cum\_na}$ 30d was subtracted from the $CO_{2\ cum}$ 30d and the result expressed as C released after 30d. This value was compared to the amount of organic carbon added with the sewage sludge. The cumulative values of CO_2 released after 30 days for the non-amended soils were transformed to C and compared to the SOC, determining the % of SOC mineralised after 30 days (% SOC 30d).

The influence of soil properties on the respiratory parameters from the soils amended with sewage sludge was determined by Pearson correlation coefficients (r). Amorphous Fe and Mn were $\log_{10}$ transformed in order to normalise the variable distributions and reduce asymmetry (Maindonald and Braun 2010). In the case of electrical conductivity, soil samples 21, 40, 50, 61 corresponding with concentration outliers (samples that appear to be isolated from the main body of data) were detected and not considered in the analysis in order to fulfil model conditions (Maindonald and Braun 2010). Statistical analyses were performed using the software SPSS v.21.0 and Microsoft Excel 2007.

3. Results

The mean values (± standard deviation) and ranges of the respiratory parameters for the amended and non-amended soils can be found in **Table 3**. **Figure 1** shows the evolution for selected soils (45, 55 and 57). Oxygen consumption rates were considerably high at the beginning of the incubation experiment for the soils amended with sewage sludge ($O_{2\ max}$ and $O_{2\ rate}$ 8d), but they declined rapidly thereafter (**Figure 1** and **Table 3**). Maximum rates were reached, on average, after 23 hours of incubation with samples ranging from 10 to 43 hours (**Table 3**). Consumed O_2 was mainly accumulated during the first days of incubation and a gradual and slight increase was observed over the incubation time (**Figure 1**). On average, more than 60% of the total cumulative O_2 at the end of the incubation period (64±4%, 50-74%) was consumed in the first 8 days (**Figure 2**). The average values for RQ was 0.52 and weakly varied across the soils (**Table 3**).

Table 3. Mean±standard deviation (SD), range and percentiles of the respiratory parameters[a] for the sewage sludge amended and non-amended soils

		$O_{2\ max}$ (µg $h^{-1}g^{-1}$)	T_{max} (h)	$O_{2\ cum}$ 8d (mg kg^{-1})	$O_{2\ rate}$ 8d (µg $h^{-1}g^{-1}$)	$O_{2\ cum}$ 30d (mg kg^{-1})	$O_{2\ rate}$ 30d (µg $h^{-1}g^{-1}$)	RQ 8d (mol CO_2 O_2^{-1})
Amended	Mean±SD	98.3±33.1	23±9	7890±1488	21.3±5.2	12224±1969	3.7±1.1	0.52±0.02
	Range	44.5-158.1	10-42	5660-11507	13.3-35.4	8671-16928	0.9-6.6	0.47-0.57
	25th-75th percentile	60.5-121.3	16-28	6569-9091	16.2-25.4	10379-13640	3.0-4.3	0.51-0.53
	Median	108.9	23	7972	21.9	12493	3.5	0.52
Non-amended	Mean±SD	6.1±4.0	17±5	589±411	1.7±1.2	1263±951	1.1±1.1	0.51±0.04
	Range	1.4-22.3	7-39	135-2376	0.5-6.7	269-5283	0.2-7.7	0.39-0.61
	25th-75th percentile	3.6-7.4	13-20	332-714	1.0-1.9	706-1396	0.6-1.2	0.49-0.53
	Median	5.3	14	483	1.4	986	0.8	0.51

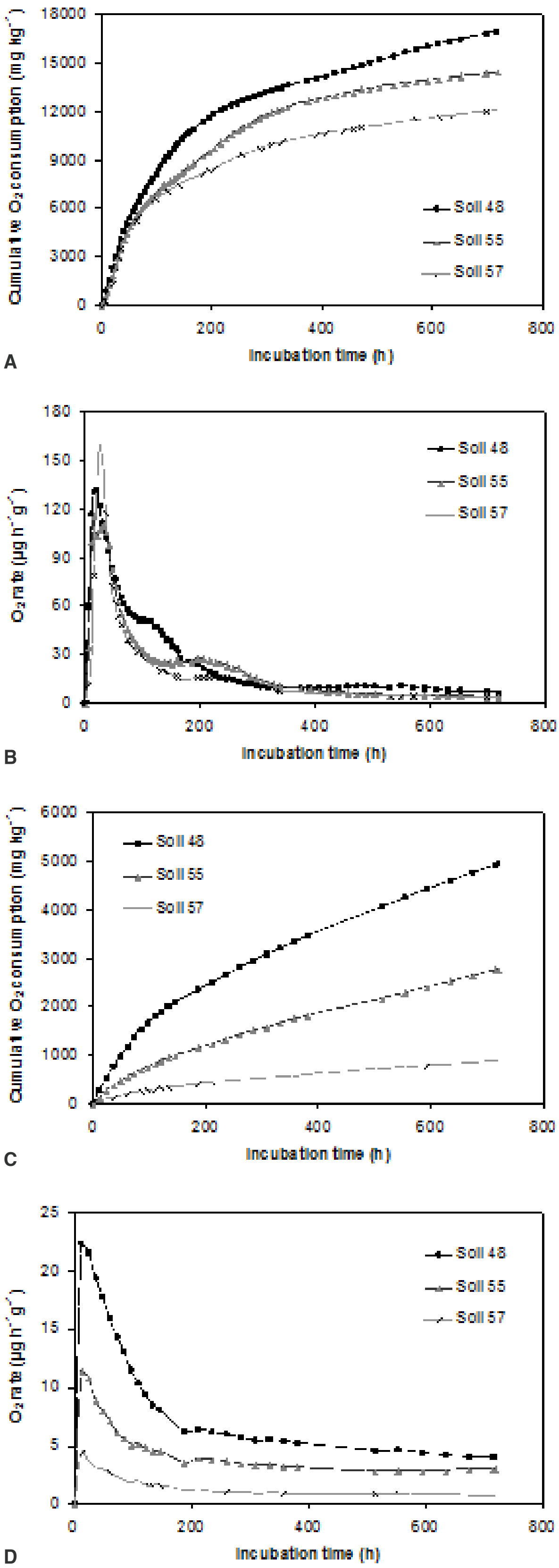

Figure 1. Cumulative O_2 consumption (**A, C**) and O_2 consumption rates (**B, D**) over 30 days of incubation for soils amended (top plots, **A** and **B**) and non-amended (bottom plots **C** and **D**) with sewage sludge.

Mineralisation patterns for the non-amended soils were similar to those described for the amended soils but observing expectable lower mineralisation rates and cumulative O_2 consumption values (Figure 1 and Table 3). Poor differences were found for the T_{max}, RQ and $O_{2\ rate}$ 30d values as a consequence of sewage sludge application (Table 3). The variation across soils for the respiratory parameters in the non-amended soils was higher than for the amended soils (an example of contrasting soils in Figure 1).

The mean (±standard deviation) and ranges (in mg kg^{-1}) for $CO_{2\ cum}$ 30d and $CO_{2\ cum\_na}$ 30d were 8340±1470, 5896-11889 and 891±751, 183-4094, respectively. Using these values, the % of organic C from EOM mineralised after 30 days was calculated and the mean (±standard deviation) and ranges for the whole set of soils was: 56±9, 41-69%. The average % of SOC mineralised after 30 days was 3±2, with values ranging from 1-12%.

Respiratory parameters were related to soil properties through Pearson correlations. Poor correlation coefficients were found between the $O_{2\ max}$, T_{max}, RQ and soil properties (data not shown). On the other hand, significant relationships were observed between the $O_{2\ cum}$ 30d and soil properties (Table 4).

The highest levels of correlation were observed between $O_{2\ cum}$ 30d and pH, carbonates and SOC. After these results, soils were ranked according to the $O_{2\ cum}$ 30d values and divided into low (lower than the 25th percentile) and high (higher than the 75th percentile) values. Soils with low $O_{2\ cum}$ 30d values were characterised by relatively low average values of pH (6.9), carbonates (4.1%), SOC (4.2 g kg^{-1}) and high values of amorphous Mn (119.1 mg kg^{-1}). Contrasting average values for these variables were found in the soils with high $O_{2\ cum}$ 30d: pH (8.5), carbonates (45%), SOC (12.4 g kg^{-1}) and amorphous Mn (42.7 mg kg^{-1}). According to these results, the cumulative $O_{2\ cum}$ 30d following

Table 4. Pearson correlations (r value) between cumulative O_2 consumption after 30 days of incubation and soil properties for soils amended with sewage sludge and after subtracting the cumulative O_2 consumption by the non-amended soils

Soil properties[a]	O_2 cum 30d (amended)	O_2 cum 30d (amended-non amended)
pH	0.51***	0.54***
EC	0.50***	0.47***
CO_3^{2-}	0.64***	0.61***
SOC	0.73***	0.48***
SOC/Nk	0.25*	0.18ns
Clay	0.21ns	0.26*
Sand	-0.35**	-0.39**
AmFe	-0.12nsb	-0.11ns
AmMn	-0.29*	-0.31*

[a]EC, electrical conductivity; CO_3^{2-}, equivalent calcium carbonate; SOC, soil organic carbon; AmFe, amorphous Fe oxides; AmMn, amorphous Mn oxides.
bns, no significant.
***, **, *: the probability level of p<0.001, 0.01 and 0.05, respectively.

sewage sludge application was conditioned by pH, carbonates, SOC and amorphous Mn.

In an attempt to evaluate if the relationships between soil properties and $O_{2\ cum}$ 30d were affected by the SOC mineralised from the non-amended soils, the differences between $O_{2\ cum}$ and $O_{2\ cum\_na}$ 30d across soils were also related to soil properties through Pearson correlations (Table 4). The contribution of SOC was weaker while the contribution of the rest of soil properties was similar.

4. Discussion

Since addition of sewage sludge means addition of a source of available C, an initial short-term O_2 flush during the first 2-3 days of incubation is characteristic of the mineralisation dynamics of the labile fraction of EOM applied in soils (Bernal et al. 1998; Haynes and Naidu 1998; Pedra et al. 2007; Huang and Chen 2009). Our results showed that a relatively high amount of O_2 was consumed during the mineralisation of available substrates. High microbial activity following the land application of sewage sludge can potentially result in negative effects for the soil-plant system (e.g. deprivation of O_2 and release of toxic substances), which can be reduced by adding organic wastes to soil some weeks before sowing (Bernal et al. 1998). The mineralisation patterns of sewage sludge in soils during the incubation time (Figure 1) were typical for organic wastes applied in soils (e.g. Hsieh et al. 1981; Bernal et al. 1998). Contrasting respiration rates at different stages of the mineralisation (Figure 1 and Table 3) were accounted for the degradation of different types of organic matter (easily and slowly biodegradable).

Oxygen uptake increases exponentially as the microbial population grows, but diminishes when the availability of the most highly biodegradable substrates becomes limited or the oxygen reduced. Thus, $O_{2\ max}$ provides information regarding the availability of easily biodegradable C.

T_{max} is dependent on the lag-time, i.e. time between the addition of substrate and the start of the exponential increase of the respiration rate (Nordgren et al. 1988) which is assumed to be indicative of changes in the physiological status of soil microorganisms (Mochizuki and Hattori 1987). Once this labile pool is decomposed, the mineralisation rates decline, revealing why weak differences were found in the $O_{2\ rate}$ 30d for amended and non-amended soils.

The RQ can reveal useful information on C metabolic pathway - organic matter mineralisation patterns (Dilly 2003). The RQ may vary depending on the composition of available substrates and the current physiology of the soil microbial communities (Dilly 2001). As expressed by RQ, both SOC and the EOM were mineralised with the same efficiency, and so RQ may be controlled by the soil microbial community of the soil.

The lower variability within the mineralisation parameters found for the amended soils, in contrast with non-amended soils, is indicative of the importance of the great mineralisation of the organic matter following the application of sewage sludge. Despite the lower variability, considerable differences were still observed across the amended soils. These differences are dependent on the mineralisation of the SOC and the different mineralisation of the EOM as conditioned by soil properties.

The lower variability found within the mineralisation parameters for the amended soils is also reflected by the % EOM 30d, which was high irrespective of the soil, contrasting with the average % of SOC mineralised after 30 days. The % EOM 30d represents an approximate value, since the increment of the CO_2 release is a consequence of the application of EOM and the mineralisation of this organic waste, but it could be potentially affected by an enhanced degradation of SOC following the application of sewage sludge (previously defined as priming effect).

The $O_{2\ max}$ is indicative of the amount of easily biodegradable C. This probably conditioned the absence of relationships between $O_{2\ max}$ and soil properties since the amount and type of EOM applied was the same in all the soils.

As discussed above, the first factor influencing the cumulative O_2 values was the large mineralisation of the EOM in the amended soils with further differences observed as function of soil type. The relationship between soil pH and $O_{2\ cum}$ 30d is not unexpected, since pH conditions the metabolic activity state and proliferation of microorganisms. Kemmitt et al. (2006) found that C mineralisation was favoured by increasing pH values. This was illustrated by Huang and Chen (2009), who examined the effect of sewage sludge application rates on the C decomposition rate in three soils with different initial pH values, showing that C mineralisation was promoted in high initial pH soils. The positive relationship between the concentration of carbonates (CO_3^{2-}) and microbial activity was conditioned by the also positive relationship between carbonates and soil pH ($r = 0.76$, $p < 0.001$). Soils with high pH and calcium carbonate content have been found to favour the contribution of inorganic C to CO_2 production (Stevenson and Verburg 2006) due to the chemical response of carbonates to the addition of organic matter forming CO_2.

The $O_{2\ cum}$ 30d values were related (negatively) to the amorphous Mn, possibly due to a mechanism of protection of the organic matter by minerals, thus preventing mineralisation (Baldock and Skjemstad 2000; Kleber et al. 2005). The positive relationship between SOC and $O_{2\ cum}$ 30d can be explained by the increased availability of labile substrates in native SOC (Steenwerth et al. 2005). This is supported by the reduction of the contribution of SOC to the $O_{2\ cum}$ 30d once the SOC mineralised in the controls was subtracted (Table 4).

5. Conclusions

The mineralisation of sewage sludge (EOM) in soils can be described by respiratory parameters obtained after a short period from the application and offer complementary information concerning environmental problems. Considering that high microbial activity was observed within the first days of incubation (with maximum O_2 flushes within the first 2 days), care must be taken in sewage sludge applications, especially during the first days following the addition to soils.

It was observed that $O_{2\ max}$, T_{max} and RQ were not related to the physical and chemical properties of this pool of contrasting agricultural soils, and that the last two parameters, together with organic matter mineralisation rates after 30 days, were weakly affected by the sludge application. In terms of the potential contribution of the sewage sludge application to the SOC pool, and irrespective of the soil, a large proportion of the EOM added to the soil was mineralised in a short period of time. Despite this fact, considerable differences were still observed across soils. Low levels of soil pH, SOC, carbonates and high levels of amorphous Mn contributed to a lower mineralisation of EOM (as expressed by $O_{2\ cum}$ 30d). Thus, soils with these characteristics may be beneficial in terms of C conservation following sewage sludge application.

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SUPPLEMENTARY MATERIAL

Table. Supplementary material 1.
Physical and chemical properties[a] of the 70 agricultural soils

Sample	Location	Crop	pH	EC (μs cm^{-1})	CO_3^{2-} (%)	SOC (g kg^{-1})	SOC/N_k	Clay (%)	Sand (%)	AmFe (mg kg^{-1})	AmMn (mg kg^{-1})
1	Villena (Alicante)	Almond	8.6	138	24.1	5.3	10.0	36	48	277	164
2	Villena (Alicante)	Cereal	8.4	255	36.2	13.0	9.3	40	22	855	131
3	Villena (Alicante)	Grape	8.6	117	6.0	3.2	8.8	16	76	222	37
4	Villena (Alicante)	Almond	8.9	64	11.5	2.7	8.2	7	91	111	11
5	Almansa (Albacete)	Cereal	8.5	98	35.5	9.1	7.5	15	69	244	53
6	Villena (Alicante)	Cereal	8.6	102	44.2	7.6	9.1	21	51	174	30
7	Campo Real (Madrid)	Cereal	8.3	221	19.2	5.7	10.0	20	41	248	203
8	Valdilecha (Madrid)	Cereal	8.2	147	2.4	7.7	10.1	20	38	370	303
9	Valdilecha (Madrid)	Cereal	8.3	122	0.2	5.4	8.5	19	33	362	139
10	Daganzo de Arriba (Madrid)	Cereal	8.3	186	1.0	6.3	9.9	20	50	285	98
11	Ribatejada (Madrid)	Cereal	5.9	43	<0.1	4.0	9.3	18	72	203	40
12	Valdetorres del Jarama (Madrid)	Cereal	5.6	54	<0.1	3.8	9.8	18	54	276	46
13	Griñón (Madrid)	Cereal	5.5	59	<0.1	2.9	8.7	10	75	289	204
14	Griñón (Madrid)	Cereal	5.7	38	<0.1	2.2	8.5	6	85	200	125
15	Getafe (Madrid)	Cereal	8.1	221	41.5	14.4	9.7	19	41	131	17
16	Corral de Calatrava (C. Real)	Cereal	5.8	42	<0.1	6.5	11.8	17	61	610	335
17	Corral de Calatrava (C. Real)	Cereal	6.0	44	<0.1	7.0	12.7	13	59	612	331
18	Puertollano (C. Real)	Cereal	8.1	186	5.9	10.1	11.1	27	41	992	418
19	Yecla (Murcia)	Almond	8.8	130	19.1	3.7	10.2	14	79	180	24
20	Yecla (Murcia)	Almond	8.6	144	16.5	5.6	10.3	10	81	223	20
21	Alfaz del Pí (Alicante)	Medlar	7.7	2370	9.3	9.1	11.7	4	41	1024	77
22	Biar (Alicante)	Grape	8.5	236	51.8	14.9	11.4	26	25	278	33
23	Sax (Alicante)	Almond	8.7	159	20.1	6.8	12.2	16	73	172	17
24	Villena (Alicante)	Cereal	9.0	107	17.1	2.7	11.3	13	80	102	14
25	Villena (Alicante)	Grape	8.7	171	17.0	6.3	10.7	10	77	159	19
26	Villena (Alicante)	Cherry	9.1	143	18.0	4.4	14.1	12	82	152	13
27	Villena (Alicante)	Grape	9.1	127	13.4	3.9	5.7	9	83	153	16
28	Caudete (Albacete)	Cereal	8.5	278	35.8	7.4	9.6	17	60	288	34
29	Caudete (Albacete)	Cereal	8.7	153	40.4	9.0	8.6	20	55	302	38
30	Almansa (Albacete)	Cereal	8.7	176	78.7	9.9	13.5	18	59	71	7
31	Almansa (Albacete)	Cereal	8.4	225	59.7	18.6	11.3	13	65	105	15
32	Almansa (Albacete)	Cereal	8.5	228	24.9	20.0	13.9	18	47	292	85
33	Almansa (Albacete)	Cereal	8.6	195	29.0	17.1	12.0	16	56	369	63

34	Almansa (Albacete)	Cereal	8.3	258	22.9	18.0	10.8	11	73	308	44
35	Almansa (Albacete)	Cereal	8.7	138	47.7	8.5	10.9	13	71	260	36
36	Almansa (Albacete)	Grape	8.8	130	30.5	6.4	11.3	17	64	185	36
37	Villena (Alicante)	Cereal	8.8	267	54.1	8.7	10.5	35	28	145	23
38	Villena (Alicante)	Cereal	8.9	194	52.4	11.8	10.3	22	30	142	16
39	Villena (Alicante)	Cereal	8.8	161	30.5	13.6	13.2	14	66	195	22
40	Villena (Alicante)	Cereal	8.1	2335	16.0	5.2	12.3	<1	40	181	44
41	Yecla (Murcia)	Grape	8.6	378	21.4	8.5	10.3	24	52	369	134
42	Agres (Alicante)	Cereal	8.4	99	59.4	6.6	8.2	31	32	192	39
43	Agres (Alicante)	Maize	8.7	106	56.4	5.7	7.4	43	20	282	52
44	Agres (Alicante)	Tomato	8.7	111	63.8	3.0	5.0	45	17	107	33
45	Jumilla (Murcia)	Grape	9.0	108	46.4	3.0	7.5	53	25	98	69
46	Almansa (Albacete)	Cereal	8.5	110	14.1	12.0	9.8	19	50	385	156
47	Higueruela (Albacete)	Grape	8.3	156	37.3	6.5	9.0	20	52	235	57
48	Ayora (Valencia)	Cereal	8.3	150	44.8	16.5	11.3	14	69	123	37
49	Ayora (Valencia)	Cereal	8.5	99	54.4	11.0	10.3	15	63	174	25
50	Almansa (Albacete)	Cereal	7.9	584	45.5	10.1	9.3	25	42	302	58
51	Benejama (Alicante)	Olive	8.4	95	24.9	8.0	10.0	21	43	381	87
52	Bocairente (Valencia)	Cherry	8.3	97	55.9	5.8	8.6	40	21	176	29
53	Bañeres (Alicante)	Grape	8.4	78	1.0	5.8	8.7	18	34	514	186
54	Benifallim (Alicante)	Almond	8.5	99	67.0	11.7	9.3	31	29	242	21
55	Torre Manzanas (Alicante)	Almond	8.6	98	72.4	11.6	12.8	17	48	136	43
56	Pozuelos de Calatrava (Ciudad Real)	Cereal	5.0	43	<0.1	5.9	8.7	11	68	991	810
57	Villa del Prado (Madrid)	Grape	6.0	24	<0.1	4.2	10.7	14	65	638	240
58	San Martín de Valdeiglesias (Madrid)	Grape	6.2	18	<0.1	7.2	11.2	11	74	608	62
59	Talamanca del Jarama (Madrid)	Cereal	6.3	44	<0.1	4.8	8.6	20	46	340	123
60	Fuente el Saz de Jarama (Madrid)	Cereal	6.4	49	<0.1	3.0	7.5	14	62	573	182
61	Aranjuez (Madrid)	Cereal	7.7	1455	8.8	6.2	8.2	34	27	421	158
62	Torrejón de la Calzada (Madrid)	Cereal	7.7	106	<0.1	2.3	9.7	13	76	181	40
63	Griñón (Madrid)	Cereal	6.7	120	<0.1	3.9	9.3	27	61	284	26
64	Santa Olalla (Toledo)	Olive	5.6	70	<0.1	5.1	8.8	28	57	362	22
65	Santa Olalla (Toledo)	Olive	6.0	67	<0.1	3.4	8.6	9	76	230	79
66	Santa Olalla (Toledo)	Pistachio	6.3	62	<0.1	2.1	12.2	7	73	170	58
67	Santa Olalla (Toledo)	Cereal	6.7	100	<0.1	6.4	11.4	12	71	148	93
68	Santa Olalla (Toledo)	Cereal	8.2	120	<0.1	4.1	9.9	12	69	153	139
69	Cadalso de los Vidrios (Madrid)	Grape	5.9	66	<0.1	5.1	11.8	8	80	919	115
70	Escalona (Toledo)	Grape	7.1	84	<0.1	3.0	9.9	10	74	455	151

[a]Soil properties: EC, electrical conductivity; CO_3^{2-}, equivalent calcium carbonate; SOC, soil organic carbon; SOC/KjN, soil organic carbon/ Kjeldahl nitrogen; AmFe, amorphous Fe; AmMn, amorphous Mn.

Table. Supplementary material 2.
Selected respiratory parameters[a] for the sewage sludge amended and non-amended soils

	Amended soils				Non-amended soils			
Sample	$O_{2\,max}$ (µg $h^{-1}g^{-1}$)	T_{max} (h)	$O_{2\,cum}$ 30d (mg kg^{-1})	RQ 8d (mol CO_2 O_2^{-1})	$O_{2\,max}$ (µg $h^{-1}g^{-1}$)	T_{max} (h)	$O_{2\,cum}$ 30d (mg kg^{-1})	RQ 8d (mol CO_2 O_2^{-1})
1	48.6	11	10917	0.48	3.1	25	867	0.51
2	50.7	42	12477	0.47	1.7	13	675	0.43
3	47.1	16	9805	0.49	1.4	13	269	0.49
4	55.7	16	9228	0.50	2.8	13	585	0.52
5	56.4	11	10615	0.49	6.1	13	1099	0.51
6	44.5	22	11649	0.48	6.1	25	1126	0.52
7	49.5	11	9961	0.48	3.5	13	704	0.51
8	45.9	12	9931	0.48	4.7	25	886	0.51
9	49.2	33	11544	0.49	2.7	13	622	0.52
10	51.9	12	11140	0.49	6.5	26	1399	0.55
11	49.4	43	10174	0.52	2.6	14	594	0.51
12	61.2	38	10352	0.53	2.4	14	602	0.52
13	60.3	33	9465	0.52	2.5	14	595	0.47
14	86.7	23	8671	0.52	2.3	14	497	0.51
15	46.0	38	12065	0.47	7.4	7	1662	0.50
16	54.3	39	9152	0.52	4.0	14	908	0.56
17	49.1	39	9829	0.52	2.8	14	597	0.51
18	55.2	13	12627	0.49	9.1	14	2022	0.56
19	46.6	13	9976	0.49	1.5	39	406	0.46
20	53.7	13	9734	0.49	4.0	15	819	0.51
21	108.5	11	14620	0.53	6.2	18	1322	0.51
22	115.2	27	14278	0.51	7.0	19	1739	0.51
23	122.3	16	13474	0.53	4.4	19	556	0.47
24	132.7	16	12452	0.55	2.9	19	790	0.51
25	135.4	22	13425	0.53	3.7	19	743	0.48
26	126.6	17	12625	0.55	3.1	19	616	0.50
27	117.0	27	12296	0.55	2.5	19	437	0.48
28	124.1	22	13007	0.53	4.5	19	661	0.46
29	118.8	27	13622	0.53	7.0	19	1306	0.50
30	121.9	33	14126	0.54	6.8	19	1422	0.52
31	133.7	22	14759	0.52	11.4	20	2233	0.52
32	109.3	28	15279	0.51	9.0	20	2685	0.54
33	128.0	17	16722	0.52	18.5	20	4419	0.56
34	136.9	23	16023	0.51	18.9	20	5283	0.55
35	111.3	23	13060	0.54	5.0	20	895	0.48
36	140.9	28	13833	0.53	5.2	20	1079	0.49
37	110.9	13	13943	0.52	5.4	20	810	0.47
38	116.2	23	13646	0.52	9.1	20	1765	0.51
39	111.8	23	14019	0.53	9.0	21	1366	0.50
40	89.8	18	12151	0.54	1.8	21	492	0.49

41	119.5	26	13995	0.52	4.0	12	712	0.47
42	109.5	16	13171	0.52	6.5	25	1076	0.48
43	102.9	11	12907	0.52	4.6	25	1009	0.50
44	100.1	16	13544	0.52	9.6	13	1785	0.53
45	132.1	16	12655	0.52	2.3	13	428	0.39
46	119.2	32	13519	0.52	9.8	13	1967	0.52
47	137.9	27	13574	0.51	7.9	13	1468	0.54
48	131.0	22	16928	0.52	22.3	13	4929	0.61
49	144.7	27	13749	0.51	11.3	13	2250	0.53
50	113.4	33	13756	0.52	6.1	13	1297	0.49
51	107.5	28	13119	0.52	5.5	14	1212	0.48
52	93.9	12	14332	0.54	10.6	14	1975	0.55
53	114.3	28	13053	0.53	5.4	26	1079	0.49
54	99.9	12	14153	0.52	11.4	26	2279	0.53
55	116.1	18	14442	0.54	11.3	14	2772	0.55
56	149.9	28	11780	0.57	3.7	14	815	0.59
57	158.1	28	12032	0.54	4.3	14	882	0.51
58	147.1	23	12926	0.54	6.1	14	1261	0.54
59	143.2	28	12509	0.55	4.5	15	795	0.53
60	119.3	29	11837	0.56	4.2	15	718	0.55
61	84.6	10	10825	0.48	5.4	24	963	0.44
62	86.4	23	9247	0.51	4.7	12	812	0.48
63	96.1	23	10458	0.51	7.6	13	1221	0.54
64	74.9	33	10055	0.55	7.1	13	1386	0.56
65	112.5	23	9340	0.53	5.2	13	866	0.48
66	94.3	14	9348	0.53	2.8	13	504	0.47
67	118.0	20	10620	0.49	7.9	13	1319	0.49
68	93.4	24	10526	0.51	7.1	13	1139	0.52
69	93.4	27	10278	0.55	4.8	13	801	0.49
70	97.2	17	10348	0.53	6.1	13	1105	0.51

[a]Respiratory parameters: $O_{2\,max}$, maximum rates; $_{T\,max}$, time to reach the maximum rate; $O_{2\,cum}$ 30d, cumulative O_2 consumption after 30 days of incubation; RQ 8d, respiratory quotient after 8 days of incubation.