RESEARCH ARTICLE

Carbon mineralization in acidic soils amended with an organo-mineral bentonite waste

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Abstract

Bentonite based organic-mineral wastes contains high concentrations of organic matter and plant nutrients and hence presents a high potential as a soil amendment. However, it also can have high salinity and high copper concentrations that may cause negative effects on microorganisms when the soil is amended with this type of wastes. In this work, the effect of soil amendment with a bentonite based winery waste on soil carbon mineralization was studied in acidic vineyard soils as an indicator of soil quality. The carbon mineralization in the waste is significantly lower and slower than that in the studied vineyard soils despite its significantly higher amount of organic carbon. However, when the bentonite winery waste was added to the soils, the carbon mineralization showed positive priming effects (increased between 78 and 337%). Therefore, reductions in the carbon mineralization, and hence changes on short-term organic matter turnover are not expected after bentonite waste amendment in acid soils.

Keywords: Bentonite, waste, organic matter, mineralization, microorganisms, soil

1. Introduction

The use of bentonite in the food industry is very common worldwide, especially in the wineries and edible oil refineries, generating big amounts of wastes rich in clays and organic matter. However, these types of organo-mineral wastes were little studied yet (Loh *et al.*, 2017). These types of wastes generally have high amounts of organic matter and plant nutrients such as K, Ca or P. Therefore, they may be used as soil amendments to increase organic matter and nutrient contents in soils (Loh *et al.*, 2017). However, these wastes may have important drawbacks as amendments because there may be high concentrations of salts and heavy metals, especially those originated in the wineries (Fernández-Calviño *et al.*, 2015). Therefore, the uncontrolled application of these wastes to the soil may negatively affect soil micro-

bial communities and hence soil ecosystems because they play a key function in ecosystem processes such as decomposition, nutrient cycling and plant symbioses (Nannipieri *et al.*, 2003).

One indicator of soil quality from a microbiological point of view is the soil organic carbon mineralization (Thomsen *et al*., 2012), a parameter related to long-term soil fertility. Therefore, studies on organic matter amendments are common and important. Organic carbon mineralization has been studied in soils amended with different types of winery wastes (Bustamante *et al.*, 2007, 2010; Mosse *et al,* 2012). The effect of wastes and other organic matter sources application to the soil on organic carbon mineralization can be quite variable, and increases (Ginocchio *et al.*, 2013; Hasbullah and Marschner, 2016) and decreases (Meli *et al*., 2002) have been reported. The short-term increases or decreases in soil organic carbon mineralization caused by the organic matter amendments are defined as positive or negative priming effects (Kuzyakov *et al.*, 2000). These priming effects can affect the organic matter turnover in the soil, and therefore, the study of organic carbon mineralization in the soil from different perspectives, such as the effects of amendments on microbial communities and on organic matter stabilization, is still an interesting research topic.

Wine clarification produces a solid waste rich in bentonite (a clay added to the wine) mixed with high amounts of organic matter and plant nutrients such as phosphorus and potassium (Arias-Estévez *et al.*, 2007; Rodríguez-Salgado *et al*., 2014). These characteristics make bentonite wastes a promising soil amendment. However, this waste also contains high concentrations of copper, and its addition to soils in high doses can lead to an excessive increase in soil pH (Fernández-Calviño *et al*., 2015). Also, the addition of clays to the soil can reduce the C-mineralization in the soil (Pal and Marschner, 2016) Therefore, the application of this waste to the soil should be performed

carefully to avoid negative effects on soil microorganisms and hence detrimental effects on the surrounding environment. Despite its potential risk for soil ecosystems, the effect of soil amendment with neither bentonite winery wastes nor other organo-mineral wastes on organic carbon mineralization has not yet been analysed.

The aim of this work is the evaluation, for first time, of organic matter mineralization in the soil in response to bentonite winery waste additions. To this, five soils with different physicochemical characteristics were amended with two different concentrations of bentonite winery waste under laboratory conditions, and the carbon mineralization was monitored for 21 days. In addition, the carbon mineralization kinetics were modelled and possible priming effects assessed.

2. Materials and Methods

2.1. Soils and bentonite winery waste characteristics

The bentonite winery waste was supplied by Adegas Cunqueiro S.L. (Castrelo de Miño, Ourense, Spain). The bentonite used for wine clarification was a sodium montmorillonite, and after this process it became a solid waste rich in clay and organic matter. Once in the laboratory, the bentonite waste was air dried, sieved (1 mm) and stored in polypropylene jars prior to analysis or addition to the soils. For soil sampling, five vineyard plots were selected in a wine-growing area (Denomination of Origin O Ribeiro). In each vineyard, 20 soil sub-samples were randomly collected at a depth of 0–20 cm and subsequently mixed into a single composite soil sample which was air-dried, sieved through a 2-mm mesh and stored in polyethylene bottles. The proportions of sand (2-0.05 mm), silt (0.05-0.002 mm) and clay (<0.002 mm) of the soils were determined by wet sieving for the size fractions > 0.05 mm (sand) and using the international pipette

method for those < 0.05 mm (silt and clay). For soils and the bentonite waste, the relevant chemical characteristics were determined as follows: the pH in water and 0.1 M KCl were measured at a solid/liquid ratio of 1:2.5 using a glass electrode connected to a pH meter, whereas the electrical conductivity was measured using a Crison 524 conductivity meter in filtered extracts obtained from 1:10 solid/liquid suspensions. Total carbon (C) and total nitrogen (N) contents were

determined in finely milled samples using a Thermo Finnigan 1112 Series NC elemental analyser. The cation exchange capacity at soil pH (eCEC) was estimated as the sum of exchangeable base cations (K, Na, Ca, and Mg) extracted with 0.2 M NH_4Cl (Sumner and Miller, 1996) and exchangeable aluminium (Al) was extracted with 1 M KCl (Bertsch and Bloom, 1996). Table 1 summarizes the general characteristics of the soils and bentonite waste.

Table 1. General characteristics (mean±standard errors) of 5 soils and bentonite waste

		S1	S2	S3	S4	S5	BW
pH		5.0±0.2	5.0±0.1	5.0±0.1	6.2±0.3	5.9±0.2	6.0±0.1
pH$_{KCl}$		4.2±0.1	3.9±0.1	4.2±0.2	5.1±0.3	4.5±0.2	5.7±0.2
EC	dS m^{-1}	0.4±0.02	0.1±0.01	0.2±0.01	0.1±0.00	0.1±0.01	19.7±1.2
Sand	%	45±1	69±4	68±5	66±3	70±5	-
Silt	%	33±2	15±1	20±3	18±2	18±2	-
Clay	%	22±2	16±1	12±1	16±3	12±1	-
C	g·kg^{-1}	45.1±3.8	34.6±2.9	24.6±1.7	20.0±1.3	7.3±0.5	171.8±10.1
N	g·kg^{-1}	3.0±0.16	2.5±0.18	1.8±0.09	1.7±0.11	0.6±0.04	20.1±1.00
C/N		15±0.3	14±0.7	14±1.0	12±0.6	12±0.4	9±0.2
eCEC	cmol$_c$ kg^{-1}	9.4±0.3	3.6±0.2	4.8±0.1	9.8±0.2	5.5±0.3	170.5±3.1
Total Cu	mg kg^{-1}	52±15	214±20	198±17	168±21	152±7	1203±86

S: soil; BW: bentonite waste; pH: pH in water; pHKCl: pH in 0.1M KCl; EC: electrical conductivity; C: total carbon; N: total nitrogen; eCEC: effective cation exchange capacity

The selected soils were sandy loam or loam in texture, presented a narrow pH range (between 5.0 and 6.2), and a relatively high variation in total carbon content (7.3-45.1 g kg^{-1}). The electrical conductivity was low in all cases (non-saline), and the eCEC variable (3.6-9.8 cmol$_c$ kg^{-1}). The bentonite waste had a pH value (6.0) in the range found for soils. However, the total carbon (172 g kg^{-1}), eCEC (171 cmol$_c$ kg^{-1}) and salinity (strongly saline) were much higher in the bentonite waste than in the soils.

2.2. Experimental design

Three days before the experiment start, the soils and bentonite waste were rewetted until they were 80% saturated, which is long enough to recover the microbial community activity (Meisner *et al.,* 2013). The soils were then mixed with two bentonite waste doses: a low waste dose that increased the original total soil organic carbon by 5% and a high waste dose that increased the original total soil organic carbon by 10%,

where the term 'original' refers to the initial organic carbon content of the non-amended soils. These concentrations were selected because the resulted bentonite waste added to the soils was in the range of previous works where changes in the general soil characteristics in soils amended with bentonite wastes were analysed (Fernández-Calviño *et al*., 2015; Rodríguez-Salgado *et al*., 2017). These concentrations in the 5 studied soils cover a range where from no significant effects to significant effects on the general soil properties. Additionally, a control without bentonite waste was used for each soil. All mixtures were performed by quadruplicate and incubated in hermetic glass bottles under darkness at 28±2 ºC.

2.3. C mineralization measurement

To measure C mineralization, a glass with 10 mL of 0.5 M NaOH solution was placed inside the hermetic glass bottles with soil and soil-waste mixtures and renovated at 2, 4, 6, 8, 11, 14, 17, and 21 days. The NaOH extracts were then titrated against HCl to estimate CO_2 released from soil. The C mineralization data were expressed with respect to the unit of total soil carbon in order to minimize the confounding influence of soil total soil organic carbon on C mineralization, as has been suggested for the study of microbiological parameters (Li *et al.*, 2009).

2.4. C mineralization modelling

The special kinetic model (Bernal *et al*., 1998) was used to describe the C mineralization pattern from cumulative data of C mineralization. The special model (Equation 1) combines first-order with zero-order mineralization models because it identifies the decomposition rates of an active pool (fast C mineralization pool) and a large resistant pool (slow C mineralization pool).

$$C = C_1 (1-e^{-kt}) + C_2 k_2 t \qquad 1$$

where C is the cumulative carbon amount mineralized at time t (mg g^{-1} soil carbon), t is the time from the beginning of the incubation (days), C_1 and C_2 are fast and slowly mineralizing C (mg g^{-1} soil carbon), respectively, and k and k_2 are the fast and slow rate constants (day^{-1}).

3. Results

The C mineralization data (expressed as mg $C-CO_2$ per gram of C) for the bentonite waste, five vineyard soils and 10 soil-bentonite waste mixtures are shown in the Figure 1. The carbon mineralization in the bentonite waste after 21 incubation days was 0.35 mg of $C-CO_2$ per gram of organic carbon, which is significantly lower than the values for the non-amended soils (1.65-5.16 mg g^{-1}). However, when bentonite waste was added to the soils to increase the initial total soil organic C by 5%, the C mineralization increased between 68 and 179%, whereas when bentonite waste was added to the soils to increase its initial total content of organic C by 10%, the C mineralization increased between 78 and 337%.

Data from Figure 1 were fitted satisfactorily by the special model (Equation 1), explaining more than 99% of the experimental results in all cases (Table 2). The fast mineralizing C (C_1) was lower than slowly mineralizing C (C_2) in the bentonite waste, whereas in the soils the C_1 was higher than C_2 in all cases. When the bentonite waste was added to the soil C_1 increased in response to the waste dose in all cases, whereas no clear response was found for C_2. The fast rate constant (k) and slow rate constant (k_2) were quite similar in the bentonite waste and studied soils, and therefore when the waste was added to the soils no clear tendencies were found.
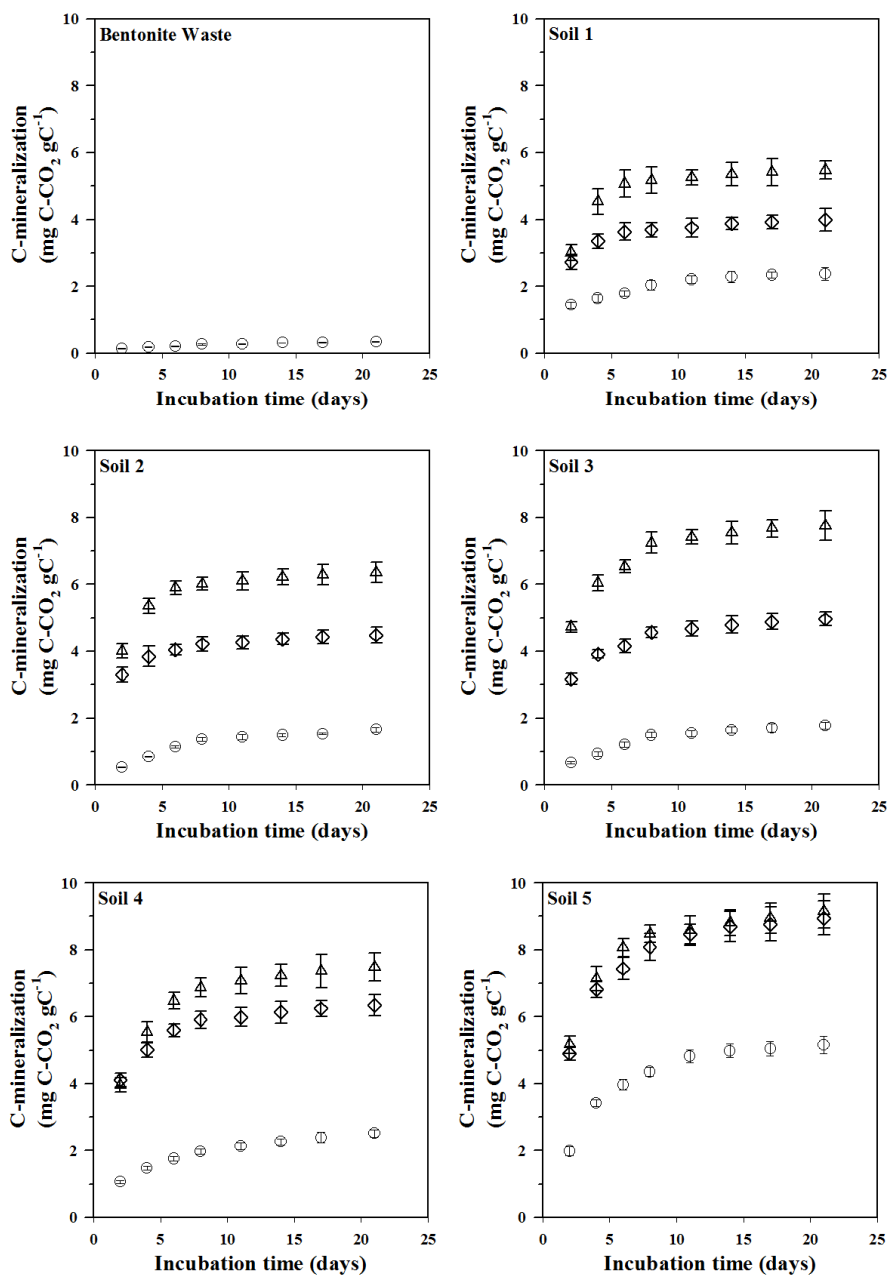
Figure 1. Cumulative C-CO$_2$ evolution in bentonite waste, soils and amended soils. Non-amended soils or bentonite waste (circles); soils amended with a bentonite waste dose exhibited an increase in the original total soil organic carbon content by 5% (diamonds) and by 10% (triangles). The error bars show the standard errors.

Table 2. Fitted parameters from the special kinetic model (C = C_1 $(1-e^{-kt})$ + C_2 k_2 t)

	C_1	C_2	k	k_2	R^2	$C_1/(C_1+C_2)$
BW	0.21±0.01	0.64±0.01	0.39±0.01	0.01±0.00	0.995	0.25
S1	1.86±0.04	1.19±0.08	0.57±0.02	0.02±0.00	0.991	0.61
S1+5%	3.58±0.04	1.04±0.05	0.68±0.02	0.02±0.00	0.999	0.77
S1+10%	5.29±0.08	0.69±0.02	0.45±0.01	0.01±0.00	0.999	0.88
S2	1.51±0.06	0.61±0.01	0.22±0.00	0.01±0.00	0.998	0.71
S2+5%	4.02±0.08	1.10±0.04	0.80±0.02	0.02±0.00	0.999	0.79
S2+10%	5.99±0.10	0.98±0.04	0.54±0.01	0.02±0.00	0.999	0.86
S3	1.63±0.03	0.63±0.03	0.22±0.01	0.01±0.00	0.997	0.72
S3+5%	4.28±0.07	1.35±0.09	0.60±0.03	0.02±0.00	0.998	0.76
S3+10%	6.97±0.06	1.46±0.10	0.51±0.02	0.02±0.00	0.998	0.83
S4	1.90±0.05	1.25±0.06	0.33±0.01	0.02±0.00	0.998	0.60
S4+5%	5.63±0.08	1.38±0.05	0.59±0.01	0.02±0.00	0.999	0.80
S4+10%	6.93±0.08	1.19±0.04	0.40±0.02	0.02±0.00	0.999	0.85
S5	4.65±0.02	1.29±0.07	0.29±0.01	0.02±0.00	0.999	0.78
S5+5%	7.96±0.11	1.65±0.07	0.45±0.02	0.03±0.00	0.999	0.83
S5+10%	8.28±0.15	1.53±0.08	0.47±0.03	0.03±0.00	0.999	0.84

S: soil; BW: bentonite waste; 5% (Soil-Bentonite waste mixtures for increase soil carbon in a 5%); 10% (Soil-Bentonite waste mixtures for increase soil carbon in a 10%)

C: cumulative carbon mineralized at time t (mg g^{-1} soil carbon); t: time from start of incubation (days); C_1 and C_2: fast and slowly mineralizing C (mg g^{-1} soil carbon); k and k_1: fast and slow rate constants (day^{-1}); R^2: coefficient of determination

4. Discussion

The C mineralization is influenced by different soil abiotic properties such as texture (Merino *et al.*, 2016), soil pH (Aciego Pietri and Brookes, 2008) or C/N ratio (Brady and Weil, 2008). However, the microbial properties such as size, activity or composition of the soil microbial biomass have low effect on the C-mineralization (Kemmitt, *et al*., 2008; Brookes *et al*., 2017). The bentonite based organo-mineral wastes may change these abiotic properties in the soil (Loh et al., 2017), but it can be opposite effect. Thus, the increased of clay content in the soil may decreased the C mineralization (Pal and Marschner, 2016), whereas the expected pH increases (Fernández-Calviño *et al*., 2015) may increase the C mineralization. The lower C/N ratio in the bentonite waste than in the soils may also contribute to C mineralization increases, but the presence of toxic substances such as high concentrations of Cu may decreased the soil respiration (Fernández-Calviño and Bååth, 2016). The C mineralization data (expressed as mg C-CO_2 per gram of C) was significantly lower in the bentonite waste than in the soils (Figure 1). Several mechanisms may be responsible of the less efficiency of carbon mineralization found in the bentonite waste with respect to the soils such as the presence of toxic substances such as Cu and salts (EC) in the waste. Fernández-Calviño and Bååth (2016) showed significant decreases of the soil respiration for total Cu concentrations higher than 250 mg kg^{-1}, whereas Rath and Rousk (2015), showed significant decreases in soil respiration for EC values over 1 dS m^{-1}. These two threshold values were clearly higher in the bentonite waste (Table 1). Also, the high presence of bentonite (clay) in the waste may cause additional C-mineralization decreases (Pal and Marschner, 2016).

When bentonite waste was added to the soils the C mineralization increased in proportions between 68 and 337% (Figure 1), suggesting a significant positive priming effect (Kuzyakov *et al.*, 2000); i.e., an increase in soil organic carbon mineralization in the amended soils relative to the carbon mineralization was observed in the soils and bentonite waste separately. In fact, the C mineralization in the soil-bentonite waste mixtures was between 75 and 370% higher than the weighted sum of the respiration observed for the soil and waste individually (Figure 2).
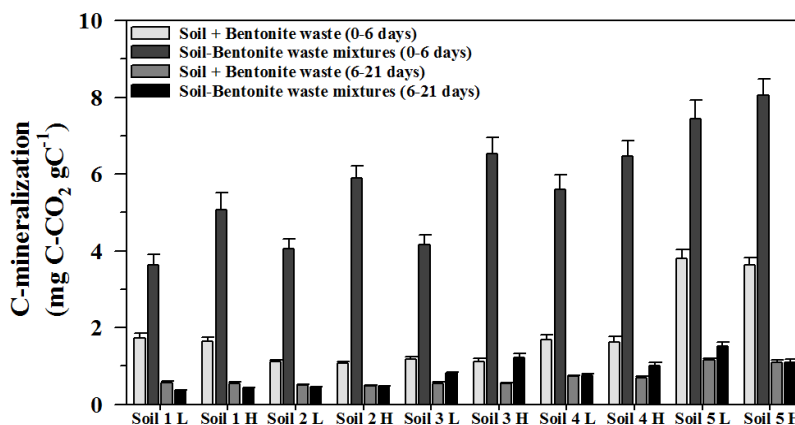
Figure 2. Comparison among C mineralization in the soil-bentonite waste mixtures with the sum of C mineralization in soils and bentonite waste separately for different periods: 0-6 days and 6-21. The codes on the x-axis are the numbers of each soil under study accompanied by the letters L or H representing the dose of bentonite waste added to increase the native total soil organic carbon content by 5% and 10%, respectively. The columns show the mean values and the error bars the standard errors.

The high positive priming effects found in the present work may be due to the presence of more bio-available organic matter in the bentonite waste than the native soil organic matter (lower C/N ratio; Table 1) because substrate availability is one of the most important factors controlling soil organic matter decomposition (Uchida *et al.*, 2012). However, as we showed previously, a lower C mineralization was found in the bentonite waste respect to the non-amended soils. This lower C mineralization may therefore be attributed to the presence of high concentrations of toxic substances in the bentonite waste for the microbial communities.

The salinity and available Cu concentrations in this type of waste are high (Table 1), and therefore, they can inhibit microbial activity in the waste. However, when the bentonite waste was mixed with an acidic soil, the salinity in the soil-bentonite waste mixtures decreased (Fernández-Calviño *et al.*, 2015), and the Cu availability decreased until it reached acceptable values (Arias-Estévez *et al.*, 2007). Also, the addition of bentonite waste to acid soils generally increase the pH (Fernández-Calviño *et al.*, 2015; Rodríguez-Salgado *et al.*, 2017), and therefore it can favour the C-mineralization (Aciego Pietri and Brookes, 2008). Therefore, the addition of bentonite waste to acidic

soils (to increase the soil organic carbon between 5 and 10%) has no negative effects on C mineralization after 21 incubation days due to the dilution of toxic substances in the soil or adsorption processes of these substances (Fernández-Calviño *et al*., 2015). This result was in accordance with a previous work where an enzymatic activity related with the C-cycle was no negatively affected by the addition of a similar bentonite waste (Rodríguez-Salgado *et al*., 2017). Thus, negative effects on short-term organic matter turnover will be not expected after soil amendment with bentonite wastes, despite negative effects on other biological properties were observed previously in an acidic soil, such as for phosphomonoesterase enzymatic activity (Rodríguez-Salgado *et al*., 2017). The cumulative quantities of C-CO$_2$ (Figure 1) were fitted satisfactorily by the special model (Equation 1), explaining more than 99% of the experimental results in all cases (Table 2). This model previously offered the most appropriate description of the cumulative C-CO$_2$ evolution data in soils (Bernal *et al*., 1998; Saviozzi *et al*., 2014). The special model is a combination of two different kinetic equations, which suggest that the organic carbon content of the soils consisted of two fractions with different degrees of biodegradability (Bernal *et al*., 1998). Table 2 shows the special model fitted parameters for bentonite waste, soils and soil-bentonite waste mixtures. The proportion of fast mineralized carbon was 25% in the bentonite waste, whereas in the non-amended soils, it ranged from 60 to 78%. These results showed that the carbon mineralization in the bentonite waste was dominated by the slow process, whereas in the non-amended soils, the fast C mineralization processes are prevalent. However, when the bentonite waste was added to the soil, the proportion of C mineralized via fast processes was even higher than that in the non-amended soils. The initial and quick C mineralization in amended soils with

organic wastes was previously observed (Bustamante *et al.*, 2007) and attributed to the amount of C present initially in soluble form, the labile C fraction (Trinsoutrot *et al*., 2000; Bernal *et al*., 1998). However, in the non-amended soils, we also observed a fast C mineralization. Another possibility is that the high C mineralization in soils and in bentonite waste amended soils may be associated to the formation of microbial hotspots promoting hot moments, i.e., short-term events inducing accelerated process rates compared to the average rates (Kuzyakov and Blagodatskaya, 2015).

In the non-amended soils, these "events" may be the rewetting process which can activate dormant microorganisms (Thomsen *et al.*, 1999), whereas in amended soils the rewetting effect may be reinforced by a high input of labile carbon in the soil supply by the bentonite waste, an important event that favours the hotspots activity and thus drives the hot moments (Kuzyakov and Blagodatskaya, 2015). However, in the bentonite waste the presence of hotspots driving hot moments may be limited by the negative effect of high concentrations of salts and copper (Table 1) on microbial communities. Therefore, the C mineralization in the bentonite waste is produced mainly via slow processes. After the initial increase of carbon mineralization, the evolution of the specific respiration tended to be similar to that of the control soils (Bustamante *el al.*, 2007), as in the present work, where the priming effects were observed mainly during the first 6 incubation days, whereas for longer incubation times, they were almost negligible (Figure 2). These results seem to be in accordance with the formation of microbial hotspots that are active during short-term hot moments and clearly indicated that the addition of bentonite can have important short-term implications in the C cycle of the vineyard soils.

5. Conclusions

The addition of a bentonite winery waste, rich in organic matter, nutrients, salinity and copper, to acid vineyard soils at amounts equivalent up to 10% of the native C, did not induced decreases on C-mineralization after 21 incubation days. Therefore negative effects on short-term organic matter turnover will be not expected after the addition of bentonite based winery wastes in acid soils.

Acknowledgements

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