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SHORT COMMUNICATION

Latitude-dependent underestimation of microbial extracellular enzyme activity in soils

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Abstract Decomposition of soil organic matter by microorganisms is a major process governing the carbon balance between soil and atmosphere which needs to be fully understood. Extracellular enzyme activity is often the limiting factor for microbial utilization of soil organic matter. Contrary to expectations, we observed that enzymatic activity rises at increasing temperatures in soils and sediments. Current climatic change will induce the increase of global mean temperatures, frequency of extreme heat events and soil temperatures during the next decades. The relevance of the increase in activity at high temperature is dependent on latitude. At latitudes around and below 40° a significant number of days per year present high temperatures. Results suggest that the hydrolytic activity of microbial extracellular enzymes is currently underestimated mainly at medium and low latitudes where soil temperatures frequently reach high values (often above 40 °C). This report contributes to understand (1) the hydrolysis of soil organic matter within a latitude-dependent scenario of global warming and (2) the role of microorganisms in processing soil organic matter and their influence in carbon cycling.

Keywords Extracellular enzyme activity · Global warming · Extreme heat events · Microbial activity · Latitude · Soil · High temperature

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Introduction

Carbon stock is much larger in soil than in the atmosphere (Conant et al. 2011; Davidson and Janssens 2006; Smith et al. 2008). The accumulation and utilization of organic matter in soils rules the carbon balance between terrestrial environments and atmosphere. Inputs from plant-derived carbon and outputs by decomposition and mineralization result in feedback mechanisms having major impacts on Earth's climate (Conant et al. 2011; Davidson and Janssens 2006; Smith et al. 2008). Factors inducing changes to that equilibrium need to be understood for accurate predictive modeling and simulations (Davidson and Janssens 2006).

Organic carbon decomposition is mainly carried out by microorganisms (Conant et al. 2011; Whitman et al. 1998). Soils contain extremely diverse and abundant microbial communities (Curtis et al. 2002; Gonzalez et al. 2012). The production of extracellular hydrolytic enzymes by microorganisms is a primary mechanism for the decomposition of soil organic matter. These enzymes break down complex organic molecules releasing smaller molecules into the environment which can be assimilated and mineralized (Asmar et al. 1994; Madigan et al. 2003). The availability of different organic substrates regulates the production of these hydrolytic enzymes (Velasco-Ayuso et al. 2011). The activity of extracellular enzymes represents the rate-limiting step in processing soil organic matter and constitutes a direct indicator of microbial production (Asmar et al. 1994; Chróst 1992; Conant et al. 2011; Velasco-Ayuso et al. 2011). Extracellular enzyme activity is commonly used to assess the functional capacity of microbial communities (Allison and Treseder 2008).

Microbial extracellular enzymatic activity in soils is currently determined at or below 30 °C (Bradford et al.

2010; Conant et al. 2011; Craine et al. 2010; Fierer et al. 2006). Generally, enzymatic activity shows maxima at a temperature slightly higher than the optimum for growth of a microorganism (Burton et al. 2002; Kube et al. 2013; Madigan et al. 2003; Tang et al. 2010). However, enzymatic activity sharply decreases at increasing temperatures beyond its optimum (Ise and Moorcroft 2006; Madigan et al. 2003). As a consequence, increasing temperatures above the experimental threshold for mesophiles (i.e., temperatures for optimum microbial activity and growth) should reflect highly diminished microbial enzymatic activities and consequently a decrease of the estimates for the expected decomposition of organic matter in soils (Conant et al. 2011; Davidson and Janssens 2006).

A few studies have pointed out the presence (Marchant et al. 2002, 2008) and potential role (Portillo et al. 2012; Santana et al. 2013) of thermophilic microorganisms in soils. This evidence suggests that soil thermophiles could show relevance decomposing organic C under periods of elevated temperature in nature. Further research on this point is required to fully realize the potential of microbial processes on C cycling under global increasing temperature scenarios.

The prediction of global processes and the consequences of climate change require a good understanding on the role of microorganisms and their activities (Conant et al. 2011; Craine et al. 2010; Davidson and Janssens 2006). Among other causes, temperature is a major factor influencing the microorganisms and microbial activity leading to the decomposition of soil organic matter (Craine et al. 2010; Parmesan and Yohe 2003). Although trends in mean temperature conditions are clearly important in understanding the effects of global warming, accumulating facts suggest that changes in the frequency distribution of extreme temperature events may also have far-reaching implications (IPCC 2007; Jentsch et al. 2007). Current evidence indicates that the frequency of hot temperatures in soils (e.g., above 40 °C) is expected to increase along the next decades.

The aim of this study was to comparatively evaluate microbial extracellular enzyme activity over a wide range of temperature conditions focusing on the effect of high temperatures. The occurrence of high temperatures at different latitudes is also analyzed to assess the potential relevance of the findings. This report contributes to a better understanding of the influence of temperature on extracellular enzyme activity by microorganisms in terrestrial environments and the temperature-dependent functionality of soil microbial communities. This study was performed during 2010–2011 at the Institute of Natural Resources and Agrobiology of Seville, Spanish Council for Research, in Seville, Spain.

Materials and methods

Sampling

Samples were collected from different environments under sterile conditions. Sampling site A corresponds to a clay soil in Southwestern Spain (Seville; location 37°21.02′N 5°59.34′W, altitude 5 m) with soil temperatures ranging from 10 °C to over 60 °C. Sandy sediments were from a wetland at Doñana National Park (DNP; Southwestern Spain). These samples were collected from the edge of three freshwater ponds: "La Dulce" (Sampling site B) (location 36°58.84′N 6°29.23′W, altitude 3 m), "Santa Olalla" (Sampling site C) (location 36°58.84′N 6°28.91′W, altitude 5 m) and "Zahillo" (Sampling site D) (location 36°59.21′N 6°30.43′W, altitude 2 m). Sandy soil (Sample site E) from DNP around "Santa Olalla" Pond was also collected. Samples from a meadow in Cambridge (United Kingdom) (Sampling site F) (location 52°11.92′N 0°7.07′E, altitude 37 m) were collected. Triplicate samples were collected from the surface except when noticed otherwise. Including in this study, different types of soils and sediments contributes to determine whether the observed results of extracellular enzymatic activity over a wide range of temperatures corresponds to a general pattern. See supplementary material for a more detailed description of the methods.

Enzymatic assays

In situ protease and glucosidase activities in natural samples were determined using fluorogenic substrates (EnzCheck green fluorescence protease assay kit, E6638; and fluorescein diglucoside, F2881; Invitrogen, Carlsbag, CA, USA) without additional treatments. Samples were suspended in phosphate buffer (pH 7.0). All samples were processed in triplicate and controls without sample, controls without fluorogenic substrate and autoclaved controls were carried out. Reactions (50 µl) were prepared in 96-well microplates with 1–2 mg sample per well. A final substrate concentration of 0.1 mM was used in this study (Marx et al. 2001). Reactions were incubated in an optical thermocycler (iQ iCycler, Bio-Rad, Hercules, California, USA) and final activity was expressed as the slope of the linear increase of fluorescence produced during the reaction time per dry weight at each temperature. Temperatures were assayed at 5 °C intervals from 5 to 95 °C. The proteolytic substrate was stable within this range and the glucoside substrate showed certain instability. Other fluorogenic substrates targeting different enzymatic activities, such as phosphatases and esterases, were tested but they were unstable at elevated temperatures. ANOVA tests were

used to determine significant differences among samples (Sokal and Rolhf 1995).

Bacterial cultures were used to evaluate enzymatic activity from known bacteria (mesophile and thermophile). *Escherichia coli* K12 was a mesophilic microorganism and two soil bacterial isolates, *Brevibacillus* sp. strain 10 (CECT7629) and *Ureibacillus* sp. strain 12 (CECT7628) (Portillo et al. 2012) were chosen as thermophilic bacteria. Optimum growth temperatures were 55 and 60 °C for strains 10 and 12, respectively. Cultures were grown in nutrient broth composed by beef extract (3 g l^{-1}), peptone (5 g l^{-1}) and sodium chloride (5 g l^{-1}).

Temperature sensitivity

Temperature sensitivity was studied using the parameter Q_{10} which was estimated according to Stone et al. (2012) as $Q_{10} = \exp(\text{slope} \times 10)$. The slopes represent the change of activity for each sample at the temperature range being considered (Stone et al. 2012). Q_{10} constitutes the change of activity resulting from a temperature variation of 10 °C and it is generally expected to obtain results of Q_{10} around 2 in biological systems (Davidson and Janssens 2006; Madigan et al. 2003). Q_{10} is probably the most frequently used parameter to evaluate the effect of temperature on biological processes and it is widely used in global modeling efforts (Conant et al. 2011; Davidson and Janssens 2006; Stone et al. 2012).

Temperature record

Temperature records were obtained at the National Oceanic and Atmospheric Administration (Washington DC, USA; http://www7.ncdc.noaa.gov/CDO/cdoselect.cmd). The number of days with a maximum temperature of 30 °C or above was counted for each year at a number of locations covering the range of latitude from 70°N to 70°S. According to our measurements, air temperatures of 30 °C and above resulted in soil temperatures above 40 °C.

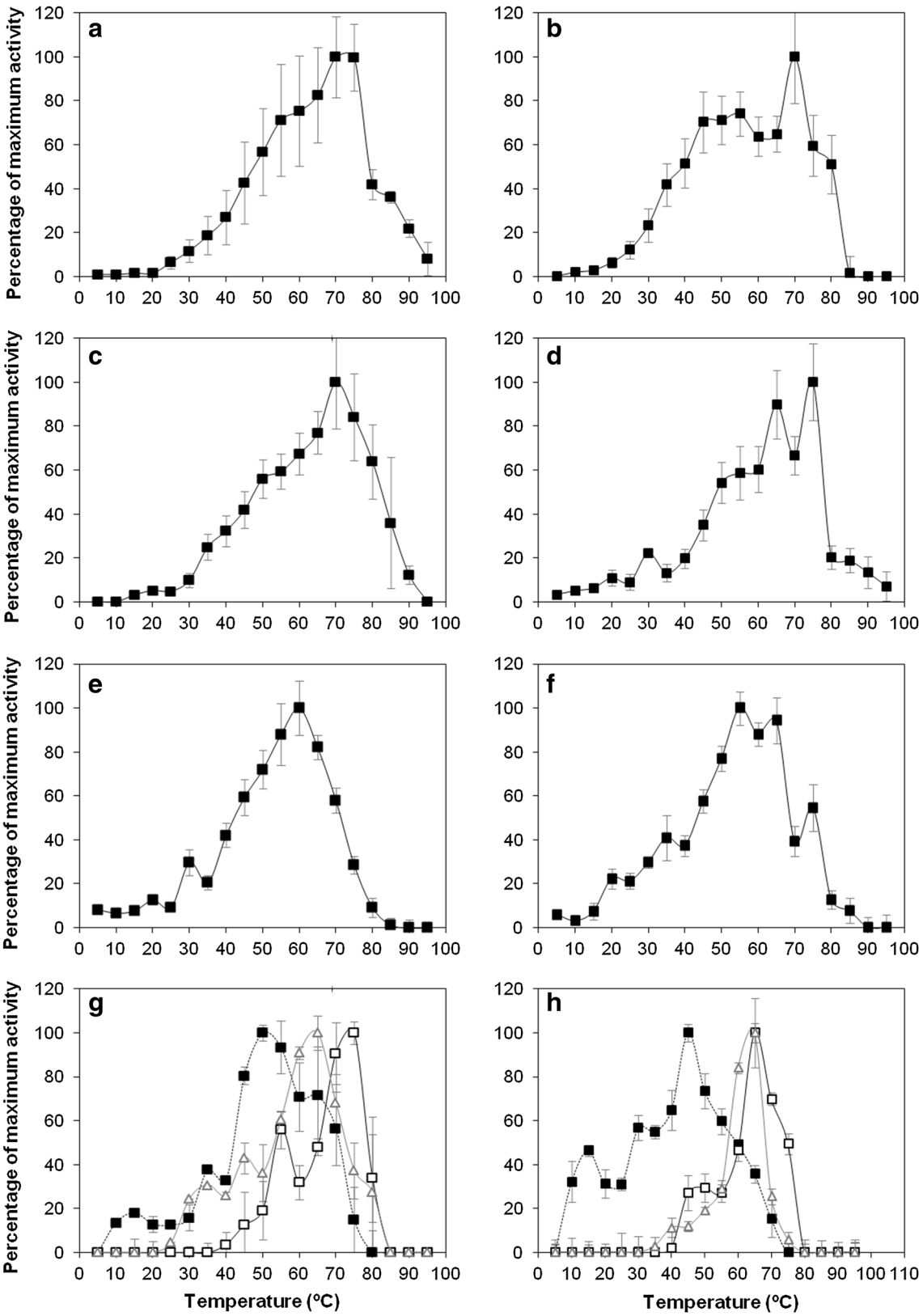
Results and discussion

Soils are among the systems with highest microbial diversity. Microbial communities in soils are highly complex and a large number of different microorganisms thrive in these environments adapting to this highly heterogeneous habitat (Curtis et al. 2002; Torsvik et al. 2002). Decomposition of soil organic matter by microorganisms is a major process governing C stocks (Davidson and Janssens 2006). Climate change expectancies suggest increasing global temperatures. Even more importantly, an increase in the frequency and intensity of extreme heat events has also been predicted (IPCC 2007). However, the effect of temperature on microbial activity remains to be fully understood (Conant et al. 2011). Extracellular hydrolysis of polymers is generally limiting the rate of organic matter decomposition by soil microorganisms (Allison and Treseder 2008; Conant et al. 2011; Velasco-Ayuso et al. 2011). In this study, the potential relevance of high temperature on extracellular enzyme activity by soil microorganisms is analyzed.

In natural samples collected at different temperatures and from different locations, protease activity maxima were detected from 45 to 75 °C and the maxima of glucosidase activity were detected around 70 °C (Fig. 1a–f). Above these threshold temperatures, enzymatic activity started to decline. In natural samples, distinct microorganisms contributed differentially to the activity detected at each temperature (Bradford et al. 2010; Conant et al. 2011). Protease and glucosidase activities from *E. coli* showed peaks of maximum activity at 45 and 50 °C, respectively (Fig. 1g, h) which corresponded to the expected results according to previous reports (Burton et al. 2002; Kube et al. 2013; Madigan et al. 2003; Tang et al. 2010). Thermophilic isolates showed peaks of maximum glucosidase activity at 75 and 60 °C for strains 10 and 12, respectively (Fig. 1g). Maximum protease activity was detected at 65 °C for thermophilic strains 10 and 12 (Fig. 1h).

Temperature sensitivity using Q_{10} were in the range 1.28–2.23 (Table 1) for the proteolytic activity in natural samples (mean 1.67; SD 0.28) over a range of 60 °C. Glucosidase activity in natural samples showed Q_{10} values around 2.1. In monospecific cultures, protease and glucosidase activities were in the ranges 1.58–2.68 and 1.56–2.31, respectively, which included mesophilic (i.e., *E. coli*) and thermophilic microorganisms (Table 1).

Figure 2 shows the average number of days reaching air temperatures equal to or above 30 °C for a variety of locations including the range of latitudes from 70°N to 70°S. The observed Gaussian distribution indicates that locations within the 40°N–40°S range experience a significant number of days with high temperature. At low latitudes, below 20°, over 200 high temperature days per year have been recorded. For example, an average of more than 100 days per year reached 30 °C or more at Seville (37°N) which represents a conservative site at the limit of latitude within the 40°N to 40°S range. Medium to low latitude zones are prone to hot summers and a predicted increase of extreme heat events (IPCC 2007; Jentsch et al. 2007) will result in frequent soil temperatures well above 40 °C. At medium and low latitudes, areas with poor, or partial, vegetation can reach over 60 °C at soil surface and well over 40 °C a few centimeters below ground (Portillo


a
b
c
d
e
f
g
h
Percentage of maximum activity
Temperature (°C)

◀**Fig. 1** Glucosidase (**a**, **b**, **g**) and protease (**c**–**f**, **h**) activities from natural samples (**a**–**f**) and cultures (**g**, **h**) at a wide spectrum of temperatures (from 5 to 95 °C). Natural samples correspond to: **a** soil sample at an outlying area of Seville, sampling site A collected at 28.0 °C, **b** sampling site A collected at 28.5 °C, **c** sediments from DNP, "Santa Olalla" Pond, sampling site C, collected from the surface at 23.9 °C, **d** sediments from DNP, "La Dulce" Pond, sampling site B, collected from the surface at 16.3 °C, **e** soil from DNP, sampling site E, collected from the surface at 12.6 °C, **f** soil from Cambridge, sampling site F, collected at 2 °C. Glucosidase (**g**) and protease (**h**) activities in cultures of *E. coli* (*filled squares, dashed line*), *Brevibacillus* sp. strain 10 (*open squares, continuous line*) and *Ureibacillus* sp. strain 12 (*open triangles, gray line*). *Bars* indicate SD

et al. 2012). Desserts can easily get above 90 °C (McCalley and Sparks 2009). High latitudes (above 40°N and S) showed practically no days at 30 °C (Fig. 2). High latitudes (above 40°N and S) present a cold climate and soil temperatures only sporadically reach those elevated temperatures (Marchant et al. 2002, 2008). These results confirm a clear latitude dependence on the potential relevance of microbial extracellular enzyme activity.

Herein, the detection of hydrolytic activities in the environment has been carried out over a wide range of temperatures (5–95 °C) unlike previous studies that determined hydrolytic activities at mesophilic temperatures,

Table 1 Temperature of maximum enzymatic activity, Q_{10}, and in situ temperature for the samples and cultures analyzed during this study

Sample/culture	Enzymatic activity	Temperature of maximum activity (°C)	Q_{10}	In situ temperature (°C)
Natural samples				
Sampling site A	Protease	75	2.23	10.1
Sampling site A	Protease	65	1.62	25.1
Sampling site A	Protease	65	1.79	11.8
Sampling site A. 6:00[a]	Protease	50	1.62	21.6
Sampling site A. 11:00[a]	Protease	55	1.33	44.4
Sampling site A. 16:00[a]	Protease	55	1.41	60.0
Sampling site A. 20:00[a]	Protease	55	1.35	39.4
Sampling site A. 24:00[a]	Protease	55	1.57	27.0
Sampling site A. 10 cm[b] 6:00[a]	Protease	50	1.72	30.8
Sampling site A. 10 cm[b] 11:00[a]	Protease	50	1.45	29.7
Sampling site A. 10 cm[b] 16:00[a]	Protease	55	1.58	34.4
Sampling site A. 10 cm[b] 20:00[a]	Protease	55	1.57	40.4
Sampling site A. 10 cm[b] 24:00[a]	Protease	50	1.63	36.3
Sampling site B.	Protease	75	1.73	16.3
Sampling site B. 5 cm[b]	Protease	65	1.73	13.4
Sampling site C.	Protease	70	1.91	23.9
Sampling site C. 5 cm[b]	Protease	70	1.48	18.6
Sampling site D.	Protease	60	1.28	20.4
Sampling site D. 5 cm[b]	Protease	50	1.42	16.7
Sampling site E.	Protease	60	1.45	12.6
Sampling site F.	Protease	45	1.99	2.0
Sampling site F.	Protease	45	2.23	0.0
Sampling site F.	Protease	55	2.22	0.0
Sampling site A.	Glucosidase	70	2.18	28.0
Sampling site A.	Glucosidase	70	2.10	28.5
Cultures				
E. coli	Protease	45	1.58	30.0[c]
Brevibacillus strain 10	Protease	65	2.68	50.0[c]
Ureibacillus strain 12	Protease	65	2.68	50.0[c]
E. coli	Glucosidase	50	1.89	30.0[c]
Brevibacillus strain 10	Glucosidase	75	2.31	50.0[c]
Ureibacillus strain 12	Glucosidase	60	1.56	50.0[c]

[a] Samples collected at a different time during a day

[b] Centimeters below soil surface

[c] Incubation temperature

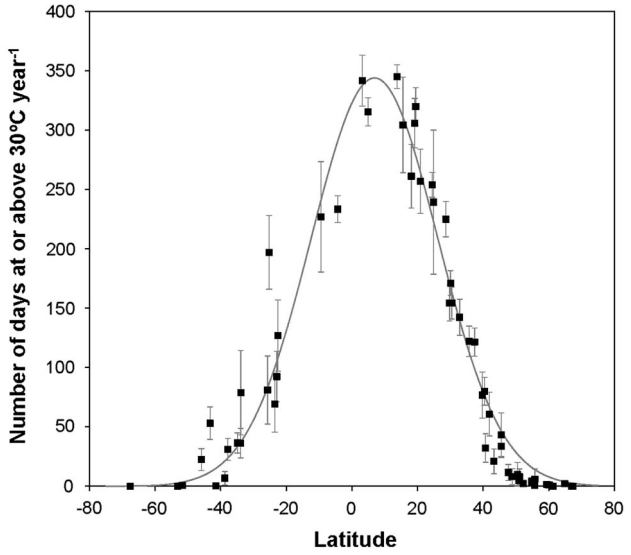


Fig. 2 Average of days reaching 30 °C and above per year as a function of latitude from S (*negative values*) to N (*positive values*). The Gaussian curve corresponds to the best fitting for the presented data set. *Bars* indicate SD

generally below 30 °C (Craine et al. 2010; Fierer et al. 2006; Ise and Moorcroft 2006; Townsend et al. 1992). Enzymatic activities in soil and sediment samples measured during the present study showed maximum rates at temperatures above 50 °C. This is in agreement to early studies in soils reporting maximum enzymatic activity around 50 °C (Ladd and Butler 1972). A first consequence of these results is that high temperatures will lead to increased estimates of microbial extracellular enzymatic activity (i.e., depolymerisation of macromolecules). An exponential increase of microbial respiration versus temperature has been reported although high temperature values were previously considered above any real or predicted climate (Townsend et al. 1992). The presence in soils of active thermophilic bacteria (Marchant et al. 2002, 2008; Portillo et al. 2012) adds on to the finding of significant soil microbial activities during high temperature conditions. The hydrolysis of polymers and microbial activity at elevated temperatures show potential ecological and global relevance.

Results have shown an increase in the proteolytic and glucolytic activities under elevated temperatures. During this study, the activity estimated at 50–70 °C represented 4–8-fold the activity determined at 20–30 °C, with an average around sixfold which corresponds to average Q_{10} values around 2 which were observed in these experiments. Considering that (1) at latitudes below 40°N and 40°S high temperatures are reached numerous days annually (Fig. 2) and (2) at these latitudes about half a day can show soil surface temperatures at or above 40 °C (Portillo et al. 2012), one could deduce the occurrence of higher microbial extracellular enzymatic activity at high temperatures than predicted from current estimates (Bradford et al. 2010; Conant et al. 2011; Craine et al. 2010; Gruber et al. 2004). Latitudes below 40°, from N to S, represent over 64 % of Earth surface with corresponds to above half the land surface of our planet. These consequences of microbial extracellular enzymatic activity in soils and sediments can reach significant levels on a global scale, and they should be considered in future modeling and predictions at local and global scales. With those considerations, conservative estimates suggest that hydrolytic enzymatic activities at medium and low latitudes under elevated temperatures could represent a doubling of previous estimates on depolymerisation of macromolecules by microorganisms at these locations and above an additional 50 % of current global estimates. Results indicate that the effect of high temperature conditions on microbial activity processing soil organic matter should be considered in future analyses and predictions on the role of microorganisms in soils.

Microbial enzymatic activity to depolymerise soil organic matter can be influenced by numerous factors (Conant et al. 2011; Davidson and Janssens 2006; Smith et al. 2008). Temperature is an important factor affecting microbial activity in soils and shows relationships with many biological, physical and chemical processes (Conant et al. 2011; Davidson and Janssens 2006). Some research have focused on the quality or degradability of organic matter in soils and the effects of climate change on its decomposition (Fierer et al. 2005; Smith et al. 2008; Wetterstedt et al. 2010). The temperature effects on availability and adsorption onto particles of soil organic matter is scarcely understood (Davidson and Janssens 2006). Recent debate has concluded that an increase in temperature should increase the rate of substrate desorption relative to adsorption of organic matter to mineral particles, meaning that substrate availability (i.e., the non-sorbed fraction) and solubility should be higher at warmer temperatures (Conant et al. 2011; Kalbitz et al. 2000). Besides, increasing water losses with temperature indicates a potential concentration of solutes while maintaining sufficient soil water moisture (Lee et al. 2008). These aspects would confer certain advantage to microbial depolymerisation under elevated temperatures with respect to moderate temperatures. Thus, climate warming, which is expected to result in more frequent extreme heat events and warmer summer seasons, should result in an increasing trend toward facilitating microbial extracellular enzymatic activity in soils.

Conclusion

In this study, microbial extracellular enzyme activity at elevated temperature has been shown to be of potential

importance on the processing of organic matter in soils, above all, when considering global warming. The relevance of thermophilic processes could gain interest due to an expected increase in length and frequency of extreme heat events predicted for the next decades. This study identifies a novel mechanism and scenario within the multiple processes and environmental constraints that govern the microbial activity and depolymerisation of organic matter in a future where climatic disruptions are expected to become increasingly pronounced as a consequence of global warming.

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