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Catalytic power of enzymes decreases with temperature: new insights for understanding soil C cycling and microbial ecology under warming.

Running head: Temperature response of enzymatic reactions

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Abstract

Most current models of soil C dynamics predict that climate warming will accelerate soil C mineralization, resulting in a long-term CO₂ release and positive feedback to global warming. However, ecosystem warming experiments show that CO₂ loss from warmed soils declines to control levels within a few years. Here, we explore the temperature-dependence of enzymatic conversion of polymerised soil organic C (SOC) into assimilable compounds, which is presumed the rate-limiting step of SOC mineralization. Combining literature review, modelling and enzyme assays, we studied the effect of temperature on activity of enzymes considering their thermal inactivation and catalytic activity. We defined the catalytic power of enzymes (E_{power}) as the cumulative amount of degraded substrate by one unit of enzyme until its complete inactivation. We show a universal pattern of enzyme's thermodynamic properties: activation energy of catalytic activity (EA_{cat}) < activation energy of thermal inactivation (EA_{inact}). By investing in stable enzymes (high EA_{inact}) having high catalytic activity (low EA_{cat}) microorganisms may maximise the E_{power} of their enzymes. The counterpart of such EAs' hierarchical pattern is the higher relative temperature sensitivity of enzyme inactivation than catalysis, resulting in a reduction of E_{power} under warming. Our findings could explain the decrease with temperature in soil enzyme pools, microbial biomass (MB) and carbon use efficiency (CUE) reported in some warming experiments and studies monitoring the seasonal variation of soil enzymes. They also suggest that a decrease of soil enzyme pools due to their faster inactivation under warming contributes to the observed attenuation of warming effect on soil C mineralization. This testable theory predicts that the ultimate response of

SOC degradation to warming can be positive or negative depending on the relative temperature response of E_{power} and microbial production of enzymes.

Introduction

Current models predict an accelerated mineralization of soil organic carbon (SOC) under warming, resulting in a long-term CO₂ release and positive carbon-climate feedback (Cox *et al.*, 2000; Knorr *et al.*, 2005a; Davidson & Janssens, 2006). However, studies along global temperature gradients suggest that degradation rates of slow-decaying SOC, which constitutes the largest SOC pool, does not vary with temperature (Trumbore *et al.*, 1996; Liski *et al.*, 1999; Giardina & Ryan, 2000). Moreover, ecosystem warming experiments show that the initial stimulation of soil respiration in response to warming disappears within a few years (Luo *et al.*, 2001; Rustad, 2001; Melillo *et al.*, 2002; Eliasson *et al.*, 2005) suggesting an acclimation of SOC mineralization to higher temperatures. An apparent acclimated response of SOC mineralization does not necessarily mean a physiological acclimation of soil microorganisms. These findings led to debates on the temperature sensitivity of SOC pools, methodological limitations to capturing the temperature response of slow-decaying SOC and on the processes leading to a disappearance of warming effects with time (Eliasson *et al.*, 2005; Fang *et al.*, 2005; Knorr *et al.*, 2005a; Reichstein *et al.*, 2005a; Zhang *et al.*, 2005; Davidson & Janssens, 2006; Bradford *et al.*, 2008; Hartley *et al.*, 2008; Allison *et al.*, 2010; Zhou *et al.*, 2012; Tucker *et al.*, 2013; Crowther *et al.*, 2016). These processes include reduction of soil substrate availability, mineral protection of SOC and microbial adaptations to warming (Conant *et al.*, 2011). However, few studies re-examined the theoretical framework of

enzyme kinetics to understand the temperature dependence of SOC degradation (Menichetti *et al.*, 2015; Razavi *et al.*, 2016).

Temperature response of SOC degradation is commonly modelled by Arrhenius and Eyring equations that are based on the energy required to initiate the reactions (Activation Energy, EA). These equations were initially designed to describe the exponential increase of chemical reactions rate with temperature. However, to degrade SOC into assimilable compounds, microorganisms produce a broad range of extracellular enzymes that catalyse the reactions. Therefore, rate of SOC degradation is driven by the size of enzymes pool and enzyme activity, each of which may display distinct temperature responses (Stearn, 1949; Segel, 1975; Daniel & Danson, 2010). An explicit consideration of enzymatic system may explain the temporal shift (attenuation) of SOC mineralization response to temperature.

While warming is generally assumed to increase enzyme activities that drive degradation, temperature could have a counteracting negative effect on enzyme pools in soils. Enzymes are perishable proteins that do not continuously accumulate in soils. Once excreted outside the cell, extracellular enzymes escape from cell control and diffuse into the soil matrix where they undergo numerous interactions with soil minerals, microorganisms and broader environmental variables such as temperature. These interactions lead to enzyme inactivation and loss of catalytic activity. Enzyme inactivation can be caused by numerous biotic and abiotic processes leading to a loss of protein conformational structure required for catalytic function (Burns & Dick, 2002; Daniel *et al.*, 2008). Among them, thermal inactivation induced by the Brownian movement and collisions between molecules is particularly important to consider when studying the effect of temperature on enzymatic activities since its intensity increases with temperature and it affects all enzymes (Vrábel *et al.*, 1997a, 1997b; Ballesteros *et al.*, 1998; Aymard &

Belarbi, 2000; O’Fágáin, 2003). Warming-induced enzyme inactivation would reduce overall enzyme activity contributing to attenuate warming effect on SOC degradation.

Here, we explore the temperature-dependence of SOC decomposition with a focus on extracellular degradation, which is presumed to be the rate-limiting step of decomposition (Schimel & Bennett, 2004). We studied this temperature-dependence in models where the reaction rate depends on the catalytic activity of enzymes and the pool of enzymes, each of which may display distinct temperature responses. The enzyme pool was simulated by considering (1) a microbial production of enzymes and (2) the thermal inactivation of these enzymes that depends on time and temperature. A thorough review of thermodynamic properties of enzymes was made to constrain model parameters. In order to link the temperature-dependence of enzymatic activities to SOC degradation we defined the catalytic power of enzymes (E_{power}), that is, the cumulative amount of substrate degraded by one unit of enzyme until its complete inactivation.

Materials and methods

Theory

According to Michaelis-Menten the velocity of enzymatic reactions can be co-limited by active enzymes (E_{act}) and substrate (C_s) concentrations:

$$Reaction\ Velocity = \frac{V_{max}}{k_{cat} \cdot E_{act}} \cdot \frac{C_s}{K_m + C_s} \quad (1)$$

where k_{cat} is the catalytic activity of enzyme, V_{max} represents the maximum velocity at saturating substrate concentration and K_m is the half-saturation constant which reflects the affinity of the enzyme for the substrate ($1/K_m$).

Here we emphasize the role of extracellular enzymes in the temperature response of SOC degradation. Therefore, for sake of simplicity, we focus on the temperature

dependence of V_{max} (Equation 1 at saturating substrate concentration) which is studied in two successive models. Model 1 describes the degradation activity catalysed by a pool of enzymes that undergoes thermal inactivation with time (batch reactor). Model 2 combines thermal inactivation and a continuous replenishment of the enzyme pool (continuous enzyme flow reactor).

Model 1: batch reactor

Thermal inactivation has been extensively studied by enzymologists focused on food industry and biotechnology applications (Boy *et al.*, 1999; Busto *et al.*, 1999; Aymard & Belarbi, 2000; O'Fágáin, 2003; Márquez & Waliszewski, 2008). Inactivation can be described by a multi-step reaction with reversible reactions between active and inactive forms of enzyme followed by an irreversible denaturation of enzymes (Daniel & Danson, 2010). Viewed as a process of continuous enzyme ageing, inactivation increases with time and temperature (Aymard & Belarbi, 2000; Zhang *et al.*, 2011).

In order to study the effect of inactivation on the temperature response of enzymatic reactions, we consider a pool of enzymes released by a non-modelled microbial biomass. This pool of active enzymes (E_{act}) undergoes a thermal inactivation and continuously decreases (Equation 2). The velocity of SOC degradation (V_{max} , Equation 3) depends on E_{act} and enzyme catalytic activity (k_{cat}). The model structure is:

$$\frac{dE_{act}}{dt} = -k_{inact} \cdot E_{act}(t) \quad (2)$$

$$\frac{dC_s}{dt} = V_{max}(t) = k_{cat} \cdot E_{act}(t) \quad (3)$$

where k_{inact} is the thermal inactivation rate constant.

The variation of the two rate constants k_{cat} and k_{inact} with temperature is given by:

$$k_{inact} = a_i \cdot e^{\frac{-EA_{inact}}{R.T}} \quad (4)$$

$$k_{cat} = a_c \cdot e^{\frac{-EA_{cat}}{R.T}} \quad (5)$$

where a_i and a_c are the pre-exponential factors of thermal inactivation and catalytic activity of enzyme respectively, EA_{inact} and EA_{cat} are the activation energies (EA) of enzyme thermal inactivation and catalytic activity respectively. This model is based on the classical model of enzyme activity used in enzymology (Segel, 1975; Daniel *et al.*, 2001).

Model 2: continuous enzyme flow reactor

In ecosystems, continuous enzyme inactivation is compensated by the microbial production of fresh enzymes. This production is limited by the amount of energy-rich substrates supplied by plants (Bingeman *et al.*, 1953; Dalenberg & Jager, 1989; Kuzyakov *et al.*, 2000; Fontaine *et al.*, 2007). To take into account this limitation, microbial enzyme production is modelled as a fixed continuous flux (ϕ). Therefore, the pool of active enzymes is determined by the enzyme inactivation and ϕ :

$$\frac{dE_{act}}{dt} = \phi - k_{inact} \cdot E_{act}(t) \quad (6)$$

The degradation velocity of SOC and the rate constants k_{cat} and k_{inact} are described in model 1.

Model analysis and simulations

Model 1

Model 1 was solved to analyse the time and temperature dependence of the reaction velocity (V_{max}) mediated by a pool of enzymes. The enzyme pool that inactivates over time was obtained by solving Equation (2):

$$E_{act}(t) = E_0 \cdot e^{-k_{inact} \cdot t} \quad (7)$$

where E_0 is the initial amount of active enzymes.

By combining Equations (7) and (3) we obtained the reaction velocity over time:

$$\frac{dC_s}{dt} = V_{max}(t) = k_{cat} \cdot E_0 \cdot e^{-k_{inact} \cdot t} \quad (8)$$

After replacing the thermo-dependent rate constants k_{cat} and k_{inact} by their expressions (Equations 4&5), we obtained the time and temperature dependence of reaction velocity:

$$V_{max}(T, t) = a_c \cdot e^{\frac{-EA_{cat}}{R \cdot T}} \cdot E_0 \cdot e^{-t \cdot \left(a_i \cdot e^{\frac{-EA_{inact}}{R \cdot T}} \right)} \quad (9)$$

V_{max} corresponds to the instantaneous activity mediated by an enzyme pool. In nature, the enzymes released by soil microorganisms degrade SOC compounds until their complete inactivation. Therefore, to understand the effect of a pool of enzymes on the total amount of degraded SOC, we must consider the cumulative activity over time. This cumulative activity, called reaction yield (RY), was obtained by integrating V_{max} over time.

$$RY(T, t) = \int_0^t V_{max}(t) \cdot dt = \frac{a_c}{a_i} \cdot e^{\frac{EA_{inact} - EA_{cat}}{R \cdot T}} \cdot E_0 \cdot \left(1 - e^{-t \cdot a_i \cdot e^{\frac{-EA_{inact}}{R \cdot T}}} \right) \quad (10)$$

The total amount of substrate degraded by the pool of enzymes, that is, the whole reaction yield, was obtained with Equation (10) considering $t \rightarrow +\infty$:

$$RY_{\infty} = E_0 \cdot \frac{a_c}{a_i} \cdot e^{\frac{EA_{inact} - EA_{cat}}{R \cdot T}} \quad (11)$$

The catalytic power of enzyme (E_{power}) corresponds to RY_{∞} per unit of enzymes initially incorporated (E_0).

$$E_{power} = \frac{a_c}{a_i} \cdot e^{\frac{EA_{inact} - EA_{cat}}{R \cdot T}} \quad (12)$$

The relative temperature sensitivity of RY_{∞} and E_{power} is given by:

$$\frac{1}{RY_{\infty}} \cdot \frac{d RY_{\infty}}{dT} = \frac{1}{E_{power}} \cdot \frac{d E_{power}}{dT} = \frac{-(EA_{inact} - EA_{cat})}{R T^2} \quad (13)$$

Model 2

Model 2 was solved at steady state of the enzyme pool ($dE_{act}/dt = 0$) to determine the ultimate (when time does not matter anymore) temperature response of the enzyme pool (E_{act}^* ; the star indicating steady state), velocity of SOC degradation (V_{max}^*) and catalytic power of enzymes (E_{power}).

We first considered that the microbial production of enzymes is constant and temperature independent (ϕ). The pool of enzymes at steady state (E_{act}^*) is given by:

$$E_{act}^* = \frac{\phi}{k_{inact}} \quad (14)$$

By replacing E_{act}^* in Equation (3) we obtained V_{max}^* :

$$\left(\frac{dC_s}{dt}\right)^* = V_{max}^* = k_{cat} \cdot E_{act}^* = \phi \cdot \frac{k_{cat}}{k_{inact}} \quad (15)$$

After replacing k_{cat} and k_{inact} by their expressions (Equations 4&5), we obtained the temperature dependence of V_{max}^* :

$$\left(\frac{dC_s}{dt}\right)^* = V_{max}^* = \phi \cdot \frac{E_{power}}{a_i} \cdot e^{\frac{EA_{inact} - EA_{cat}}{R \cdot T}} \quad (16)$$

Therefore, in a continuous enzyme flow reactor, the catalytic power of enzyme (E_{power}) acting in the reactor is obtained by the ratio between the flux of degraded substrate $\left(\frac{dC_s}{dt}\right)^*$ and the flux of enzymes entering the reactor (ϕ).

If the microbial production of enzymes changes with temperature ($\phi(T)$), the temperature response of SOC degradation is given by:

$$\left(\frac{dC_s}{dt}\right)^* = \phi(T) \cdot E_{power}(T) \quad (17)$$

The temperature sensitivity of SOC degradation expressed in terms of the relative sensitivities of $\phi(T)$ and $E_{power}(T)$ is given by:

$$\frac{d\left(\frac{dC_s}{dt}\right)^*}{dT} = \phi(T) \cdot E_{power}(T) \cdot \left(\frac{1}{\phi(T)} \cdot \frac{d\phi(T)}{dT} + \frac{1}{E_{power}} \cdot \frac{dE_{power}}{dT} \right) \quad (18)$$

Model 2 was also analysed dynamically to describe the transition between two steady states. A 5°C increase was simulated over time for a system previously at steady state with a temperature of 10°C. These analyses were first made considering a constant flux (ϕ) of enzymes, which does not depend on temperature. In ecosystems, enzyme production may vary with temperature due to temperature effect on microbial activities and plant allocation of substrates to microorganisms. To account for this variation, we also made simulations with model 2 by considering a 40% increase or decrease of enzyme production with warming. For example, an increase in ϕ can be explained by a temperature stimulation of plant photosynthesis and exudation of substrates to microorganisms. A reduction of ϕ can be due to an adverse effect of warming on microbial physiology (Allison *et al.*, 2011).

Simulations were made for three enzymes representative of oxidative and hydrolytic enzymes: a peroxidase, a chitinase and a β -glucosidase. For brevity, methods and results for β -glucosidase are presented in Appendix S1 and simulations of model 1 are presented for the peroxidase only. The thermodynamic properties of enzymes were determined by performing lab enzyme assays (Table 1 and Appendix S1).

Lab assays

Lab assays were performed for a chitinase from *Trichoderma viride* (N-acetyl- β -glucosaminidase, NAGase, EC 3.2.1.14, Sigma-Aldrich C8241) and a peroxidase from *horseradish* (EC 1.11.1.7, Sigma-Aldrich P8125). The activation energies and the pre-

exponential factors of thermal inactivation (EA_{inact} and a_i) and catalysis (EA_{cat} and a_c) of these enzymes (Table 1) were determined in two distinct experiments.

Temperature response of enzyme catalytic activity (k_{cat}): determination of EA_{cat} and a_c

The temperature response of k_{cat} was determined in short-term enzyme assays conducted at various temperature: from 13 to 45°C for peroxidase and from 10 to 63°C for chitinase. The enzyme assay is presented in details for each enzyme below.

NAGase assay - A solution of N-acetyl- β -glucosaminidase containing 0.012 U.mL⁻¹ was prepared in sodium acetate buffer (50 mM, pH 5 at 25°C). One enzyme unit (U) is the amount of enzyme that catalyses the release of 1 μ mol.min⁻¹ of *p*-nitrophenol at pH 5 and 20°C. For each temperature of reaction (10, 20, 36, 40, 50, 60 and 63°C), the catalysed reaction was performed in a polyethylene 96-wells (0.5 mL) microplate continuously shaken on a heidolph titramax 1000 plate shaker. Wells received 50 μ L 50mM acetate buffer and 150 μ L 2.5 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide as a substrate. The microplate was pre-heated 5 minutes in an incubator before adding 50 μ L of the NAGase solution with a multi-channel pipet to start the reaction. Two wells without enzyme were also incubated as substrate controls. Kinetics of reaction was monitored over 16 minutes by adding every 4 minutes 20 μ L 1M NaOH in 3 wells to stop the reaction and develop the yellow colour of *p*-nitrophenol released. 200 μ L of each triplicate were pipetted into a quartz microplate and the intensity of the yellow colour of *p*-nitrophenol released was measured at 405 nm with a spectrophotometer (BioTek Power Wave XS2).

Peroxidase assay - A solution of peroxidase containing 0.18 U.mL⁻¹ was prepared in potassium phosphate buffer (100 mM, pH 5 at 25°C). One enzyme unit (U) is the amount of enzyme that catalyses the oxidation of 1 μ mol.min⁻¹ of ABTS at pH 5 and 20°C. For each temperature of reaction (13, 16, 20, 25, 30, 35, 40 and 45°C), the rate of reaction was

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determined by continuous enzyme assay in a 96-wells quartz microplate placed in the spectrophotometer pre-heated at the specified temperature. 6 wells received 20 μ L 100 mM potassium phosphate buffer, 174 μ L 9.1 mM ABTS as a substrate (previously pre-heated in a water bath) and 10 μ L 0.3% (w/w) H₂O₂. The microplate was pre-heated 5 minutes in the spectrophotometer before adding 20 μ L of peroxidase solution with a multi-channel pipet to start the reaction. Two wells without enzyme were also incubated as substrate controls. The oxidation rate of ABTS was determined by continuous measurement of absorbance at 405 nm during 2 minutes.

Temperature response of thermal inactivation of enzymes (k_{inact}): determination of EA_{inact} and a_i

The temperature response of k_{inact} was determined by incubating solutions of each enzyme at different temperatures. The enzyme residual activity was measured at different times over the incubation according to the expected rate of inactivation depending on the enzyme and the incubation temperature. The enzyme incubation is presented in detail for each enzyme below.

NAGase incubation - 1.8 mL of NAGase solution (0.012 U.mL⁻¹) in 50 mM sodium acetate buffer was pipetted in 2 mL pyrex vial stoppered with a septum. For each temperature treatment, 8 vials were incubated in a water bath at the specified temperature. To measure the residual activity of the incubated solution, a vial was collected from water bath and immediately cooled at 0°C before the residual activity was assayed at 30°C with method described above. A total of 7 to 8 assays performed at consecutive times described the kinetics of thermal enzyme inactivation. Incubations were performed at 20, 30, 40, 50, 60 and 65°C for time varying from 22 minutes to 7.41 hours depending on the rate of enzyme inactivation.

Peroxidase incubation - A solution of peroxidase (0.56 U.mL^{-1}) in 100 mM potassium phosphate buffer was sterilized by filtering at $0.22\mu\text{m}$ to avoid microbial growth during incubation. 0.5 mL of enzyme solution was pipetted in 2 mL autoclaved Pyrex vials, which were stoppered with a sterile septum. For each temperature treatment (30, 50, 60, 65, 70 and 75°C), 15 vials were incubated in a water bath. To measure the residual activity of the incubated solution, 3 vials were collected from water bath and immediately cooled at 0°C before the residual activity was assayed at 30°C with method described above. A total of 5 to 6 assays performed at consecutive times described the kinetics of thermal enzyme inactivation. Incubations were performed for time varying from 7 minutes to 3 days depending on the rate of enzyme inactivation.

Calculation of enzyme parameters

For each temperature, the thermal inactivation rate constant (k_{inact}) was estimated from the logarithm of the proportion of enzyme activity remaining over time, according to equation $\ln(A/A_0) = -k_{inact}.t$ where A_0 is the initial enzyme activity of the solution and A is the activity after incubation for a defined period of time.

The activation energies and the pre-exponential factors of thermal inactivation (EA_{inact} and a_i) and catalysis (EA_{cat} and a_c) were calculated from the slopes and intercepts of $\ln(k_{inact})$ vs. $1/T$ and $\ln(k_{cat})$ vs. $1/T$ respectively. The slopes and intercepts were determined by linear regression analysis according to equation $\ln(k) = \ln(a) - (EA/R).(1/T)$ where R is the universal gas constant and T is the absolute temperature.

Literature review

The model analysis indicated that the effect of temperature on reaction velocity and catalytic power of enzyme depends on the thermodynamic properties of each enzyme. To determine whether a universal pattern exists among enzymes, a thorough review of

activation energies for catalysis and inactivation processes was made (Table 2). This data compilation covers thirty-three different types of enzymes originating from a wide range of organisms with contrasted temperature growth optima (2-75°C).

Results

Batch reactor (Model 1)

Reaction velocity (V_{max})

Fig. 1a shows that V_{max} has temperature optima. Below the temperature optima, the effect of temperature on the enzyme catalytic activity is higher than on the decrease of enzyme pool through thermal inactivation, resulting in increase in V_{max} . Above the optima, the decrease in the enzyme pool exceeds the increase of enzyme activity leading to a decrease in V_{max} .

V_{max} decreases over time due to enzyme inactivation. The slower decrease of V_{max} at low temperature is explained by the better preservation of enzyme from thermal inactivation. This better preservation also induces a shift of temperature optima to lower values with time. These results imply that the temperature response of V_{max} changes with time. The effect of a given temperature change on V_{max} can even be opposite depending on time considered. For example, from 300 to 310°K V_{max} increases at day 1 whereas it decreases at day 6.

Reaction yield (RY) and catalytic power of enzymes (E_{power})

Fig. 1b shows that the cumulative amount of degraded substrate (RY) mediated by the pool of enzymes increases with time until a plateau. The accumulation rate of degraded substrate is maximal at the beginning and decelerates with time due to enzyme

inactivation. The plateau corresponds to the maximum amount of degraded substrate (RY_{∞}), that is, when the pool of enzymes is entirely inactivated.

The relative temperature sensitivity of RY_{∞} is monotonous, that is, the yield continuously increases or decreases with temperature (Equation 13). The direction of the RY_{∞} response is determined by the sign of the delta of activation energies between the enzyme inactivation and the catalysis ($\Delta EA = EA_{inact} - EA_{cat}$). RY_{∞} continuously decreases with temperature if $EA_{inact} > EA_{cat}$ and continuously increases if $EA_{inact} < EA_{cat}$. The intensity of the response is determined by the magnitude of ΔEA .

The two thermodynamic parameters EA_{inact} and EA_{cat} are controlled by the molecular architecture and behaviour of enzymes, which may vary among enzymes. To determine whether a universal pattern exists among enzymes, we collated published values of activation energy of catalysis and inactivation processes (Table 2). The large range of studied enzymes exhibited contrasting thermodynamic properties with EA_{cat} ranging from 13 to 88 kJ mol⁻¹ and EA_{inact} ranging from 69 to 311 kJ mol⁻¹. Despite these contrasting properties, all enzymes showed higher values of EA_{inact} than EA_{cat} . This universal pattern signifies that inherent enzyme properties must lead to a negative response of the whole reaction yield to temperature.

Taking into account the universal pattern $EA_{inact} > EA_{cat}$, we simulated the dynamics of reaction yield and its response to temperature using Equation (10) (Fig. 1b). Simulations show that the response of reaction yield to temperature reverses with time, switching from positive in the short-term to negative in the long-term. Therefore the catabolic power of enzymes, defined as the maximum amount of substrate that can be catabolized per unit of enzyme (RY_{∞} / E_0), decreases with temperature.

Enzyme flow reactor (Model 2)

Steady state analysis

At steady state, the SOC degradation is the arithmetical product of microbial production of enzymes (ϕ) and catalytic power of enzymes (E_{power}) (Equation 17). Therefore, the temperature response of SOC degradation is determined by the relative temperature sensitivities of ϕ and E_{power} (Equation 18). Similar to model 1, the universal enzyme pattern (Table 2) leads to a continuous decrease of E_{power} with temperature (Fig. 2). Therefore, if $\phi(T)$ remains constant or decreases with temperature, the SOC degradation is reduced under warming (Equation 18 and Fig. 3a,c). If $\phi(T)$ increases with temperature, the relative temperature sensitivities of $\phi(T)$ and $E_{power}(T)$ have opposite signs and the temperature response of SOC degradation can be negative or positive (Equation 18 and Fig. 3b,d).

Transitory response

In order to describe the transitory response to warming, a 5°C increase was simulated over time for three enzymes (peroxidase, chitinase and β -glucosidase) previously acclimated to 10°C (Fig. 3 and Appendix S1). The effect of warming on enzymatic SOC degradation was successively studied with microbial enzyme production (ϕ) constant (Fig. 3a,c) and 40% increased under warming (Fig. 3b,d). For each enzyme, SOC degradation (V_{max}) immediately increases after warming due to the instantaneous positive response of enzyme catalytic activity. This is followed by a slow and persistent decrease of V_{max} and enzyme pool (Appendix S2) due to thermal inactivation of enzymes. With ϕ constant, V_{max} decreases towards a new steady state value that is lower than before warming. This reduction of V_{max}^* is caused by the decrease of E_{power} under warming. With ϕ increased under warming, the over-production of enzymes offsets the reduction of E_{power}

leading to either positive or negative response of V_{max}^* depending on enzyme type. For enzymes having low relative temperature sensitivity of E_{power} (chitinase; low ΔEA), the increased ϕ compensates for the reduction in E_{power} leading to positive response of V_{max}^* to warming. On the contrary, for enzyme with high relative temperature sensitivity of E_{power} (peroxidase; high ΔEA), the reduction in E_{power} dominates leading to negative response of V_{max}^* to temperature. Finally, the time for enzyme pool and V_{max}^* to reach a new steady state can take several years depending on enzyme type (Fig. 3 & Appendix S2).

Discussion

E_{power} : a relevant enzyme trait to characterize temperature effect on substrate degradation

It is frequently taught that enzymes have a single and well-defined temperature optimum reflecting the organism's adaptation to their thermal environment. Determined by measuring the short-term temperature response of reaction velocity (V_{max}) in enzyme assays or soil incubations, the optimum temperature of an enzyme is considered as a trait characterizing the optimal temperature condition of bio-catalysed reactions. However, as already demonstrated by others (Stearn, 1949; Barton, 1979; Daniel *et al.*, 2001), the temperature optimum of the reaction shifts with time towards colder temperatures (Fig. 1a). This time shift is explained by the slower thermal-inactivation of enzymes and longer persistence of enzyme activity at cold temperatures. Therefore, the temperature optimum is not a suitable enzyme trait since its value depends on assay duration and does not inform on the long-term effect of temperature on enzymatic activity.

The enzymes released by microorganisms catalyse substrate degradation until their complete inactivation. Therefore, our findings indicate that a more relevant enzyme trait to characterize temperature effect on substrate degradation is the catalytic power of enzymes

(E_{power}). The E_{power} corresponds to the cumulative amount of substrates degraded by one unit of enzymes until its complete inactivation. The E_{power} is determined by the thermodynamics properties of enzymes (EA_{inact} , EA_{cat} and the pre-exponential factors a_i and a_c) depending on their molecular structure and not on assay duration. The sign and magnitude of the delta between activation energies (ΔEA) give the direction and magnitude of the E_{power} response to temperature (Equation 13). Our literature review shows a universal pattern among enzymes with systematically higher values of EA_{inact} than EA_{cat} . This signifies that the enzyme thermal inactivation process has higher relative temperature sensitivity than enzyme catalytic process. Under these conditions, our model predicts that the catalytic power of enzymes decreases with temperature (Fig. 1b and Fig. 2). This prediction can be tested in i) batch reactors by measuring the reaction yield generated by a pool of enzymes incubated at different temperatures and ii) continuous flow reactors by measuring the reaction velocity at steady state for different temperatures.

Is the universal pattern of enzyme thermodynamic properties the consequence of a microbial evolutionary process?

The universal pattern among enzymes of $EA_{inact} > EA_{cat}$ might result from evolutionary selection of microbial species with the highest performance enzymes. Indeed, low EA_{cat} combined with high EA_{inact} leads to high catalytic activity of enzymes (k_{cat}) and high persistence of the active form of enzymes (low k_{inact}). This results in a high E_{power} of enzymes maximising the return on investment in enzyme production by organisms. The counterpart of such EAs' hierarchical pattern is the higher relative temperature sensitivity of thermal inactivation than catalysis resulting in a negative temperature effect on E_{power} .

EA_{cat} and EA_{inact} are limited to range of values (Table 2) suggesting that the evolution process reducing EA_{cat} and increasing EA_{inact} is limited by physical or/and ecological

constraints. As an example of physical limit, the enzyme flexibility necessary for substrate catalysis is constrained by a need for rigidity to maintain enzyme stability and functionality (Zavodszky *et al.*, 1998; D'Amico *et al.*, 2002). Moreover, there might be a trade-off in C and nutrients investment between the amount of produced enzymes and the building of super enzymes with high E_{power} . Given the heterogeneous distribution of microorganisms and substrates in soils (Dungait *et al.*, 2012), it might be more profitable for microorganisms to invest in large amount of enzymes with moderate E_{power} because it increases chance for enzymes to reach substrate.

The lower catalytic power of enzymes (E_{power}) under warm temperatures predicted by our theory (Fig. 2) could be attenuated by an adaptation of microorganisms and their enzymes. This adaptation could imply the production of isoenzymes with enlarged ΔEA to maintain their E_{power} (Equation 12). As explained above, the variation of this ΔEA is limited due to different constraints that narrow the possibility of adaptation. Nevertheless, there is evidence for biogeographical patterns in enzyme traits, especially when studying extreme thermal environments (Zavodszky *et al.*, 1998; Huston *et al.*, 2000; Feller, 2003). Many studies have observed that hot-adapted microorganisms produce more stable enzymes (higher EA_{inact}) at the cost of lower catalytic activity (higher EA_{cat}) compared to their mesophilic counterpart (Zavodszky *et al.*, 1998; Feller, 2003). These changes do not necessarily increase the catabolic power of enzymes since it depends on ΔEA . Therefore, the observed biogeographical change in enzyme traits could be explained by other reasons than maintaining the catabolic power of enzymes. For example, due to the physical separation of microorganisms and substrates, hot-adapted microorganisms could produce stable enzymes to let enough time for these enzymes to diffuse and reach substrates before their inactivation. In contrast, the release of stable enzymes in cold environment may prevent microorganisms from adjusting soil enzyme activities to their needs over time due

to enzyme persistence. The nutritional needs of microorganisms fluctuate with time due to the fluctuation of soil resources ("hot moments" Kuzyakov & Blagodatskaya, 2015) and respect of their elemental composition (Sturner & Elser, 2002). The persistence of enzymes that fail to respond to microbial needs lead to accumulation of nutrients that become toxic or are lost. Therefore, cold-adapted microorganisms could take advantage of producing of enzymes of low stability but with high catalytic activity.

Perspectives

As with any theoretical work, our study has limitations that require further research. In nature, enzymatic systems are complex including many physical and biological processes altering enzyme pools and enzyme properties that are not considered in this study. For example, enzymes in soil are quickly immobilized on soil particles (clays, humic colloids) (Kandeler, 1990; Burns *et al.*, 2013), which changes their thermodynamics properties (Nannipieri *et al.*, 2002; Acevedo *et al.*, 2010; Menezes-Blackburn *et al.*, 2011). Immobilisation generally increases the thermal stability of enzymes (Higher EA_{inact}) and can maintain or decrease the catalytic activity (similar or higher EA_{inact}). Further research is required to understand how immobilisation of soil enzymes modulates the responses seen in our model. Moreover, we only considered the temperature response of V_{max} corresponding to the case of extracellular enzymes that work at saturating substrate concentration. For enzymes that could face low substrate concentration, the substrate affinity ($1/K_m$) and its dependence to temperature must be considered. Given that K_m increases with temperature, thus slowing down the enzymatic reaction (Equation 1; Stone *et al.*, 2012; Blagodatskaya *et al.*, 2016), the consideration of this parameter should not qualitatively change our predicted negative temperature effect on E_{power} . The strength of this theory is to show that the temperature responses (transitory and long-term) of

biocatalyzed reactions can be better understood by considering the catalytic power of enzymes.

Implications for soil C modelling, microbial physiology and soil-climate interactions

Most current models depict SOC degradation with first-order kinetics ($k.C$) where the temperature dependence of the decay rate constant (k) is modelled by the Arrhenius equation. These models predict that degradation increases with temperature and the relative sensitivity of the response is higher for recalcitrant (high EA_{cat}) than labile substrate (Sierra, 2012). This modelling is suitable for describing the temperature response of chemical reactions without catalyst or with stable catalysts (e.g. metals). However, SOC degradation, at least for a large part (Kéroul *et al.*, 2016), relies on activity of enzymes that undergoes thermal inactivation. By considering the perishable character of enzymes, we show that temperature response of SOC degradation can be positive or negative, depending on the relative temperature sensitivities of microbial enzyme production and catalytic power of enzymes. For a given microbial enzyme production, the relative temperature sensitivity of substrate degradation depends on enzyme properties (ΔEA) and not only on substrate degradability (EA_{cat}) (Equation 13). Given the interdependence of EA_{cat} and EA_{inact} , it is probable that the correlation between substrate degradability and temperature sensitivity is weak or inexistent explaining the lack of consensus on this topic (Knorr *et al.*, 2005b; Reichstein *et al.*, 2005b; Davidson & Janssens, 2006; Fang *et al.*, 2006).

The lower E_{power} under warming (Fig. 1b and Fig. 2) implies that microorganisms must invest a higher quantity of carbon to degrade similar amount of soil substrates, leaving less C for growth (Allison, 2014). This finding could explain the decrease in

microbial biomass (MB) and carbon use efficiency (CUE) reported in numerous warming experimental studies (Devêvre & Horwáth, 2000; Bradford *et al.*, 2008; Steinweg *et al.*, 2008; Allison *et al.*, 2010; Manzoni *et al.*, 2012; Frey *et al.*, 2013; Tucker *et al.*, 2013). Moreover, the E_{power} response to temperature varies among enzymes depending on their thermodynamics (Equation 13), which may explain why the temperature effect on CUE changes with the type of substrate (Frey *et al.*, 2013).

Several ecosystem warming experiments show that CO₂ loss from warmed soils declines to control levels (Liski *et al.*, 1999; Melillo *et al.*, 2002) or even below within a few years (Luo *et al.*, 2001). We find that the decrease of soil enzyme pools due to their higher rate of inactivation under warming can contribute to this long-term temporal shift of SOC degradation response to temperature (Fig. 3 and Appendix S2). This contribution is supported by the fact that the time for enzyme pool to reach a new steady state can take several years. Consistently, the rare observations of enzyme pools in long-term warming experiments tends to show a negative temperature effect on enzyme pools (Zhou *et al.*, 2012; Schindlbacher *et al.*, 2015). Moreover, monitoring of soil enzyme activities over seasons in arctic soils show for many enzymes a strong decrease in enzyme pools with the increase in soil temperature during spring and summer (Wallenstein *et al.*, 2009; Sistla & Schimel, 2013). Other processes may act in concert to attenuate the warming effect on SOC degradation such as reduction of easily degradable C, mineral protection of SOC and shifts in microbial physiology and/or community structure (Conant *et al.*, 2011). Our theory also indicates that if microbial enzyme production does not vary with warming, the ultimate response of SOC degradation is negative. If microbial enzyme production increases with warming, the ultimate response can be negative or positive depending on the relative temperature sensitivities of enzymes production and E_{power} . These findings emphasize the need for more studies characterizing microbial production of enzymes, soil

enzyme pools and properties (k_{cat} , k_{inact} , K_m)(Zhou *et al.*, 2012; Ali *et al.*, 2015; Menichetti *et al.*, 2015; Razavi *et al.*, 2016) to understand the long-term effect of warming on ecosystem carbon flux.

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Conflict of Interest

The authors declare no conflict of interest.

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Tables

Table 1: Thermodynamic properties of the two studied enzymes and model parameters of models 1 & 2. One enzyme unit (U) is defined as the amount of enzyme that catalyses the release of 1 μmol of reaction product per minute at 20°C and pH5.

Symbol	Definition	Enzyme system		Dimension
		Chitinase	Peroxidase	
a_c	pre-exponential factor of enzyme catalytic activity	$1317 \cdot 10^3$	2796	$\mu\text{mol min}^{-1} \text{U}^{-1}$
a_i	pre-exponential factor of enzyme thermal inactivation	$2.28 \cdot 10^{10}$	$2.76 \cdot 10^{26}$	min^{-1}
EA_{cat}	activation energy of enzyme catalytic activity	34.3	19.4	kJ
EA_{inact}	activation energy of enzyme thermal inactivation	75.2	180.3	kJ
ΔEA	$EA_{inact} - EA_{cat}$	40.9	160.9	kJ
ϕ	flux of enzymes entering the flow reactor before warming	$1 \cdot 10^{-3}$	$1.5 \cdot 10^{-6}$	U min^{-1}
$\phi_{5^\circ\text{C warming}}$	flux of enzymes entering the flow reactor under warming	$1.4 \cdot 10^{-3}$	$2.1 \cdot 10^{-6}$	U min^{-1}

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Table 2 : Activation energies of catalysis (EA_{cat}) and inactivation (EA_{inact}) reactions for 33 enzymes produced by different organisms. T_{growth} corresponds to the growth temperature optimum of the source organism. EA values are derived by fitting assay data measured in non-limiting substrate conditions with models based on Eyring (^a) or Arrhenius equations (^b).

Organism	Enzyme	Organism T_{growth} (°C)	Activation energy of		Reaction class	References
			catalysis EA_{cat} (kJ.mol ⁻¹)	inactivation EA_{inact} (kJ.mol ⁻¹)		
<i>Nc</i>	Tannase	-	17 ^b	85 ^b	Hydrolase	(Nie <i>et al.</i> , 2012)
<i>Aspergillus niger</i>	Tannase	-	23 ^b	69 ^b	Hydrolase	(Yu & Li, 2006)
<i>Trichoderma reesei</i>	Xylanase	-	32 ^b	311 ^b	Hydrolase	(Cobos & Estrada, 2003)
<i>Ganoderma lucidum</i>	Lipase	-	13 ^b	79 ^b	Hydrolase	(Bhatti & Amin, 2013)
<i>Trichoderma viride</i>	Chitinase	-	34 ^b	75 ^b	Hydrolase	Table 1
<i>Agrobacterium sp.</i>	β -Glucosidase	-	34 ^a	96 ^a	Hydrolase	Appendix S1
<i>ASB HK47</i>	Alkaline phosphatase	15	55 ^a	90 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>Wheat germ</i>	Acid Phosphatase	20	79 ^a	95 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>P. dulcis</i>	β -Glucosidase	20	63 ^a	95 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>P. fluorescens</i>	ATPase	25	74 ^a	92 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>S. cerevisiae</i>	α -Glucosidase	28	71 ^a	90 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>B. taurus</i>	Alkaline phosphatase	39	57 ^a	97 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>G. steatothermophilus</i>	α -Glucosidase	55	64 ^a	96 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>T. aquaticus</i>	α -Glucosidase	70	88 ^a	103 ^a	Hydrolase	(Daniel <i>et al.</i> , 2008)
<i>C. saccharolyticus</i>	β -Glucosidase	70	81 ^a	98 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>Thermus sp. RT41a</i>	Alkaline phosphatase	75	72 ^a	99 ^a	Hydrolase	(Lee <i>et al.</i> , 2007)
<i>R. glutinis</i>	Ammonia-Lyases	24	80 ^a	97 ^a	Lyase	(Lee <i>et al.</i> , 2007)
<i>S. scrofa</i>	Fumarase	39	60 ^a	92 ^a	Lyase	(Lee <i>et al.</i> , 2007)
<i>Potato tubers</i>	5-lipoxygenase	-	28 ^b	110 ^b	Oxidase	(Lagutina <i>et al.</i> , 1990)
<i>Beef liver</i>	Catalase	-	18 ^b	230 ^b	Oxidoreductase	(Sizer, 1944)
<i>Horesradish</i>	Peroxydase	-	19 ^b	180 ^b	Oxidoreductase	Table 1
<i>M. profunda</i>	Dihydrofolate reductase	2	67 ^a	93 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. psychrophilus</i>	3-isopropylmalate dehydrogenase	20	72 ^a	100 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. subtilis</i>	3-isopropylmalate dehydrogenase	30	73 ^a	94 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. cereus</i>	Dihydrofolate reductase	30	66 ^a	90 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>C. utilis</i>	Glutamate dehydrogenase	31	57 ^a	94 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. taurus</i>	Malate dehydrogenase	39	53 ^a	85 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. taurus</i>	Glutamate dehydrogenase	39	63 ^a	93 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>E. coli</i>	Malate dehydrogenase	40	55 ^a	96 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>G. steatothermophilus</i>	Dihydrofolate reductase	55	67 ^a	97 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. caldovelox</i>	3-isopropylmalate dehydrogenase	70	76 ^a	101 ^a	Oxidoreductase	(Lee <i>et al.</i> , 2007)
<i>B. taurus</i>	γ -Glutamyl transferase	39	63 ^a	98 ^a	Transferase	(Lee <i>et al.</i> , 2007)
<i>Thermoplasma acidophilum</i>	Citrate synthetase	70	88 ^a	103 ^a	Transferase	(Daniel & Danson, 2013)

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Figure captions

Fig. 1 : Temperature and time responses of (a) reaction velocity (V_{max}) and (b) reaction yield (RY) mediated by a pool of enzymes that inactivates with time under non-limiting substrate conditions. RY corresponds to the cumulative amount of degraded substrate with time by a pool of enzymes. Values simulated by Equations (9) & (10) with an initial enzyme concentration $E_0=0.1 \text{ U L}^{-1}$ (1 enzyme Unit catalyses a velocity of $1 \mu\text{mol ABTS min}^{-1}$ at 20°C pH 5). Enzyme thermodynamic parameters were determined for horseradish peroxidase (Table 1).

Fig. 2 : Temperature response of the catalytic power of enzyme. E_{power} corresponds to the cumulative amount of substrate degraded by one unit of enzyme until its complete inactivation. Simulation was performed for peroxidase using enzyme parameters in Table 1.

Fig. 3 : Transitory responses of reaction velocity predicted by model 2 after a 5°C increase applied to a system previously at steady state with a temperature of 10°C . Simulation were made for two enzymes, chitinase (a,b) and peroxidase (c,d), by considering a constant or 40% increased flux of enzymes (ϕ) with warming. Parameters values used for simulations are presented in Table 1.