Functional Ecology

Soil enzymes in response to climate warming: mechanisms and feedbacks

Author names: Nicolas Fanin[a], Maria Mooshammer[b], Marie Sauvadet[cd], Cheng Meng[e], Gaël Alvarez[f], Laëtitia Bernard[g], Isabelle Bertrand[g], Evgenia Blagodatskaya[hi], Lucie Bon[a], Sébastien Fontaine[f], Shuli Niu[e], Gwenaelle Lashermes[j], Tania L. Maxwell[a], Michael Weintraub[k], Lisa Wingate[a], Daryl Moorhead[k], Andrew Nottingham[l].

Affiliations: [a]INRAE, Bordeaux Sciences Agro, UMR 1391 ISPA, 71 avenue Edouard Bourlaux, CS 20032, F33882 Villenave-d'Ornon cedex, France

[b]Department of Environmental Science, Policy, and Management, University of California Berkeley, Berkeley, CA, USA

[c]CIRAD, UPR GECO, F97285 Le Lamentin, Martinique, France

[d]CIRAD, GECO, Univ Montpellier, Montpellier, France

[e]Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

[f]INRAE, Université Clermont Auvergne, VetAgro Sup, UMR Ecosystème Prairial, 63000 Clermont Ferrand, France

[g]INRAE, IRD, CIRAD, Institut Agro, Univ Montpellier, UMR Eco&Sols, Montpellier, France

[h]Department of Soil Ecology, Helmholtz Centre for Environmental Research – UFZ, Halle, Saale, Germany

[i]Agro-Technological Institute, RUDN University, Moscow, Russia

[j]INRAE, Université de Reims Champagne-Ardenne, UMR A 614 FARE, 51097 Reims, France

[k]Department of Environmental Sciences, University of Toledo, 2801 W. Bancroft St., Toledo, Ohio 43606-3390 USA

[l]School of Geography, University of Leeds, Leeds, UK

Corresponding author: nicolas.fanin@inrae.fr, ORCID: https://orcid.org/0000-0003-4195-855X, phone: +33 (0)5.57.12.25.16, fax: +33 (0)5 57 12 24 20.

Article type: Review

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2435.14027

Acknowledgements: The authors acknowledge the editors Pablo Garcia Palacios and Ji Chen for their invitation to the special issue about '*microbes, soil carbon storage and climate change*'. This was work was supported by ANR CARTON (ANR-19-CE32-0006) and by the RUDN University Strategic Academic Leadership Program.

Conflict of Interest: Shuli Niu is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper. The authors declare no conflict of interest.

Authors' contributions: N.F. conceived the research idea, drawn the figures and wrote the first structure of the manuscript in close consultation with A.N. All authors contributed vigorously to the discussion and writing of this paper.

Data availability statement: There are no new data associated with this manuscript.

NICOLAS FANIN (Orcid ID : 0000-0003-4195-855X)

DR LAETITIA BERNARD (Orcid ID : 0000-0002-7057-6182)

DR SHULI NIU (Orcid ID : 0000-0002-2394-2864)

DR TANIA L. MAXWELL (Orcid ID : 0000-0002-8413-9186)

DR DARYL L. MOORHEAD (Orcid ID : 0000-0002-3460-5302)

DR ANDREW NOTTINGHAM (Orcid ID : 0000-0001-9421-8972)

Article type : Review

Title

Soil enzymes in response to climate warming: mechanisms and feedbacks

Summary (350 words)

1. Soil enzymes are central to ecosystem processes because they mediate numerous reactions that are essential in biogeochemical cycles. However, how soil enzyme activities will respond to global warming is uncertain. We reviewed the literature on mechanisms linking temperature effects on soil enzymes and microbial communities, and outlined a conceptual overview on how these changes may influence soil carbon fluxes in terrestrial ecosystems.

2. At the enzyme scale, although temperature can have a positive effect on enzymatic catalytic power in the short-term (i.e., *via* the instantaneous response of activity), this effect can be countered over time by enzyme inactivation and reduced substrate affinity. At the microbial scale, short term warming can increase enzymatic catalytic power *via* accelerated synthesis and microbial turnover, but shifts in microbial community composition and growth efficiency may mediate the effect of warming in the long-term.

3. Although increasing enzyme activities may accelerate labile carbon decomposition over months to years, our literature review highlights that this initial stage can be followed by the following phases: (i) a reduction in soil carbon loss, due to changing carbon-use efficiency

among communities or substrate depletion, which together can decrease microbial biomass and enzyme activity; (ii) an acceleration of soil carbon loss, due to shifts in microbial community structure and greater allocation to oxidative enzymes for recalcitrant carbon degradation. Studies that bridge scales in time and space are required to assess if there will be an attenuation or acceleration of soil carbon loss through changes in enzyme activities in the very long term.

4. We conclude that soil enzymes determine the sensitivity of soil carbon to warming, but that the microbial community and enzymatic traits that mediate this effect change over time. Improving representation of enzymes in soil carbon models requires long-term studies that characterize the response of wide-ranging hydrolytic and oxidative enzymatic traits – catalytic power, kinetics, inactivation – and the microbial community responses that govern enzyme synthesis.

Keywords: Carbon storage, Carbon-use efficiency, Climate change, Microbial ecology, Soil extracellular enzymes, Temperature sensitivity.

1. - Introduction

Atmospheric temperature has increased by more than 1°C since the 1900s, and is predicted to increase by another 2.7°C by 2100 (IPCC, 2021). The consequence of this global warming for soil carbon (C) storage is among the most important questions highlighted by many intergovernmental reports, notably because soils are the biggest sink of C in terrestrial ecosystems (Shukla et al., 2019). Given that microbes contribute significantly to organic matter cycling and long-term C stabilization in soils (Garcia-Palacios et al., 2021), it is essential to assess the direction and magnitude of global warming impacts on microbial communities and soil C cycling rates (Allison et al., 2010). Multiple lines of evidence suggest that rising temperatures may increase soil microbial activity across a variety of soil types and ecosystems (Chen et al., 2015; Xu & Yuan, 2017). Increased microbial activity can translate into accelerated decomposition rates, which in turn can release soil-derived CO_2 into the atmosphere and decrease soil C storage, contributing to a positive feedback on global warming (Bardgett et al., 2008). However, the highly uncertain responses of microbial communities to warming renders low confidence in the projections of carbon–climate feedbacks in global models (Sulman et al., 2018).

Soil enzymes, produced mainly by microorganisms, are one of the main limiting factors controlling the degradation of soil organic matter (Burns et al., 2013). Enzymes are generally present within microbial cells, associated with the microbial cell's plasma membrane or periplasmic space, or grouped into multi-enzyme extracellular complexes (cellulosomes). They are also present external to microbial cells, excreted into the aqueous soil solution, or stabilized in soils through interactions with organic matter and clay minerals (Fig. 1). Soil enzymes depolymerize high molecular weight organic compounds into smaller oligomers or monomers that are recognized by cell-wall receptors and transported into microbial cells. Because understanding organic matter degradation at a very fine scale is often necessary to estimate ecosystem functions at higher spatial scales (Allison et al., 2010; Bradford et al., 2021), studying changes in overall enzyme activities can help predict biogeochemical processes related to C, nitrogen (N), phosphorus (P) and sulfur cycling in terrestrial ecosystems.

A critical knowledge gap is the fine-scale factors controlling the temperature

sensitivity of enzymes. Changes in the backbone structure of isoenzymes and their high flexibility in the conformation of active sites allow enzymes to maintain high activity across a range of temperatures (Feller & Gerday, 2003). Moreover, the majority of cold-adapted enzymes exhibit high reaction rates (k_{cat}) by decreasing their energy of activation (EA_{cat}) at the expense of stability (Box 1) (Siddiqui & Cavicchioli, 2006). However, there are further potentially important factors to consider that have, until recently, been overlooked, either due to limitations in methodological approaches and/or absence of concrete evidence. Among them, thermal inactivation, catalytic power and adsorption-desorption mechanisms may all influence the response of enzymatic organic matter depolymerization to temperature (Alvarez et al., 2018). Changes in the structure, biomass and activity of microbial communities may also have repercussions on enzyme allocation or carbon use efficiency (CUE) (Geyer et al., 2016). However, whether microbes adapt to warming through physiological adjustments, where community 'adaptation' could arise at the species level or *via* community compositional change, is still under debate (Carey et al., 2016; Romero-Olivares et al., 2017; Walker et al., 2018).

Another major knowledge gap is how enzyme activities respond to experimental warming in field studies at the global scale, and how this response may affect soil organic carbon (SOC) stocks from short (days to months), to medium (years to decades), to long-term (centuries to millennia). Recent meta-analyses have demonstrated that enzyme responses to temperature vary with the duration and magnitude of warming (Chen et al., 2018; Meng et al., 2020). Generally, warming strongly increases the activity of hydrolases (e.g., cellulase) that catalyze the hydrolysis of glycosidic bonds in the short term, while oxidoreductases (e.g., ligninase) involved in the oxidative degradation of recalcitrant molecules often increase in the medium term (Chen et al., 2020). However, a survey of data from the literature highlights tremendous variations in enzyme responses to warming within the same climatic region, ranging from positive to negative (Table S1). Differences in mean annual temperature, soil moisture, oxygen, iron and C availability may all contribute to explaining this variability within and among studies (Xiao et al., 2018; Wen et al., 2019; Meng et al., 2020). This emphasizes the importance for improved understanding of both the environmental context and fine-scale mechanisms to better predict responses of enzymes to warming and their

impacts on SOC stocks at large scales.

In this review, our main objective is to highlight the potential effect of warming on soil enzymes, and how this, in turn, may affect SOC stocks across spatiotemporal scales. To this end, we first developed a lexicon of definitions to clarify and harmonize the main concepts and ideas across various disciplines encompassing enzymology, biogeochemistry, microbial ecology and soil ecology (Box 1). Because we hypothesize that fine-scale biochemical mechanisms may help explain variation in SOC cycling and stocks at large spatial scales, we review the effects of temperature on enzyme activity at the enzyme scale (Section 2) and at the microbial scale (Section 3). We highlight the main sources of uncertainties and propose new conceptual frameworks in each of these two sections. We then evaluate the potential repercussions of altered enzyme activities on SOC storage (Section 4). Finally, we provide new directions for improved integration of soil enzymes in models (Section 5) and identify key research priorities for further investigation (Section 6).

2. - Effects of temperature on enzyme activity at the enzyme scale

2.1 - Generalities about K_m, V_{max} and other factors in relation with temperature

In soils, enzyme-substrate complexes react to convert substrates (e.g., organic molecules) into products (Fig. 2A), releasing the enzyme to potentially catalyze more reactions. The velocity of this reaction is traditionally viewed as a saturating function of substrate concentration (C_s) and is often described by the Michaelis-Menten equation (Michaelis & Menten, 1913) (see also Section 5 for other related equations):

$$\text{Reaction Velocity, V (T)} = V_{max}\,(\text{T}) \cdot \frac{C_s}{K_m\,(\text{T}) + C_s} \qquad (1)$$

where V_{max} is the reaction velocity when the substrate concentration is not limiting and K_m is the half-saturation constant reflecting the affinity ($1/K_m$) of the enzyme for the substrate (Box 1). Both parameters are sensitive to temperature (T) and to determine its effect on reaction velocity, the temperature responses of both V_{max} and K_m are usually measured in short-term assays (from minute to hours). Under these conditions, V_{max} increases with temperature to an optimum, above which the reaction velocity decreases due to thermal inactivation of enzymes

(Fig. 2B). The parameter K_m also increases with temperature, indicating a reduction of enzyme affinity for substrate at higher temperatures (Razavi et al., 2015; Ma et al., 2017).

Short-term temperature responses of enzyme activities are often used to predict long-term responses of biocatalyzed reactions to warming (Davidson et al., 2012). For example, warming is expected to increase soil enzyme activities and C mineralization if the temperature optima of soil enzymes (V_{max}) exceed the temperatures usually observed *in situ* (Knorr et al., 2005). This prediction would be particularly true for organic-rich soils where reaction velocity is controlled more by the temperature response of V_{max} than K_m, as long as the substrate is accessible to the enzymes. However, an increase in K_m with temperature can compensate for an increase in V_{max} when substrate is limiting, leading to a weak net impact of temperature on reaction velocity (Razavi et al., 2015; Blagodatskaya et al., 2016). Therefore, the theory predicts that organic-matter poor soils are less sensitive to warming.

The short-term temperature responses of V_{max} and K_m are useful to assess the instantaneous potential activity in soils, but they are inadequate to describe long-term effects. For example, several ecosystem experiments observed a decline in CO_2 loss from warmed soils within a few years (Liski et al., 1999; Melillo et al., 2017), suggesting that C mineralization is driven by changes in enzyme activity and the sizes of C and enzymes pools, all of which may display distinct temperature responses over time. Consistently, enzyme assays conducted over long periods showed that temperature optima of reactions shifted to lower temperatures (Daniel et al., 2001; Alvarez et al., 2018). This shift can be explained by a slower thermal-inactivation of enzymes and longer persistence of enzyme activity at cold temperatures, which may also be affected by changes over time in the activity and composition of the microbes that synthesize them (see Section 3). These observations imply that, in field-scale studies and natural systems, the temperature optima of soil enzyme activity can vary over time.

A recent analysis of the temperature dependence of enzymatic systems demonstrated that the instantaneous temperature response of V_{max} is insufficient to model the long-term temperature response of bio-catalyzed reactions (Alvarez et al., 2018). The study identified that, by confounding the instantaneous V_{max} with cumulative activity over time, the positive

effect of warming on enzymatic reactions was overestimated. Therefore, describing the temperature responses of enzymatic reactions must include their time dependence.

2.2 - The catalytic power of enzymes and its response to temperature

The variable V_{max} describes the instantaneous enzymatic activity mediated by an enzyme pool. In nature, however, the enzymes released by microorganisms catalyze biochemical reactions until their complete inactivation, unless another factor limits the reaction. The total amount of matter processed by a pool of enzymes (e.g., soil C respired) is the cumulative activity of the enzyme pool until its complete degradation or turnover (Alvarez et al., 2018). The cumulative activity mediated by a single unit of enzymes is defined as its catalytic power (E_{power} in mole UE^{-1}) (Box 1). The standard E_{power} measured in normalized conditions (i.e., soil-free buffered solutions and excess of substrates) is determined by the following equation (Alvarez et al. 2018):

$$E_{power}(\mathrm{T}) = \frac{k_{cat}(\mathrm{T})}{k_{inact}(\mathrm{T})} \qquad (2)$$

where k_{cat} is the specific catalytic activity of the enzymes ($k_{cat} = V_{max}$ mediated by one unit of enzyme) and k_{inact} is the thermal inactivation rate. The parameters k_{cat} and k_{inact} usually increase with increasing temperature, but k_{inact} is assumed to have a steeper slope than k_{cat} for a wide range of enzymes (Fig. 2B) (Daniel et al., 2001; Alvarez et al., 2018). The relative temperature sensitivity of the E_{power} is determined by:

$$\frac{1}{E_{power}(\mathrm{T})} \cdot \frac{\mathrm{d}\,E_{power}(\mathrm{T})}{dT} = -\frac{(EA_{inact} - EA_{cat})}{RT^2} \qquad (3)$$

Thus, the catalytic power of enzymes monotonically varies with increasing temperature, depending on the sign of the difference in activation energies between enzyme inactivation and catalysis (EA_{inact} - EA_{cat}). Values of EA_{inact} and EA_{cat} vary greatly among enzymes, reflecting the flexibility of enzyme conformation structure and adaptation to thermal environment (Daniel et al., 2001; Alvarez et al., 2018). However, a universal pattern showing higher temperature sensitivity of inactivation than catalysis ($EA_{inact} > EA_{cat}$) for a wide range

of enzymes has been shown (Alvarez et al., 2018). Therefore, warming has a negative effect on the catalytic power of enzymes, which could explain the observed attenuation of warming effects on soil C mineralization as well as decreases in soil enzyme pools, microbial biomass and CUE reported in numerous warming experiments (Allison et al., 2010; Frey et al., 2013; Tucker et al., 2013).

2.3 - Temperature effects on enzyme activity through diffusive and adsorption/desorption processes

Microbes and their substrates are often spatially separated, implying that soil enzymatic activities are limited by the diffusion of enzymes and substrates (Fig. 1). Therefore, the responses of instantaneous and cumulative enzyme activities also depend on the effects of temperature on diffusive processes. The diffusion of water and solutes in a soil matrix increases with temperature due to higher Brownian movements and lower water viscosity (i.e., Stokes-Einstein law) (González Sánchez et al., 2008; Mon et al., 2016). The greater diffusion in soil under warming may thus promote encounters between enzymes and substrates, thereby increasing instantaneous and cumulative enzyme activities. Moreover, the temperature sensitivities of water and solute diffusion of soil minerals (EA ranging from 15 to 25 KJ) are on the same order of magnitude as the E_{power} of many enzymes (EA ranging from 15 to 279 KJ) (González Sánchez et al., 2008; Mon et al., 2016; Alvarez et al., 2018). However, the contribution of diffusion processes to the temperature responses of soil enzyme activity and mineralization rates has been overlooked and may further depend on soil moisture availability. In particular, although increasing temperature may increase diffusion when soil moisture is high, a decrease in soil water availability in response to warming may in turn decrease soil enzyme activities (Zuccarini et al., 2020).

Temperature may also affect soil enzymatic activities by affecting adsorption and desorption processes. Most enzymes and substrates adsorb onto soil particles and can be released due to changes in environmental conditions (Gianfreda & Bollag, 1996). For example, the equilibrium between adsorption and desorption shifts toward desorption with increasing temperature, because adsorption reactions are exergonic and have lower activation energies (Ten Hulscher & Cornelissen, 1996). Enzyme adsorption has been shown to reduce

their catalytic activity but increase their functional persistence due to the protection of clay minerals against degradation (Gianfreda & Bollag, 1996; Menezes-Blackburn et al., 2011). Desorption of enzymes and substrates increase the reaction velocity in the short-term by increasing catalytic activity (k_{cat}) and substrate concentration (Nannipieri et al., 1996; Wallenstein et al., 2011). In the medium-term, a lower enzyme persistence reducing the reaction velocity may decrease E_{power}. Collectively, these results indicate that the E_{power} of enzymes in soil can differ from the standard E_{power} measured in solution with excess substrate (Alvarez et al., 2018), and highlights the need for further studies estimating the temperature response of E_{power} under natural soil conditions. Furthermore, adsorption and desorption typically occur in solution, so that impacts of warming on soil water content may override temperature effects on these processes in drier soils, and this interaction should be considered as research priorities in future experiments.

3. - Effects of temperature on enzyme activity at the microbial scale

Further to the generally short-term direct effects of temperature on enzyme kinetics (Section 2), indirect effects can occur over the short- to long-term *via* changes in microbial physiology and microbial community structure (Fig. 3). In soils, temperature responses represent aggregated and emergent processes of the microbial community, where individual microbial populations may differentially respond to temperature changes. In the short term (i.e., instantaneous temperature response), physiological responses are the result of the combined effects on the enzymes involved in cell metabolism (i.e., anabolic and catabolic activities) and on adjustments in cellular physiology and metabolism through altered gene expression within individuals (i.e., acclimation) (Donhauser et al., 2021). In the long term, changes in temperature lead to shifts in microbial traits (i.e., community adaptation) that impact growth and survival through compositional changes of the microbial community (Malik et al., 2020). In this section, we discuss the effects of temperature on microbial biomass, CUE, microbial community structure and substrate-induced changes on microbial activity.

3.1 - Temperature effect on microbial biomass and activity

In general, an increase in temperature is expected to promote microbial activity and growth

(Fig. 3A) (Singh et al., 2010; Burns et al., 2013; Cavicchioli et al., 2019). Such an increase in microbial biomass, in turn, may increase enzyme synthesis because of both constitutive production and greater resource demand (Baldrian et al., 2013). However, elevated temperatures may also induce shifts in microbial growth strategy, with fewer resources allocated to enzyme production (Allison, 2014), resulting in a neutral response or even decreased enzymatic activities with an increase in temperature (Burns et al., 2013; Jaskulak & Grobelak, 2020). For instance, the activity of several extracellular enzymes decreased after almost three decades of warming (Liu et al., 2021a), leading to a lower investment in enzymes per unit of biomass (i.e., specific enzyme activity) (Fig 3B). This inconsistency has led to the conclusion that the effect of warming on enzymes are not universal, and that a finer understanding of the context of substrate decomposition is necessary to reveal the mechanisms of temperature control on enzyme synthesis (Singh et al., 2010).

3.2 - Temperature effect on carbon-use efficiency

Microbial carbon use efficiency (CUE) (or growth yield) provides a framework to connect microbial physiological changes to altered extracellular enzyme production (Box 1) (Geyer et al., 2016; Sinsabaugh et al., 2016) (Fig. 3A). According to theoretical considerations, microbial CUE is expected to decrease with increasing temperature (Mainzer & Hempfling, 1976; Hall & Cotner, 2007) (Fig. 3B), as respiration is considered to have a higher temperature sensitivity than growth (Allison et al., 2010). Although this pattern has often been confirmed experimentally and *via* modeling (Allison et al., 2010; Manzoni et al., 2012; Tucker et al., 2013; Allison, 2014; Alvarez et al., 2018), other studies found no effect (Hagerty et al., 2014; Walker et al., 2018; Simon et al., 2020), or even positive effects of increasing temperature on CUE, which may be the result of compositional shifts in the community at warmer temperatures in the longer-tern (Zheng et al., 2019) (see below). The rate-yield tradeoff conceptual framework suggests that microbes with greater investment in resource acquisition have lower CUE and *vice versa* (Allison, 2014). Alternatively, microbes that have a greater enzymatic capacity should process complex resources more rapidly but also incur relatively greater respiratory costs that reduce CUE. A decreased investment in enzyme production by microorganisms at higher temperatures may thus mask the expected

decrease of CUE (Allison, 2014; Cavicchioli et al., 2019). This should occur when respiratory costs increase faster than the benefits of enzyme production as temperatures rise.

3.3 - Temperature effect on microbial community structure and stoichiometry

The variable response of enzyme allocation and CUE to temperature may also depend on shifts in the microbial community structure (Domeignoz-Horta et al., 2020; Pold et al., 2020). Temperature-altered community structure may be linked to extracellular enzymatic capacity through the concept of microbial life history strategies (Malik et al., 2020). Microbial guilds may vary strongly in their functional abilities to produce enzymes (e.g., copiotrophic *versus* oligotrophic bacteria and fungi), both in terms of the types of enzymes (i.e., hydrolases versus oxidoreductases), and their costs of production (i.e., backbone structure of enzyme and metabolic costs) (Allison et al., 2010; Allison, 2014). Therefore, temperature-induced changes in the relative proportion of bacteria and fungi within the community can have consequences for enzyme allocation and CUE (Keiblinger et al., 2010; Reischke et al., 2014).

Enzyme activity can be affected by changes in the microbial community composition and their stoichiometric nutrient requirements. Several studies have found that Fungal:Bacterial (F:B) ratios increase in response to warming (Pritchard, 2011; Yuste et al., 2011), although increased cold resistance for fungal compared to bacterial growth has also been observed (Pietikäinen et al., 2005). An increase in the F:B ratio, in turn, is expected to increase community-level CUE and lower N-related enzyme allocation because fungi have lower nutrient requirements per C unit than bacteria (Keiblinger et al., 2010). Shifts in the microbial community composition resulting in an increased F:B ratio should also increase the C_{mic}:N_{mic} biomass ratio (Singh et al., 2010; Bragazza et al., 2013; Liu et al., 2021b), because fungi often present higher stoichiometric C:N:P ratios (Fanin et al., 2013; Mooshammer et al., 2014). As such, shifts in enzyme allocation due to changes in stoichiometric requirements often occur simultaneously with decreases in CUE (Sinsabaugh & Shah, 2012; Sinsabaugh et al., 2016; Manzoni et al., 2021) (Fig. 3B). However, these relationships may also depend on changes in substrate recalcitrance (Sinsabaugh & Shah, 2012; Margida et al., 2020); for example, whether microorganisms meet their C-demands from organic N compounds like proteins (Mori, 2020).

3.4 - Temperature effects on substrate availability

Warming will also have indirect effects on microbial communities by modifying resource availability and quality, in addition to the soil physical environment, where complex interactions and feedbacks occur between microbes, plants and soil (Bardgett et al., 2008; Singh et al., 2010). For instance, long-term warming can lead to depletion of the soil labile C pool (Singh et al., 2010; Burns et al., 2013; Walker et al., 2018) and immobilization of N (Sinsabaugh et al., 2017; Gao & Yan, 2019; Terrer et al., 2021), which in turn can increase N limitation to microbial activity (Singh et al., 2010; Liu et al., 2021b) and decrease organic matter quality (Pritchard, 2011; Bragazza et al., 2013). Changes in substrate availability and quality may also have consequences for the biomass and structure of microbial communities (Cavicchioli et al., 2019) and microbial community CUE (Keiblinger et al., 2010; Sinsabaugh et al., 2014), with efficiency declining as nutrient availability decreases and as substrate recalcitrance increases (Mooshammer et al., 2014; Margida et al., 2020). Taken together, these results highlight the need for considering both direct and indirect effects of temperature on microbial communities and their substrates to accurately predict the effects of warming on enzyme activities.

4 - Consequences of warming on soil carbon stocks

The sensitivity of soil C decomposition to warming (Fig. 4) can be viewed from the perspective of the temperature responses of enzymatic traits (k_{cat}, k_{inact}, K_m, E_{power}; Section 2, Fig. 2). These traits are further modified *via* the temperature responses of microbial community composition, growth and activity; in addition to organic matter inputs, availability and composition (related to plant productivity) and abiotic factors including mineral-stabilisation (Section 3, Fig. 3). The manner in which these enzymatic traits influence soil C under warming is strongly dependent on time-scale. We subsequently frame our discussion around short-term (days to months), medium-term (years to decades) and longer-term (centuries to millennial) effects of enzymes on soil C under warming (Fig. 4).

4.1 - The response of soil carbon and enzymes to short-term warming

Soil warming experiments consistently show an acceleration of soil CO_2 emission over the short-term (e.g., < 2 years) (Romero-Olivares et al., 2017). This short-term CO_2 emission increase is widely understood to be the result of increased microbial metabolic activity and increased catalytic activity (V_{max}) of enzymes present in the soil matrix which, together, increase the degradation of assimilable and labile organic C substrates (Phase 1; Fig. 4). This short-term sensitivity is well described by Arrhenius kinetics (see Section 2), which predicts that enzymatic activation energies (i.e., Q_{10} of V_{max}) are higher in cooler climates and for less reactive and more recalcitrant substrates (Davidson & Janssens, 2006). Arrhenius theory for enzymatic reactions is consistent with broad observations of increased enzyme activity and soil CO_2 emission in warming experiments (Table S1 and references therein). The theory is also consistent with observations of greater temperature sensitivity at higher latitudes and cooler climates, for both soil enzymes (e.g., for K_m in German *et al.*, 2012) and soil CO_2 emission (Carey et al., 2016), and by short-term incubation experiments showing increased Q_{10} for more recalcitrant substrates (Knorr et al., 2005; Craine et al., 2010). The support for Arrhenius theory to describe the temperature sensitivity of soil enzyme catalytic activity and CO_2 emission, has resulted in its widespread application in Earth System models to represent the sensitivity of soil C to warming (Todd-Brown et al., 2013) (see also Section 5 hereafter).

Importantly, however, Arrhenius theory often cannot explain soil C cycle responses to warming observed *in situ* and in long-term field experiments (Melillo et al., 2017; Nottingham et al., 2020). The theory does not predict enzymatic reaction responses due to changes in the microbial community (Karhu et al., 2014), *via* changes in plant inputs to soil (Melillo et al., 2011) or *via* abiotic processes and destabilisation of mineral-associated C (Doetterl et al., 2015). The theory is also inconsistent with reports of greater Q_{10} of V_{max} for hydrolytic enzymes than for oxidative enzymes (Nottingham et al., 2016; Tan et al., 2020), suggesting a greater short-term temperature sensitivity for more labile organic matter rather than more recalcitrant lignocellulose compounds (although the sensitivity of recalcitrant compounds appears to be greater in the longer-term) (Melillo et al., 2017; Chen et al., 2020). Further evidence that Arrhenius theory is insufficient to explain soil C cycling responses under field conditions comes from estimates for the short-term temperature sensitivity of soil respiration across global ecosystems (e.g., Q_{10} of 1.3-3.3, median 2.4) (Raich & Schlesinger,

1992), that consistently exceed the temperature sensitivity reported for hydrolytic enzymes (Q_{10} ranging by 1.5-2.3 across latitudinal gradients) (German et al., 2012; Allison et al., 2018). These differences in the observed temperature sensitivity of enzymatic V_{max} and CO_2 emission also reflect additional influences on enzymatic traits under field conditions, including substrate supply and moisture, that increase the apparent temperature sensitivity of respiration (Davidson et al., 2006). Furthermore, under field conditions, site-specific differences in nutrient availability and in enzyme pool sizes involved in C and nutrient-degradation can affect the magnitude and time-scale of the increase in enzyme activity and related soil CO_2 emission (i.e., altering the slope of Phase 1; Fig. 4).

4.2 - The response of soil carbon and enzymes to medium-term warming

From annual to decadal time-scales, soil C and the catalytic power of soil enzymes is increasingly influenced by changes in the composition and physiology of microbial communities, of plant communities and substrate inputs to soil, and by changes in the soil abiotic or geophysical environment. These medium-term effects of warming appear to occur in two distinct phases in the literature. Warming over the medium-term can result in a decline in enzyme activity and CO_2 emission due to substrate depletion (Phase 2a, Fig. 4), or an increase in activity and CO_2 emission *via* microbial community changes and increased capacity for lignin degradation (Phase 2b, Fig. 4). Although effects on enzyme systems *via* both substrate depletion and community change can occur concurrently, these two phases may also switch over time (Melillo et al., 2017). Regardless, the contribution of each of the two phases depend on initial C availability and C inputs (Walker et al., 2018; Terrer et al., 2021), which may also explain why the effects of warming on soil C stocks are strongly context-dependent.

The observed medium-term decline in the stimulation of soil CO_2 emissions following warming (Phase 2a; Fig. 4) (Romero-Olivares et al., 2017), has been explained by substrate limitation to decomposers (Hartley et al., 2007; Walker et al., 2018), exacerbated by increases in enzyme substrate affinity (K_m), which further constrains reaction rates and subsequent CO_2 emission (Razavi et al., 2015). Substrate depletion leads to a decline in microbial biomass and enzyme activities, which contributes to the attenuation of warming-induced soil CO_2

release over time (Walker et al., 2018). Another explanation for this medium-term decline in CO_2 emission is a decline in microbial CUE (Tucker et al., 2013), when the temperature sensitivity of respiration is greater than that of growth (Manzoni et al., 2012). This microbial CUE decline under warming has been further linked to a loss of enzyme catalytic power (E_{power}) because the temperature sensitivity of enzyme deactivation under warming is greater than that of synthesis (Alvarez et al., 2018). Together, these factors contribute towards a lower impact of warming *via* enzyme-mediated reactions in the medium-term (Phase 2a; Fig. 4).

Warming over decadal time-scales can also affect soil enzyme systems *via* changes in soil communities and can result in additional large losses of soil C (Phase 2b; Fig. 4). For instance, following 27 years of soil warming in a temperate forest, persistent losses of soil C occurred alongside a change in the microbial community composition and a four-fold increase in ligninase activity (Melillo et al., 2017). Similarly, 12 years of warming in a prairie ecosystem led to an increase in the respiration of slow-cycling C pools, microbial community change and increased abundance of genes involved in degrading complex organic matter (Feng et al., 2017). Alternatively, in a tropical forest ecosystem, 5 years of warming by translocating soil across a mountain gradient led to a decline in labile soil C pools, community composition change and increased activity of hydrolytic and oxidative enzymes (Nottingham et al., 2019). Indeed, this pattern of increased activity of lignin-degrading enzymes under warming is commonly observed in experiments, as reported in meta-analyses (Chen et al., 2018; Meng et al., 2020).

The increased enzymatic activity under year-to-decadal warming appears to be, in turn, related to increased efficiency of growth and/or CUE of the community (Feng et al., 2017; Melillo et al., 2017). In contrast to short-term warming experiments where CUE often declines, studies across biogeographical climate gradients have reported increases with warmer temperatures over the long-term. For example, a modelling study using a global soil data set found increased microbial CUE in warmer climates (Ye et al., 2019) and a study where soil microbial growth was measured across climate gradients found that growth was temperature adapted (i.e., relatively faster growth at higher temperatures for soils from warmer climates) (Bååth, 2018), as similarly observed for bacterial growth in montane

tropical forest soils after 11 years of warming via translocation (Nottingham et al., 2021). However, decadal-scale response of CUE may also be context dependent (e.g., on site or substrate). For example, in a temperate forest following 20 years of soil warming, CUE decreased overall (Li et al., 2019) but increased for the degradation of recalcitrant C substrates (Frey et al., 2013). Such physiological adaptation to warming of microbial community activity has been explained by changes in the community composition (Donhauser et al., 2020). For example, increased soil fungal:bacterial ratios, as observed under warming (Yuste et al., 2011), have been associated with higher community-level CUE (Keiblinger et al., 2010). Thus, CUE may decline in the short-term but, *via* compositional changes, increase in the longer-term (Fig. 3B), increasing metabolic and enzymatic activity and with negative implications for soil C stocks (Garcia-Palacios et al., 2021).

4.3 - The response of soil carbon and enzymes to long-term warming

Over century to millennial time-scales, soil C turnover and enzyme activities appear at quasi-equilibrium with climate and plant inputs, based on the observation of greater soil C accumulation at cooler temperatures across global temperature gradients (Post et al., 1982). Soil enzymatic traits reflect this equilibrium of soil C turnover, with higher activity of hydrolytic enzymes in ecosystems with greater C turnover (e.g., higher net primary production) and a shift in enzyme efficiency due to the temperature-adaptation of both microbial communities and the isoenzymes they synthesize (Wallenstein et al., 2011; Bååth, 2018). However, great uncertainty lies in whether such relationships are relevant to the warming predicted for the coming decades (Garcia-Palacios et al., 2021). On the one hand, rapid decadal warming may cause a persistent acceleration of enzyme activities and destabilization of soil C (Phase 3; Fig. 4). This soil C loss could be further exacerbated by priming effects, especially where warming increases NPP or coincides with increased atmospheric CO_2 (Terrer et al., 2021), whereby increased plant C-inputs to soil stimulates microbial activity and enzyme synthesis for nutrient acquisition, in the process degrading soil organic matter (Blagodatskaya & Kuzyakov, 2008). On the other hand, as observed across these long-term gradients in temperature, an equilibrium of C turnover may eventually occur whereby soil C loss is balanced by inputs from plants or is mediated by acclimation responses

of microbes and the isoenzymes they synthesise. Reconciling these countervailing effects requires further empirical information on the response of microbial communities and soil enzymes from field experiments at wide spatial and temporal scales.

5 - Integrating soil enzymes into models to predict temperature effects on soil C cycling

Experimental evidence shows a strong dependence of enzyme activity on soil C, which varies over time (Fig. 4). Given this strong dependency, how effectively have soil enzymes been represented in models to predict warming effects on soil C? The rationale, development, and limitations of enzyme-driven decomposition models have been discussed in several recent reviews (Manzoni & Porporato, 2009; Todd-Brown et al., 2012; Wieder et al., 2015). In brief, adding temperature-sensitive, enzymatic processes increases the potential realism of simulated ecosystem-level responses but requires more model parameters and supporting data (Sulman et al., 2018; Wang & Allison, 2019). Herein, we focus attention on quantifying the fine scale activities of extracellular enzymes responsible for the catalysis of dead organic matter and possible responses to temperature as well as key environmental constraints.

5.1 - Conceptual foundations

The ecoenzymatic stoichiometric theory provides an underlying conceptual framework for enzyme-based decomposition models and a central equation quantifying relationships between fundamental controls (Sinsabaugh & Shah, 2012):

$$EEA_{C:X} = \frac{A_{C:X}}{CUE} \cdot \frac{B_{C:X}}{L_{C:X}} \qquad (4)$$

The extracellular enzyme activities (EEA) associated with the acquisition of C and other (X) nutrients (C:X), are determined by the stoichiometry of microbial biomass ($B_{C:X}$) and available substrate ($L_{C:X}$), constrained by resource use efficiencies for C (CUE) and X (A_X). Decay rates for particular substrates can be approximated by EEA assuming these activities scale with the catalysis of these substrates.

Enzyme-driven models typically use the Michaelis-Menten (MM) equation to estimate the catalysis of soluble substrates by soluble enzymes (see Section 2), the Reverse

Michaelis-Menten (RMM) equation for insoluble substrates catalyzed by soluble enzymes, or the Equilibrium Chemistry Approximation (ECA) equation that integrates both reactions (Tang, 2015; Tang & Riley, 2019; Wang & Allison, 2019):

$$\frac{\mathrm{dS}}{\mathrm{dt}} = \frac{(V_{max} \cdot C_s \cdot \mathrm{EEA})}{(K_m + C_s + \mathrm{EEA})} \qquad (5)$$

The ECA equation (eq. 5) saturates on both substrate (C_s) and extracellular enzyme activities (EEA) whereas the MM equation saturates on C_s and the RMM saturates on EEA, with the relative merits of each equation reviewed elsewhere (Wang & Post, 2013; Moorhead & Weintraub, 2018; Tang & Riley, 2019). Additional syntheses have shown that the kinetic coefficients (V_{max} and K_m) scale with microbial biomass, metabolism, stoichiometry and resource availability (Sinsabaugh et al., 2014; Sinsabaugh et al., 2015), consistent with the ecoenzymatic stoichiometric theory.

Within this modeling framework, the most direct effects of warming include changes in enzyme and/or substrate concentrations and catalysis rates per unit enzyme (Davidson & Janssens, 2006; Pold et al., 2017). These effects are likely to manifest as changes in the apparent kinetics of enzyme-catalyzed reactions, e.g., V_{max} or K_m (Fig. 1, Box 1) and are usually simulated as Q_{10} or Arrhenius functions modifying overall reaction rates (dS/dt) or the underlying kinetic coefficients (Davidson et al., 2012; Sihi et al., 2016):

$$p = \mathrm{a} \cdot \mathrm{e}^{\left(\frac{-EA_{cat}}{[\mathrm{R} \cdot \mathrm{T}]}\right)} \qquad (6)$$

where the parameter (p) is estimated as an Arrhenius function given a coefficient (a), activation energy (EA_{cat}), universal gas constant (R) and temperature (T). Although this combination of thermodynamic controls (eq. 6) on biochemical mechanisms (eq. 5) seems straightforward, interactions between key controls (eq. 4) are a prominent feature of contemporary enzyme-driven decomposition models.

5.2 - Temperature effects on substrate-enzyme interactions

Earlier discussions of temperature effects on the kinetic coefficients (V_{max} and K_m) of enzyme-substrate reactions (Section 2.1) and the diffusion and adsorption of enzymes in soils

(Section 2.3), may be especially relevant to simulating the catalysis of insoluble substrates because their surface features influence enzyme adsorption and activity (Jeoh et al., 2017; Nill & Jeoh, 2020) in ways that soluble substrates do not. For example, Kari et al., (2017) showed that the kinetic parameters for cellulase-cellulose hydrolysis were determined by the density of surface binding sites instead of the mass of cellulose. Binding sites also are constrained by structural features of the cellulose fibril, such as the degree of polymerization and links to hemicellulose and lignin (Jeoh et al., 2017; Kari et al., 2017; Nill & Jeoh, 2020). It is not clear how temperature affects the mechanisms of enzyme adsorption on solid substrates because reports are inconsistent and complicated by non-productive binding to both target and non-target substrates (Baig, 2020). However, both the ECA and RMM equations can explicitly represent the availability and saturation of binding sites, as well as temperature effects on kinetic parameters.

In addition to temperature effects on individual enzyme-substrate interactions, several decomposition models also include multiple substrate pools, which can exhibit differential sensitivities to temperature (Davidson & Janssens, 2006; Allison et al., 2018; Alvarez et al., 2018). Two of the most common forms of substrate control are their relative resistances to decay and nutrient contents. For example, microorganisms may preferentially use less recalcitrant substrates with higher resource use efficiencies (e.g., CUE in eq. 4) and thus generate higher enzyme activities for those substrates (Margida et al., 2020). However, substrates with higher activation energies (eq. 6) can have higher temperature sensitivity, thus altering the relative decay rates of various substrates as temperatures change (Davidson & Janssens, 2006). Differences in substrate nutrient content also affects their relative decay rates as microbes balance stoichiometric needs (eq. 4), such as C and N from multiple substrates (Manzoni et al., 2021). Again, temperature changes can differently affect enzymes associated with C versus N acquisition (Lehmeier et al., 2013; Tan et al., 2020), potentially altering the balance of C and N-acquisition. Models that consider both stoichiometry and recalcitrance of substrates must include potential shifts in microbial demands and concomitant enzyme activities (see Section 3) with temperature, as overall resource limitations vary between different forms of C and nutrients (Sinsabaugh & Shah, 2011).

5.3 - Current modeling challenges

Several recent models use enzyme activities to simulate soil organic C dynamics. Most include relatively few types of enzymes or substrates that represent broad classes of both. One of the simplest is the MEND model (Microbial-ENzyme-mediated Decomposition; Wang, Post & Mayes 2013) which uses MM equations to simulate the activities of two generic enzyme pools produced by microorganisms, one that degrades particulate organic C and another that degrades mineral-associated organic C. However, even relatively simple models are difficult to calibrate (Schimel & Weintraub, 2003; Todd-Brown et al., 2012; Wieder et al., 2015; Sulman et al., 2018; Wang & Allison, 2019), particularly when parameters are used to estimate aggregated processes (Wang & Post, 2012). In subsequent studies, Li et al., (2019) and Jian et al., (2020) used data from field and laboratory experiments, respectively, to refine estimates of MEND parameters, and in turn predict changes in soil C with warming. This approach produced reasonable results but risks the pitfalls of aggregation schemes discussed by Bradford et al., (2021), in that underlying controls can be masked by the aggregation. For MEND and models using similar substrate definitions (see above reviews), this is a likely problem because organic matter varies in chemical composition and needs different enzymes to degrade. Fatichi et al., (2019) addressed this limitation in part by dividing the particulate organic C pool into polysaccharide and polyphenol components that were degraded by different enzymes. However, polysaccharides and polyphenols, particularly lignocellulose, do not decay independently and interact to influence patterns of enzyme expression (Margida et al., 2020).

In contrast to models that simulate activities of only a few enzymes, the DEMENT model (Decomposition Model of Enzymatic Traits; Allison 2012) selects traits for a population of microorganisms from an array of enzyme types driving MM kinetics operating on a range of substrates to establish communities, which in turn drive decomposition as a consequence of the selected traits. The model has been used to evaluate the effects of drought tolerance and temperature on decomposition (Allison & Goulden, 2017; Pold et al., 2019), and compare the efficacy of the MM, RMM and ECA equations (Wang & Allison, 2019). DEMENT greatly reduces the likelihood of obscuring microbial-level controls on emergent system behavior, such as decomposition, and provides a framework that might be able to

integrate synergisms among enzymes. However, assumed relationships for the underlying tradeoffs between traits may represent aggregative responses that are not consistent across trait combinations. The model also operates at a spatially explicit microbial scale that is not directly applicable to global scale C fluxes. However, it evaluates microbial-scale behaviors that are directly relevant to broad scale patterns in soil C. Thus, DEMENT is a process-level tool that may be used to evaluate causative relationships at fine scales (Bradford et al., 2021).

Although we focused on fine-scale modelling of soil enzyme activity herein, a fundamental challenge to simulating the effects of climate warming on soil enzymes is that enzyme-catalyzed reactions occur at the scale of molecular interactions whereas questions about soil warming usually focus on broader scales in time and space. Section 4 explained that short-, medium-, and long-term responses of soil enzyme activities to warming differ in context and controls and thus, the models addressing different scales need different formulations (Wieder et al., 2015; Sulman et al., 2018; Wang & Allison, 2019). This contrast illustrates the conundrum discussed by Bradford et al. (2021) in that aggregating processes across scales risks masking important underlying mechanisms, but simulating detailed processes across broad scales requires knowledge and parameter sets that seldom exist (Todd-Brown et al., 2012). Current modeling efforts seek to balance these two constraints given the question of interest defining modeling goals (e.g., MEND, DEMENT).

6. - Scientific advances, synergies and research priorities

Given the various lines of theory and experimental evidence that underpin our understanding of how temperature affects both simple enzyme systems and soil processes *in situ*, scaling responses across spatial and temporal scales remains a challenge. This problem of scaling limits our ability to yield quantitative predictions regarding the magnitude and sometimes even the direction of feedbacks between climate change and soils. Furthermore, current models are effectively restricted to fine scales and are prone to overestimating enzyme responses when compared to experimental field data. It is therefore clear that we lack empirical understanding of the interrelated biotic and abiotic constraints on soil enzymes. Recent studies have attempted to address this problem, for example by characterizing guilds within the microbial community that are inherently associated with different enzymatic traits

that may correlate with soil C storage traits such as CUE (Hagerty et al., 2018; Malik et al., 2020). However, modelling the response of microbial community guilds to the diverse feedbacks of climatic disturbances is not easy, as the complexity of networked interactions and feedbacks at the molecular and community level are still poorly understood and challenging to represent in current Earth system models. Thus, improved representation of enzymes in soil C models is needed and we propose three key research priorities that may help predict the warming effect on soil enzymes and soil C stocks from the short to the long-term.

(i) Bridging scales in time and space

To improve model predictions, further study of direct (e.g., *via* response of V_{max}, K_m, E_{power}) and indirect (e.g., *via* CUE and community changes) drivers of enzymes and soil C under warming are needed. In particular, more studies are required using standardized methods that bridge scales in time and space, encompassing ecosystem properties (e.g., across gradients in NPP and rainfall) and soils (e.g., across gradients in soil weathering) where the relative importance of diffusion and desorption on enzyme catalytic power may widely differ. This breadth of spatial- and temporal-scales can be achieved by combining laboratory incubation studies assessing short-term responses at high spatial replication (Craine et al., 2010; Bradford et al., 2019), alongside *in situ* warming experimental studies and natural temperature gradients assessing long-term responses (Blagodatskaya et al., 2016; Nottingham et al., 2016; Melillo et al., 2017; Walker et al., 2018). Within this framework, wide biogeographical representation is required with improved standardisation of methods. For example, there are several remaining methodological challenges in the quantification of oxidative enzymes - including the applied substrate and buffer conditions (Bach et al., 2013) - and the separation of biotic and abiotic contributions to their activity (e.g., Sanchez-Julia & Turner, 2021). Addressing these methodological issues will improve analytical power across these studies and, in turn, our understanding across these wider scales.

(ii) Identifying functional traits using an 'omics' approach

Because enzymes correspond to genes across various lineages of living organisms, using

'omics' data may help link phylotypes to specific enzyme activities. For instance, Feng *et al*. (2017) recently demonstrated that the diversity of C-degradation genes declined with warming at the expense of microbial genes involved in degrading complex organic compounds, suggesting shifts in microbial guilds as substrate quality decreases. In both terrestrial and marine environments, specific microbial species or microbial guilds are correlated with particular habitats, C storage traits, nutrient status, or even different gas emissions to the atmosphere (Clemmensen et al., 2013; Chu et al., 2020). Such trait gradients could then be augmented with a systems biology or 'omics' approach linking organismal and functional gene diversities, e.g., for enzymes to link metabolism to terrestrial ecosystem function. Thus, combining metabarcoding, metagenomics and metatranscriptomics data alongside metabolite and protein analyses could provide valuable information for enzyme-driven Earth system models (Trivedi et al., 2016). Such a genome-scale description could be used to discover new genomes or genes associated with variations in functional traits such as CUE or community-scale Q_{10} values. Identifying key organisms or genes varying across different ecological niches could provide a bridge to using metabolic network as a proxy for emulating biogeochemical cycles and deciphering mechanistic interactions between species.

(iii) Visualizing emerging patterns at the global scale using biogeography

One major transformation in ecology and soil science is being driven by the recent availability of '*big data*' in large public databases covering different temporal and spatial scales for thousands of organisms and processes, spanning from genes to ecosystems. In this context, there are a growing number of studies that have emulated the distribution of organisms such as bacteria, fungi, soil fauna and plants over the land surface, using models constructed from geo-referenced inventories, describing the presence of species and abiotic or biotic characteristics that describe the 'niche' occupied by these species (e.g., Tedersoo et al., 2014). These niches are constructed from open access georeferenced datasets that are becoming increasingly available, describing climate, soil properties and land use obtained from experiment measurements, remotely sensed products and even outputs from climate or Earth System models. For example, such datasets have been used to understand the emergent drivers of symbiotic relationships between plants and belowground communities, and of

ecosystem C storage (Steidinger et al., 2019). Thus, adopting a 'niche-level' approach may pave the way to elucidating important general emerging features of metabolic (i.e., of enzyme systems) and community interactions across different biomes (Chu et al., 2020).

7. - Conclusions

The action of soil enzymes underpins the terrestrial C cycle, and biogeochemical cycling more broadly, by transforming organic matter to assimilable forms for biotic uptake and growth. Despite the fundamental nature of these processes and our long-standing recognition of their importance (Burns, 1978), only relatively recently has information emerged to demonstrate their importance at larger scales (Sulman et al., 2018), and how they may alter terrestrial C storage under climatic change in the coming decades (Melillo et al., 2017; Chen et al., 2020). However, extrapolating molecular-scale protein-substrate interactions to the global-scale brings new challenges associated with scaling, which can be addressed by the implementation of experiments spanning wide spatio-temporal scales, new approaches to characterize coupled microbial community and enzymatic traits, and big data approaches to increase analytical power and standardized methods to better inform models. Together these approaches will lead us to a step-change in our understanding of how soil enzymes affect terrestrial C dynamics under a changing climate.

8 - References

Allison, S. (2012) A trait‐based approach for modelling microbial litter decomposition. *Ecology Letters, 15*, 1058-1070. https://doi.org/10.1111/j.1461-0248.2012.01807.x

Allison, S.D. (2014) Modeling adaptation of carbon use efficiency in microbial communities. *Frontiers in Microbiology, 5*, 571. https://doi.org/10.3389/fmicb.2014.00571

Allison, S.D. & Goulden, M.L. (2017) Consequences of drought tolerance traits for microbial decomposition in the DEMENT model. *Soil Biology and Biochemistry, 107*, 104-113. https://doi.org/10.1016/j.soilbio.2017.01.001

Allison, S.D., Romero-Olivares, A.L., Lu, Y., Taylor, J.W. & Treseder, K.K. (2018) Temperature sensitivities of extracellular enzyme V-max and K-m across thermal environments. *Global Change Biology, 24*, 2884-2897. https://doi.org/10.1111/gcb.14045

Allison, S.D., Wallenstein, M.D. & Bradford, M.A. (2010) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience, 3*, 336-340. https://doi.org/10.1038/ngeo846

Alvarez, G., Shahzad, T., Andanson, L., Bahn, M., Wallenstein, M.D. & Fontaine, S. (2018) Catalytic power of enzymes decreases with temperature: New insights for understanding soil C cycling and microbial ecology under warming. *Global Change Biology, 24*, 4238-4250. https://doi.org/10.1111/gcb.14281

Bååth, E. (2018) Temperature sensitivity of soil microbial activity modeled by the square root equation as a unifying model to differentiate between direct temperature effects and microbial community adaptation. *Global Change Biology, 24*, 2850-2861. https://doi.org/10.1111/gcb.14285

Bach, C.E., Warnock, D.D., Van Horn, D.J., Weintraub, M.N., Sinsabaugh, R.L., Allison, S.D. & German, D.P. (2013) Measuring phenol oxidase and peroxidase activities with pyrogallol, L-DOPA, and ABTS: effect of assay conditions and soil type. *Soil Biology and Biochemistry, 67*, 183-191. https://doi.org/10.1016/j.soilbio.2013.08.022

Baig, K. (2020) Interaction of enzymes with lignocellulosic materials: causes, mechanism and influencing factors. *Bioresources and Bioprocessing, 7*, 1-19. https://doi.org/10.1186/s40643-020-00310-0

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T. & Valášková, V. (2013) Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. *Soil Biology and Biochemistry, 56*, 60-68. https://doi.org/10.1016/j.soilbio.2012.01.020

Bardgett, R.D., Freeman, C. & Ostle, N.J. (2008) Microbial contributions to climate change through carbon cycle feedbacks. *The ISME journal, 2*, 805-814. https://doi.org/10.1038/ismej.2008.58

Blagodatskaya, E. & Kuzyakov, Y. (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils, 45*, 115-131. https://doi.org/10.1007/s00374-008-0334-y

Blagodatskaya, E., Blagodatsky, S., Khomyakov, N., Myachina, O. & Kuzyakov, Y. (2016) Temperature sensitivity and enzymatic mechanisms of soil organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro. *Scientific Reports, 6*, 22240. https://doi.org/10.1038/srep22240

Bradford, M.A., McCulley, R.L., Crowther, T.W., Oldfield, E.E., Wood, S.A. & Fierer, N. (2019) Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation. *Nature Ecology & Evolution, 3*, 223-+. https://doi.org/10.1038/s41559-018-0771-4

Bradford, M.A., Wood, S.A., Addicott, E.T., Fenichel, E.P., Fields, N., González-Rivero, J., Jevon, F.V.,

Maynard, D.S., Oldfield, E.E. & Polussa, A. (2021) Quantifying microbial control of soil organic matter dynamics at macrosystem scales. *Biogeochemistry*, 1-22. https://doi.org/10.1007/s10533-021-00789-5

Bragazza, L., Parisod, J., Buttler, A. & Bardgett, R.D. (2013) Biogeochemical plant–soil microbe feedback in response to climate warming in peatlands. *Nature Climate Change, 3*, 273-277. https://doi.org/10.1038/nclimate1781

Burns, R.G. (1978) Enzyme activity in soil: some theoretical and practical considerations. *Soil enzymes*.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N. & Zoppini, A. (2013) Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry, 58*, 216-234. https://doi.org/10.1016/j.soilbio.2012.11.009

Carey, J.C., Tang, J.W., Templer, P.H., Kroeger, K.D., Crowther, T.W., Burton, A.J., Dukes, J.S., Emmett, B., Frey, S.D., Heskel, M.A., Jiang, L., Machmuller, M.B., Mohan, J., Panetta, A.M., Reich, P.B., Reinsch, S., Wang, X., Allison, S.D., Bamminger, C., Bridgham, S., Collins, S.L., De Dato, G., Eddy, W.C., Enquist, B.J., Estiarte, M., Harte, J., Henderson, A., Johnson, B.R., Larsen, K.S., Luo, Y., Marhan, S., Melillo, J.M., Peuelas, J., Pfeifer-Meister, L., Poll, C., Rastetter, E., Reinmann, A.B., Reynolds, L.L., Schmidt, I.K., Shaver, G.R., Strong, A.L., Suseela, V. & Tietema, A. (2016) Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings of the National Academy of Sciences of the United States of America, 113*, 13797-13802. https://doi.org/10.1073/pnas.160536511

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W. & Classen, A.T. (2019) Scientists' warning to humanity: microorganisms and climate change. *Nature reviews microbiology, 17*, 569-586. https://doi.org/10.1038/s41579-019-0222-5

Chen, J., Elsgaard, L., van Groenigen, K.J., Olesen, J.E., Liang, Z., Jiang, Y., Lærke, P.E., Zhang, Y., Luo, Y. & Hungate, B.A. (2020) Soil carbon loss with warming: New evidence from carbon‐degrading enzymes. *Global Change Biology, 26*, 1944-1952. https://doi.org/10.1111/gcb.14986

Chen, J., Luo, Y., Xia, J., Jiang, L., Zhou, X., Lu, M., Liang, J., Shi, Z., Shelton, S. & Cao, J. (2015) Stronger warming effects on microbial abundances in colder regions. *Scientific Reports, 5*, 1-10. https://doi.org/10.1038/srep18032

Chen, J., Luo, Y.Q., Garcia-Palacios, P., Cao, J.J., Dacal, M., Zhou, X.H., Li, J.W., Xia, J.Y., Niu, S.L., Yang, H.Y., Shelton, S., Guo, W. & van Groenigen, K.J. (2018) Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. *Global Change Biology, 24*, 4816-4826.

https://doi.org/10.1111/gcb.14394

Chu, H., Gao, G.-F., Ma, Y., Fan, K. & Delgado-Baquerizo, M. (2020) Soil microbial biogeography in a changing world: recent advances and future perspectives. *MSystems, 5*, e00803-00819. https://doi.org/10.1128/mSystems.00803-19

Clemmensen, K., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R., Wardle, D. & Lindahl, B. (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science, 339*, 1615-1618. https://doi.org/10.1126/science.1231923

Craine, J.M., Fierer, N. & McLauchlan, K.K. (2010) Widespread coupling between the rate and temperature sensitivity of organic matter decay. *Nature Geoscience, 3*, 854-857. https://doi.org/10.1038/Ngeo1009

Daniel, R.M., Danson, M.J. & Eisenthal, R. (2001) The temperature optima of enzymes: a new perspective on an old phenomenon. *Trends in biochemical sciences, 26*, 223-225. https://doi.org/10.1016/S0968-0004(01)01803-5

Davidson, E.A. & Janssens, I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature, 440*, 165-173. https://doi.org/10.1038/nature04514

Davidson, E.A., Janssens, I.A. & Luo, Y.Q. (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q(10). *Global Change Biology, 12*, 154-164. https://doi.org/10.1111/j.1365-2486.2005.01065.x

Davidson, E.A., Samanta, S., Caramori, S.S. & Savage, K. (2012) The Dual Arrhenius and Michaelis–Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. *Global Change Biology, 18*, 371-384. https://doi.org/10.1111/j.1365-2486.2011.02546.x

Doetterl, S., Stevens, A., Six, J., Merckx, R., Van Oost, K., Pinto, M.C., Casanova-Katny, A., Munoz, C., Boudin, M., Venegas, E.Z. & Boeckx, P. (2015) Soil carbon storage controlled by interactions between geochemistry and climate. *Nature Geoscience, 8*, 780-783. https://doi.org/10.1038/Ngeo2516

Domeignoz-Horta, L.A., Pold, G., Liu, X.-J.A., Frey, S.D., Melillo, J.M. & DeAngelis, K.M. (2020) Microbial diversity drives carbon use efficiency in a model soil. *Nature communications, 11*, 1-10. https://doi.org/10.1038/s41467-020-17502-z

Donhauser, J., Niklaus, P.A., Rousk, J., Larose, C. & Frey, B. (2020) Temperatures beyond the community optimum promote the dominance of heat-adapted, fast growing and stress resistant bacteria in alpine soils. *Soil Biology and Biochemistry, 148*, 107873. https://doi.org/10.1016/j.soilbio.2020.107873

Donhauser, J., Qi, W., Bergk‐Pinto, B. & Frey, B. (2021) High temperatures enhance the microbial genetic

potential to recycle C and N from necromass in high‐mountain soils. *Global Change Biology, 27*, 1365-1386. https://doi.org/10.1111/gcb.15492

Fanin, N., Fromin, N., Buatois, B. & Hättenschwiler, S. (2013) An experimental test of the hypothesis of non‐homeostatic consumer stoichiometry in a plant litter–microbe system. *Ecology Letters, 16*, 764-772. https://doi.org/10.1111/ele.12108

Fatichi, S., Manzoni, S., Or, D. & Paschalis, A. (2019) A mechanistic model of microbially mediated soil biogeochemical processes: a reality check. *Global Biogeochemical Cycles, 33*, 620-648. https://doi.org/10.1029/2018GB006077

Feller, G. & Gerday, C. (2003) Psychrophilic enzymes: hot topics in cold adaptation. *Nature reviews microbiology, 1*, 200-208. https://doi.org/10.1038/nrmicro773

Feng, W.T., Liang, J.Y., Hale, L.E., Jung, C.G., Chen, J., Zhou, J.Z., Xu, M.G., Yuan, M.T., Wu, L.Y., Bracho, R., Pegoraro, E., Schuur, E.A.G. & Luo, Y.Q. (2017) Enhanced decomposition of stable soil organic carbon and microbial catabolic potentials by long-term field warming. *Global Change Biology, 23*, 4765-4776. https://doi.org/10.1111/gcb.13755

Frey, S.D., Lee, J., Melillo, J.M. & Six, J. (2013) The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change, 3*, 395-398. https://doi.org/10.1038/Nclimate1796

Gao, W. & Yan, D. (2019) Warming suppresses microbial biomass but enhances N recycling. *Soil Biology and Biochemistry, 131*, 111-118. https://doi.org/10.1016/j.soilbio.2019.01.002

Garcia-Palacios, P., Crowther, T.W., Dacal, M., Hartley, L.P., Reinsch, S., Rinna, R., Rousk, J., van den Hoogen, J., Ye, J.S. & Bradford, M.A. (2021) Evidence for large microbial-mediated losses of soil carbon under anthropogenic warming. *Nature Reviews Earth & Environment*. https://doi.org/10.1038/s43017-021-00178-4

German, D.P., Marcelo, K.R.B., Stone, M.M. & Allison, S.D. (2012) The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. *Global Change Biology, 18*, 1468-1479. https://doi.org/10.1111/j.1365-2486.2011.02615.x

Geyer, K.M., Kyker-Snowman, E., Grandy, A.S. & Frey, S.D. (2016) Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry, 127*, 173-188. https://doi.org/10.1007/s10533-016-0191-y

Gianfreda, L. & Bollag, J. (1996) Influence of natural and anthropogenic factors on enzyme activity in soil. *Soil Biochemistry Vol. 9* (eds G. Stotzky & J.M. Bollag), pp. 123-193. Marcel Dekker, New York.

González Sánchez, F., Jurányi, F., Gimmi, T., Van Loon, L., Unruh, T. & Diamond, L.W. (2008) Translational diffusion of water and its dependence on temperature in charged and uncharged clays: A neutron scattering study. *The Journal of chemical physics, 129*, 174706. https://doi.org/10.1063/1.3000638

Hagerty, S.B., Allison, S.D. & Schimel, J.P. (2018) Evaluating soil microbial carbon use efficiency explicitly as a function of cellular processes: implications for measurements and models. *Biogeochemistry, 140*, 269-283. https://doi.org/10.1007/s10533-018-0489-z

Hagerty, S.B., Van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W., Kolka, R.K. & Dijkstra, P. (2014) Accelerated microbial turnover but constant growth efficiency with warming in soil. *Nature Climate Change, 4*, 903-906. https://doi.org/10.1038/nclimate2361

Hall, E.K. & Cotner, J.B. (2007) Interactive effect of temperature and resources on carbon cycling by freshwater bacterioplankton communities. *Aquatic Microbial Ecology, 49*, 35-45. https://doi.org/10.3354/ame01124

Hartley, I.P., Heinemeyer, A. & Ineson, P. (2007) Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology, 13*, 1761-1770. https://doi.org/10.1111/j.1365-2486.2007.01373.x

IPCC (2021) Summary for Policymakers. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (eds V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu & B. Zhou). Cambridge University Press.

Jaskulak, M. & Grobelak, A. (2020) Soil enzymes in a changing climate. *Climate Change and Soil Interactions* (eds M.N.V. Prasad & M. Pietrzykowski), pp. 731-749. Elsevier, Amsterdam.

Jeoh, T., Cardona, M.J., Karuna, N., Mudinoor, A.R. & Nill, J. (2017) Mechanistic kinetic models of enzymatic cellulose hydrolysis—a review. *Biotechnology and bioengineering, 114*, 1369-1385. https://doi.org/10.1002/bit.26277

Jian, S., Li, J., Wang, G., Kluber, L.A., Schadt, C.W., Liang, J. & Mayes, M.A. (2020) Multi-year incubation experiments boost confidence in model projections of long-term soil carbon dynamics. *Nature communications, 11*, 1-9. https://doi.org/10.1038/s41467-020-19428-y

Karhu, K., Auffret, M.D., Dungait, J.A.J., Hopkins, D.W., Prosser, J.I., Singh, B.K., Subke, J.A., Wookey, P.A., Agren, G.I., Sebastia, M.T., Gouriveau, F., Bergkvist, G., Meir, P., Nottingham, A.T., Salinas, N. &

Hartley, I.P. (2014) Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature, 513*, 81-84. https://doi.org/10.1038/nature13604

Kari, J., Andersen, M., Borch, K. & Westh, P. (2017) An inverse Michaelis–Menten approach for interfacial enzyme kinetics. *Acs Catalysis, 7*, 4904-4914. https://doi.org/10.1021/acscatal.7b00838

Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K. & Richter, A. (2010) The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. *FEMS microbiology ecology, 73*, 430-440. https://doi.org/10.1111/j.1574-6941.2010.00912.x

Knorr, W., Prentice, I.C., House, J.I. & Holland, E.A. (2005) Long-term sensitivity of soil carbon turnover to warming. *Nature, 433*, 298-301. https://doi.org/10.1038/nature03226

Lehmeier, C.A., Min, K., Niehues, N.D., Ballantyne IV, F. & Billings, S.A. (2013) Temperature-mediated changes of exoenzyme-substrate reaction rates and their consequences for the carbon to nitrogen flow ratio of liberated resources. *Soil Biology and Biochemistry, 57*, 374-382. https://doi.org/10.1016/j.soilbio.2012.10.030

Li, J., Wang, G., Mayes, M.A., Allison, S.D., Frey, S.D., Shi, Z., Hu, X.M., Luo, Y. & Melillo, J.M. (2019) Reduced carbon use efficiency and increased microbial turnover with soil warming. *Global Change Biology, 25*, 900-910. https://doi.org/10.1111/gcb.14517

Liski, J., Ilvesniemi, H., Mäkelä, A. & Westman, C.J. (1999) CO□ emissions from soil in response to climatic warming are overestimated: the decomposition of old soil organic matter is tolerant of temperature. *Ambio*, 171-174.

Liu, X.J.A., Pold, G., Domeignoz-Horta, L.A., Geyer, K.M., Caris, H., Nicolson, H., Kemner, K.M., Frey, S.D., Melillo, J.M. & DeAngelis, K.M. (2021a) Soil aggregate-mediated microbial responses to long-term warming. *Soil Biology and Biochemistry, 152*, 108055. https://doi.org/10.1016/j.soilbio.2020.108055

Liu, Z., Liu, X., Wu, X., Bian, R., Liu, X., Zheng, J., Zhang, X., Cheng, K., Li, L. & Pan, G. (2021b) Long-term elevated CO_2 and warming enhance microbial necromass carbon accumulation in a paddy soil. *Biology and Fertility of Soils, 57*, 673-684. https://doi.org/10.1007/s00374-021-01557-1

Ma, X.M., Razavi, B.S., Holz, M., Blagodatskaya, E. & Kuzyakov, Y. (2017) Warming increases hotspot areas of enzyme activity and shortens the duration of hot moments in the root-detritusphere. *Soil Biology & Biochemistry, 107*, 226-233. 10.1016/j.soilbio.2017.01.009

Mainzer, S.E. & Hempfling, W.P. (1976) Effects of growth temperature on yield and maintenance during

glucose-limited continuous culture of Escherichia coli. *Journal of bacteriology, 126*, 251-256. https://doi.org/10.1128/jb.126.1.251-256.1976

Malik, A.A., Martiny, J.B., Brodie, E.L., Martiny, A.C., Treseder, K.K. & Allison, S.D. (2020) Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME journal, 14*, 1-9. https://doi.org/10.1038/s41396-019-0510-0

Manzoni, S., Chakrawal, A., Spohn, M. & Lindahl, B. (2021) Modelling microbial adaptations to nutrient limitation during litter decomposition. *Frontiers in Forests and Global Change, 4*, 64. https://doi.org/10.3389/ffgc.2021.686945

Manzoni, S. & Porporato, A. (2009) Soil carbon and nitrogen mineralization: theory and models across scales. *Soil Biology and Biochemistry, 41*, 1355-1379. https://doi.org/10.1016/j.soilbio.2009.02.031

Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Agren, G.I. (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist, 196*, 79-91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

Margida, M.G., Lashermes, G. & Moorhead, D.L. (2020) Estimating relative cellulolytic and ligninolytic enzyme activities as functions of lignin and cellulose content in decomposing plant litter. *Soil Biology and Biochemistry, 141*, 107689. https://doi.org/10.1016/j.soilbio.2019.107689

Melillo, J.M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.M. & Tang, J. (2011) Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 9508-9512. https://doi.org/10.1073/pnas.1018189108

Melillo, J.M., Frey, S.D., DeAngelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P., Pold, G., Knorr, M.A. & Grandy, A.S. (2017) Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science, 358*, 101-104. https://doi.org/10.1126/science.aan2874

Menezes-Blackburn, D., Jorquera, M., Gianfreda, L., Rao, M., Greiner, R., Garrido, E. & De la Luz Mora, M. (2011) Activity stabilization of Aspergillus niger and Escherichia coli phytases immobilized on allophanic synthetic compounds and montmorillonite nanoclays. *Bioresource technology, 102*, 9360-9367. https://doi.org/10.1016/j.biortech.2011.07.054

Meng, C., Tian, D.S., Zeng, H., Li, Z.L., Chen, H.Y.H. & Niu, S.L. (2020) Global meta-analysis on the responses of soil extracellular enzyme activities to warming. *Science of the Total Environment, 705*. https://doi.org/10.1016/j.scitotenv.2019.135992

Michaelis, L. & Menten, M.L. (1913) Die kinetik der invertinwirkung. *Biochem. z, 49*, 352.

Mon, E.E., Hamamoto, S., Kawamoto, K., Komatsu, T. & Moldrup, P. (2016) Temperature effects on solute diffusion and adsorption in differently compacted kaolin clay. *Environmental Earth Sciences, 75*, 562. https://doi.org/10.1007/s12665-016-5358-2

Moorhead, D.L. & Weintraub, M.N. (2018) The evolution and application of the reverse Michaelis-Menten equation. *Soil Biology and Biochemistry, 125*, 261-262. https://doi.org/10.1016/j.soilbio.2018.07.021

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S. & Richter, A.A. (2014) Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Frontiers in Microbiology, 5*, 22. https://doi.org/10.3389/fmicb.2014.00022

Mori, T. (2020) Does ecoenzymatic stoichiometry really determine microbial nutrient limitations? *Soil Biology and Biochemistry, 146*, 107816. https://doi.org/10.1016/j.soilbio.2020.107816

Nannipieri, P., Sequi, P. & Fusi, P. (1996) Humus and enzyme activity. *Humic substances in terrestrial ecosystems*, pp. 293-328. Elsevier, Amsterdam.

Nill, J.D. & Jeoh, T. (2020) The role of evolving interfacial substrate properties on heterogeneous cellulose hydrolysis kinetics. *ACS Sustainable Chemistry & Engineering, 8*, 6722-6733. https://doi.org/10.1021/acssuschemeng.0c00779

Nottingham, A.T., Hicks, L.C., Meir, P., Salinas, N., Zimmermann, M. & Bååth, E. (2021) Annual to decadal temperature adaptation of the soil bacterial community after translocation across an elevation gradient in the Andes. *Soil Biology and Biochemistry, 158*, 108217. https://doi.org/10.1016/j.soilbio.2021.108217

Nottingham, A.T., Meir, P., Velasquez, E. & Turner, B.L. (2020) Soil carbon loss by experimental warming in a tropical forest. *Nature, 584*, 234-237. https://doi.org/10.1038/s41586-020-2566-4

Nottingham, A.T., Turner, B.L., Whitaker, J., Ostle, N., Bardgett, R.D., McNamara, N.P., Salinas, N. & Meir, P. (2016) Temperature sensitivity of soil enzymes along an elevation gradient in the Peruvian Andes. *Biogeochemistry, 127*, 217-230. https://doi.org/10.1007/s10533-015-0176-2

Nottingham, A.T., Whitaker, J., Ostle, N.J., Bardgett, R.D., McNamara, N.P., Fierer, N., Salinas, N., Ccahuana, A.J.Q., Turner, B.L. & Meir, P. (2019) Microbial responses to warming enhance soil carbon loss following translocation across a tropical forest elevation gradient. *Ecology Letters, 22*, 1889-1899. https://doi.org/10.1111/ele.13379

Pietikäinen, J., Pettersson, M. & Bååth, E. (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS microbiology ecology, 52*, 49-58. https://doi.org/10.1016/j.femsec.2004.10.002

Pold, G., Domeignoz-Horta, L.A., Morrison, E.W., Frey, S.D., Sistla, S.A. & DeAngelis, K.M. (2020) Carbon use efficiency and its temperature sensitivity covary in soil bacteria. *MBio, 11*, e02293-02219. https://doi.org/10.1128/mBio.02293-19

Pold, G., Grandy, A.S., Melillo, J.M. & DeAngelis, K.M. (2017) Changes in substrate availability drive carbon cycle response to chronic warming. *Soil Biology and Biochemistry, 110*, 68-78. https://doi.org/10.1016/j.soilbio.2017.03.002

Pold, G., Sistla, S.A. & DeAngelis, K.M. (2019) Metabolic tradeoffs and heterogeneity in microbial responses to temperature determine the fate of litter carbon in simulations of a warmer world. *Biogeosciences, 16*, 4875-4888. https://doi.org/10.5194/bg-16-4875-2019

Post, W.M., Emanuel, W.R., Zinke, P.J. & Stangenberger, A.G. (1982) Soil carbon pools and world life zones. *Nature, 298*, 156-159. https://doi.org/10.1038/298156a0

Pritchard, S. (2011) Soil organisms and global climate change. *Plant Pathology, 60*, 82-99. https://doi.org/10.1111/j.1365-3059.2010.02405.x

Raich, J.W. & Schlesinger, W.H. (1992) The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus Series B-Chemical and Physical Meteorology, 44*, 81-99. https://doi.org/10.1034/j.1600-0889.1992.t01-1-00001.x

Razavi, B.S., Blagodatskaya, E. & Kuzyakov, Y. (2015) Nonlinear temperature sensitivity of enzyme kinetics explains canceling effect - a case study on loamy haplic Luvisol. *Frontiers in Microbiology, 6*. https://doi.org/10.3389/fmicb.2015.01126

Reischke, S., Rousk, J. & Bååth, E. (2014) The effects of glucose loading rates on bacterial and fungal growth in soil. *Soil Biology and Biochemistry, 70*, 88-95. https://doi.org/10.1016/j.soilbio.2013.12.011

Romero-Olivares, A.L., Allison, S.D. & Treseder, K.K. (2017) Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. *Soil Biology & Biochemistry, 107*, 32-40. https://doi.org/10.1016/j.soilbio.2016.12.026

Sanchez-Julia, M. & Turner, B.L. (2021) Abiotic contribution to phenol oxidase activity across a manganese gradient in tropical forest soils. *Biogeochemistry, 153*, 33-45. https://doi.org/10.1007/s10533-021-00764-0

Schimel, J.P. & Weintraub, M.N. (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry, 35*, 549-563. https://doi.org/10.1016/S0038-0717(03)00015-4

Shukla, P., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H., Roberts, D., Zhai, P., Slade, R., Connors, S. & Van Diemen, R. (2019) IPCC, 2019: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.

Siddiqui, K.S. & Cavicchioli, R. (2006) Cold-adapted enzymes. *Annual Review of Biochemistry, 75*, 403-433. https://doi.org/10.1146/annurev.biochem.75.103004.142723

Sihi, D., Gerber, S., Inglett, P.W. & Inglett, K.S. (2016) Comparing models of microbial–substrate interactions and their response to warming. *Biogeosciences, 13*, 1733-1752. https://doi.org/10.5194/bg-13-1733-2016

Simon, E., Canarini, A., Martin, V., Séneca, J., Böckle, T., Reinthaler, D., Pötsch, E.M., Piepho, H.-P., Bahn, M. & Wanek, W. (2020) Microbial growth and carbon use efficiency show seasonal responses in a multifactorial climate change experiment. *Communications biology, 3*, 1-10. https://doi.org/10.1038/s42003-020-01317-1

Singh, B.K., Bardgett, R.D., Smith, P. & Reay, D.S. (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature reviews microbiology, 8*, 779-790. https://doi.org/10.1038/nrmicro2439

Sinsabaugh, R.L., Belnap, J., Findlay, S.G., Shah, J.J.F., Hill, B.H., Kuehn, K.A., Kuske, C.R., Litvak, M.E., Martinez, N.G. & Moorhead, D.L. (2014) Extracellular enzyme kinetics scale with resource availability. *Biogeochemistry, 121*, 287-304. https://doi.org/10.1007/s10533-014-0030-y

Sinsabaugh, R.L., Moorhead, D.L., Xu, X. & Litvak, M.E. (2017) Plant, microbial and ecosystem carbon use efficiencies interact to stabilize microbial growth as a fraction of gross primary production. *New Phytologist, 214*, 1518-1526. https://doi.org/10.1111/nph.14485

Sinsabaugh, R.L. & Shah, J.J.F. (2011) Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. *Biogeochemistry, 102*, 31-43. https://doi.org/10.1007/s10533-010-9482-x

Sinsabaugh, R.L. & Shah, J.J.F. (2012) Ecoenzymatic Stoichiometry and Ecological Theory. *Annual Review of Ecology, Evolution, and Systematics, Vol 43, 43*, 313-343. https://doi.org/10.1146/annurev-ecolsys-

071112-124414

Sinsabaugh, R.L., Shah, J.J.F., Findlay, S.G., Kuehn, K.A. & Moorhead, D.L. (2015) Scaling microbial biomass, metabolism and resource supply. *Biogeochemistry, 122*, 175-190. https://doi.org/10.1007/s10533-014-0058-z

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L. & Follstad Shah, J.J. (2016) Stoichiometry of microbial carbon use efficiency in soils. *Ecological Monographs, 86*, 172-189. https://doi.org/10.1890/15-2110.1

Steidinger, B.S., Crowther, T.W., Liang, J., Van Nuland, M.E., Werner, G.D., Reich, P.B., Nabuurs, G.-J., de-Miguel, S., Zhou, M. & Picard, N. (2019) Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature, 569*, 404-408. https://doi.org/10.1038/s41586-019-1128-0

Sulman, B.N., Moore, J.A., Abramoff, R., Averill, C., Kivlin, S., Georgiou, K., Sridhar, B., Hartman, M.D., Wang, G. & Wieder, W.R. (2018) Multiple models and experiments underscore large uncertainty in soil carbon dynamics. *Biogeochemistry, 141*, 109-123. https://doi.org/10.1007/s10533-018-0509-z

Tan, X., Machmuller, M.B., Huang, F., He, J., Chen, J., Cotrufo, M.F. & Shen, W. (2020) Temperature sensitivity of ecoenzyme kinetics driving litter decomposition: The effects of nitrogen enrichment, litter chemistry, and decomposer community. *Soil Biology and Biochemistry, 148*, 107878. https://doi.org/10.1016/j.soilbio.2020.107878

Tang, J. (2015) On the relationships between the Michaelis–Menten kinetics, reverse Michaelis–Menten kinetics, equilibrium chemistry approximation kinetics, and quadratic kinetics. *Geoscientific Model Development, 8*, 3823-3835. https://doi.org/10.5194/gmd-8-3823-2015

Tang, J. & Riley, W.J. (2019) Competitor and substrate sizes and diffusion together define enzymatic depolymerization and microbial substrate uptake rates. *Soil Biology and Biochemistry, 139*, 107624. https://doi.org/10.1016/j.soilbio.2019.107624

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q. & Suija, A. (2014) Global diversity and geography of soil fungi. *Science, 346*. https://doi.org/10.1126/science.1256688

Ten Hulscher, T.E. & Cornelissen, G. (1996) Effect of temperature on sorption equilibrium and sorption kinetics of organic micropollutants-a review. *Chemosphere, 32*, 609-626. https://doi.org/10.1016/0045-6535(95)00345-2

Terrer, C., Phillips, R.P., Hungate, B.A., Rosende, J., Pett-Ridge, J., Craig, M.E., van Groenigen, K., Keenan, T.F., Sulman, B.N. & Stocker, B. (2021) A trade-off between plant and soil carbon storage under elevated CO2. *Nature, 591*, 599-603. https://doi.org/10.1038/s41586-021-03306-8

Todd-Brown, K.E., Hopkins, F.M., Kivlin, S.N., Talbot, J.M. & Allison, S.D. (2012) A framework for representing microbial decomposition in coupled climate models. *Biogeochemistry, 109*, 19-33. https://doi.org/10.1007/s10533-011-9635-6

Todd-Brown, K.E.O., Randerson, J.T., Post, W.M., Hoffman, F.M., Tarnocai, C., Schuur, E.A.G. & Allison, S.D. (2013) Causes of variation in soil carbon predictions from CMIP5 Earth system models and comparison with observations. *Biogeosciences, 10*, 1717–1736. https://doi.org/10.5194/bgd-9-14437-2012

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Zhou, J. & Singh, B.K. (2016) Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. *The ISME journal, 10*, 2593-2604. https://doi.org/10.1038/ismej.2016.65

Tucker, C.L., Bell, J., Pendall, E. & Ogle, K. (2013) Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Global Change Biology, 19*, 252-263. https://doi.org/10.1111/gcb.12036

Walker, T.W., Kaiser, C., Strasser, F., Herbold, C.W., Leblans, N.I., Woebken, D., Janssens, I.A., Sigurdsson, B.D. & Richter, A. (2018) Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. *Nature Climate Change, 8*, 885-889. https://doi.org/10.1038/s41558-018-0259-x

Wallenstein, M., Allison, S., Ernakovich, J., Steinweg, J.M. & Sinsabaugh, R. (2011) Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates. *Soil Enzymology* (eds G. Shukla & A. Varma), pp. 245-258. Springer Berlin Heidelberg.

Wang, B. & Allison, S.D. (2019) Emergent properties of organic matter decomposition by soil enzymes. *Soil Biology and Biochemistry, 136*, 107522. https://doi.org/10.1016/j.soilbio.2019.107522

Wang, G. & Post, W.M. (2012) A theoretical reassessment of microbial maintenance and implications for microbial ecology modeling. *FEMS microbiology ecology, 81*, 610-617. https://doi.org/10.1111/j.1574-6941.2012.01389.x

Wang, G. & Post, W.M. (2013) A note on the reverse Michaelis–Menten kinetics. *Soil Biology and Biochemistry, 57*, 946-949. https://doi.org/10.1016/j.soilbio.2012.08.028

Wang, G., Post, W.M. & Mayes, M.A. (2013) Development of microbial‐enzyme‐mediated decomposition

model parameters through steady‐state and dynamic analyses. *Ecological Applications, 23*, 255-272. https://doi.org/10.1890/12-0681.1

Wen, Y., Zang, H., Ma, Q., Evans, C.D., Chadwick, D.R. & Jones, D.L. (2019) Is the 'enzyme latch'or 'iron gate'the key to protecting soil organic carbon in peatlands? *Geoderma, 349*, 107-113. https://doi.org/10.1016/j.geoderma.2019.04.023

Wieder, W.R., Allison, S.D., Davidson, E.A., Georgiou, K., Hararuk, O., He, Y., Hopkins, F., Luo, Y., Smith, M.J. & Sulman, B. (2015) Explicitly representing soil microbial processes in Earth system models. *Global Biogeochemical Cycles, 29*, 1782-1800. https://doi.org/10.1002/2015GB005188

Xiao, W., Chen, X., Jing, X. & Zhu, B. (2018) A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry, 123*, 21-32. https://doi.org/10.1016/j.soilbio.2018.05.001

Xu, W. & Yuan, W. (2017) Responses of microbial biomass carbon and nitrogen to experimental warming: a meta-analysis. *Soil Biology and Biochemistry, 115*, 265-274. https://doi.org/10.1016/j.soilbio.2017.08.033

Ye, J.S., Bradford, M.A., Dacal, M., Maestre, F.T. & Garca-Palacios, P. (2019) Increasing microbial carbon use efficiency with warming predicts soil heterotrophic respiration globally. *Global Change Biology, 25*, 3354-3364. https://doi.org/10.1111/gcb.14738

Yuste, J.C., Penuelas, J., Estiarte, M., Garcia‐Mas, J., Mattana, S., Ogaya, R., Pujol, M. & Sardans, J. (2011) Drought‐resistant fungi control soil organic matter decomposition and its response to temperature. *Global Change Biology, 17*, 1475-1486. https://doi.org/10.1111/j.1365-2486.2010.02300.x

Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A. & Wanek, W. (2019) Growth explains microbial carbon use efficiency across soils differing in land use and geology. *Soil Biology and Biochemistry, 128*, 45-55. https://doi.org/10.1016/j.soilbio.2018.10.006

Zuccarini, P., Asensio, D., Ogaya, R., Sardans, J. & Peñuelas, J. (2020) Effects of seasonal and decadal warming on soil enzymatic activity in a P‐deficient Mediterranean shrubland. *Global Change Biology, 26*, 3698-3714. https://doi.org/10.1111/gcb.15077

Figure 1 - Location of enzymes in soils and their importance for carbon and nutrient cycling. Soil enzymes are often characterized by their maximal velocity (V_{max}), i.e., the maximum reaction rate at saturating substrate concentration for a given temperature, and the Michaelis-Menten constant (K_m), i.e., the half-saturation constant (V_{max} / 2) which reflects the binding affinity (1 / K_m) of an enzyme for a substrate. Because enzymes are highly variable in their forms and location in soils, we referred to enzyme activities throughout the manuscript. All the figures were created with BioRender.com and Powerpoint.

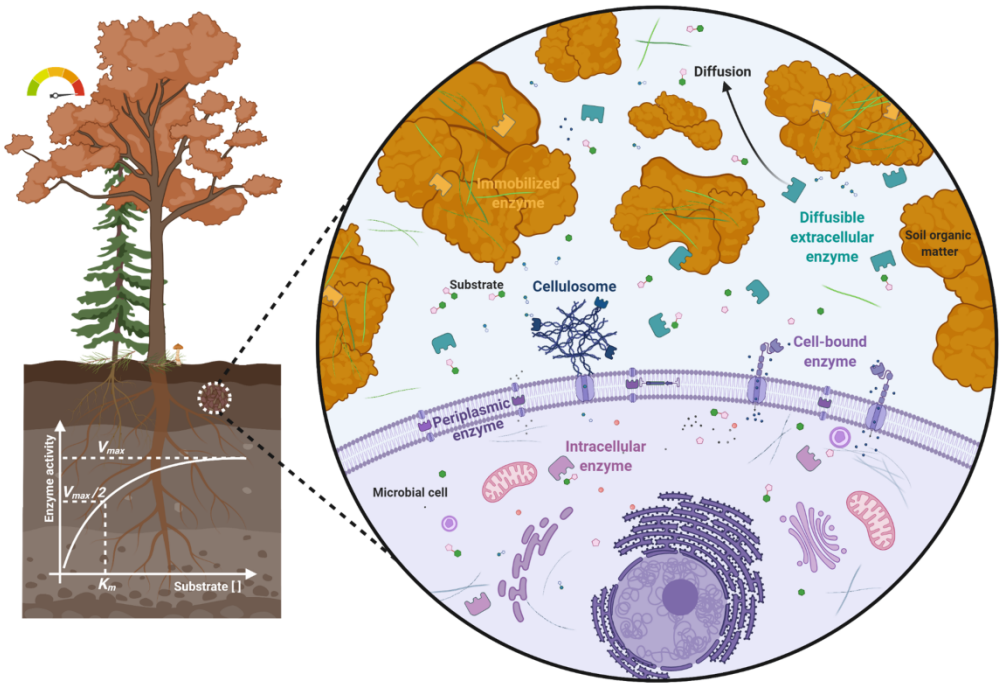
Figure 2 - Effects of temperature at the enzyme scale. A) Enzyme-substrate relationships and associated parameters, and **B)** responses of enzyme parameters to temperature (adapted from Ma et al., 2017). The relationship between rate of reaction and concentration of substrate depends on the affinity of the enzyme for its substrate (1 / K_m). The active site is a region of an enzyme where substrate molecules bind and undergo a chemical reaction that generates products and releases the enzyme. The maximum reaction rate and number of times each enzyme converts substrate to product per unit time are defined by V_{max} and k_{cat}, respectively. The cumulative amount of substrate degraded by a unit of enzyme E_{power} depends on k_{cat}, but also on thermal inactivation of enzymes k_{inact}. The total period of time needed to metabolize the substrate at a given concentration is the substrate turnover time. Finally, the Q_{10} temperature coefficient is a measure of the rate of change in enzyme activity as a consequence of increasing the temperature by 10°C. The optimum temperature is defined as the temperature at which enzymes best facilitate reactions. Temperature interval as a whole (i.e., from low and high temperatures) may vary for psychrophilic, mesophilic and thermophilic communities.

Figure 3 - **Effects of temperature at the microbial scale. A)** Importance of microbial parameters in enzyme-substrate relationships (adapted from Schimel & Weintraub, 2003), and **B)** responses of microbial parameters to temperature. Microbes use all available C. Because efficiency of new biomass C produced per unit of organic resource C consumed depends strongly on the structure of microbial communities, their requirements and activity will influence enzyme allocation and specific enzyme activity per unit of microbial biomass. Decomposition of litter or soil organic carbon is a function of enzyme concentration which depends on CUE, community composition (which can also directly influence CUE at the community scale), microbial maintenance and growth, and enzyme allocation.

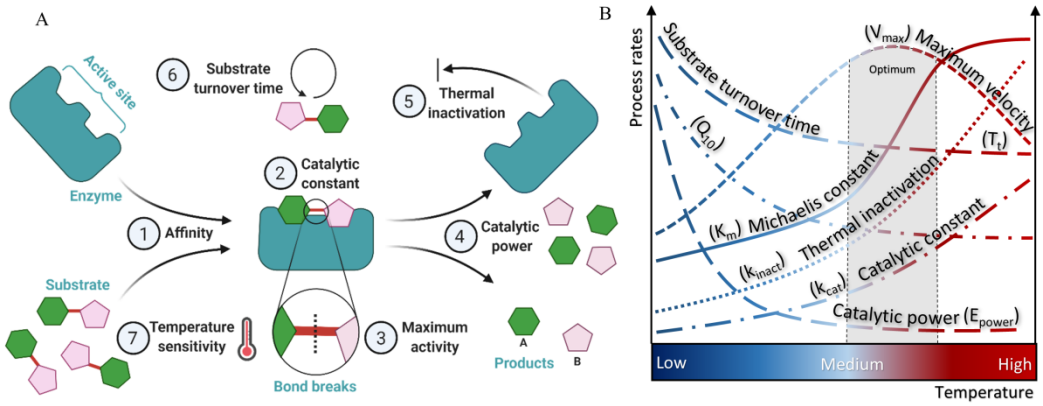
Figure 4 Effects of temperature on soil carbon stocks at different temporal scales. Temperature may affect C inputs through rhizodeposition and necromass, which in turn may affect microbial strategies: yield, resource acquisition and stress tolerance (adapted from Malik et al., 2020). Interactions between microbial communities, chemical complexity and availability of organic matter may in turn affect the pool of labile *versus* recalcitrant carbon at different temporal scales. In the short-term, microbial communities will produce more acquisitive C-related enzymes in response to warming which will mainly affect the labile C pool (Phase 1). This first phase is quickly followed by one of the two Phases 2a or 2b. Physiological adaptations or substrate depletion decrease microbial biomass and activity and lead to a reduction in soil C loss (Phase 2a). On the other hand, shifts in microbial community structure and allocation to oxidative enzymes may accelerate soil C loss through its impact on the recalcitrant C pool (Phase 2b). One of the most important questions for soil ecologists and modelers in the 21st century is whether there will be an attenuation or acceleration of soil C in the very long term (Phase 3). Note: The effects of temperature on soil C stocks are dynamic and soil C stocks fluctuate constantly (i.e., increase or decrease) over time.

Box 1 – Summary of definitions used in this article.

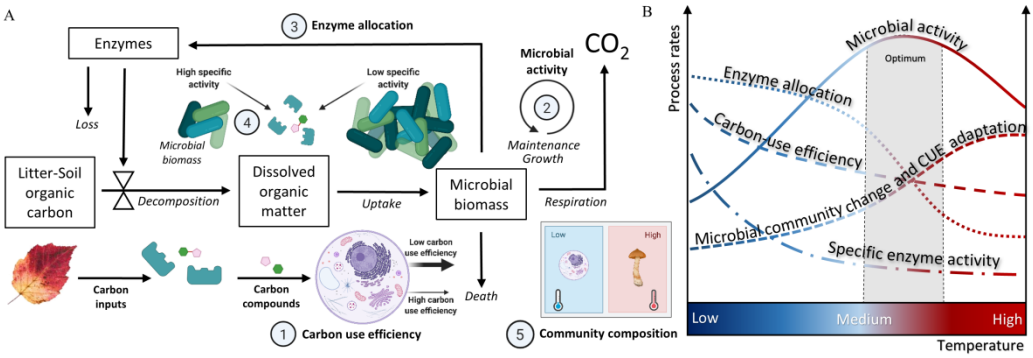
Term	Unit	Definition
Activation energy (EA_{cat})	kJ mol^{-1}	Activation energy of enzyme catalytic activity
Activation energy inactivation (EA_{inact})	kJ mol^{-1}	Activation energy of enzyme inactivation
Carbon use efficiency (CUE)	unitless	Measure of the partitioning of assimilated C into microbial growth or respiration
Catalytic constant (k_{cat})	nmol min^{-1} U^{-1}	Catalytic constant for the conversion of substrate into product
Catalytic power of enzyme (E_{power})	mol U^{-1}	Cumulative amount of substrate degraded by one unit of enzyme until its complete inactivation
Enzyme production	mol kg^{-1}	Total quantity of enzymes produced by microbes
Maximum reaction velocity (V_{max})	nmol g^{-1} h^{-1}	Maximum reaction rate at saturating substrate concentration for a given temperature
Michaelis constant (K_m)	mol g^{-1}	Half-saturation constant (V_{max} / *2*) which reflects the binding affinity (1 / K_m) of enzyme for a substrate
Temperature sensitivity (Q_{10})	unitless	Relative response of an enzymatic reaction rate to a temperature increase of 10°C
Thermal inactivation (k_{inact})	min^{-1}	Thermal inactivation rate constant
Specific enzyme activity	nmol g^{-1}	Enzyme activity by unit of protein, microbial biomass or soil organic carbon
Substrate turnover time	h^{-1}	Period of time needed to metabolize a substrate



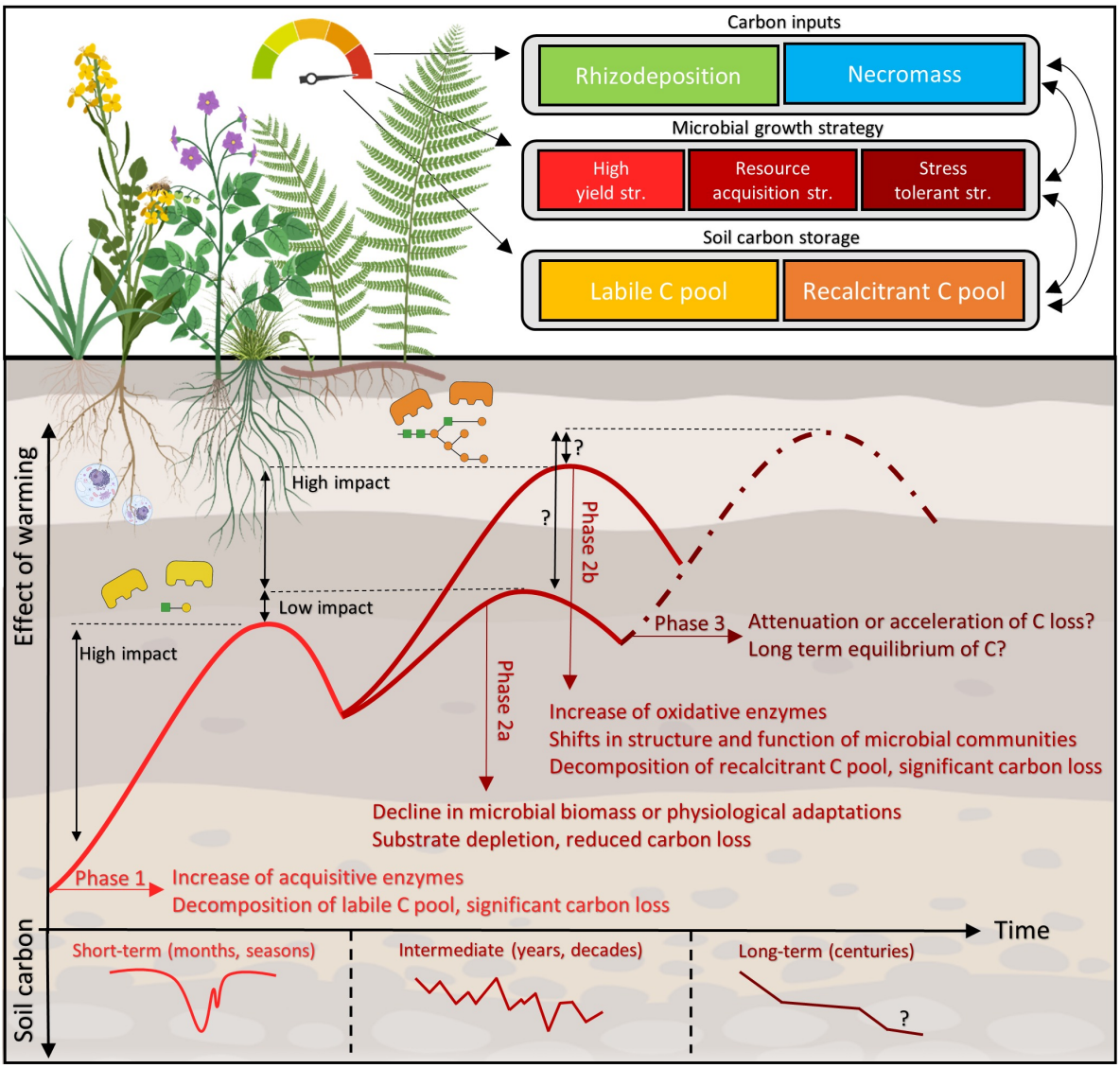
fec_14027_f1.png



fec_14027_f2.png



fec_14027_f3.png



fec_14027_f4.png