Chapter 13
Controls on the Temperature Sensitivity of Soil Enzymes: A Key Driver of *In Situ* Enzyme Activity Rates

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13.1 Introduction

Soil microorganisms are surrounded by organic matter that is rich in carbon and nutrients that are required for growth and cell maintenance. However, microbes cannot directly transport these macromolecules into the cytoplasm. Rather, they rely on the activity of a myriad of enzymes that they produce and release into their environment. These enzymes depolymerize organic compounds and generate soluble oligomers and monomers that are then recognized by cell wall receptors and transported across the outer membrane and into the cell. Thus, the activities of extracellular enzymes are critical to soil functioning and for maintenance of the vast biodiversity of organisms in soils.

The activity of glucosidases, phosphatases, phenol oxidases, and other enzymes that degrade the principal components of detrital organic matter have been extensively studied from many perspectives. In early studies, the physical and kinetic characteristics of soil enzymes were a major topic (Bremner and Zatua 1975; McClaugherty and Linkins 1990; Frankenberger and Tabatabai 1991b). More recent studies have concentrated on the ecological significance of soil enzyme activity as a mediator of nutrient cycling. Yet, the fundamental role of temperature

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in regulating enzyme activities under field conditions has been examined in relatively few studies. In theory, the temperature sensitivity of enzyme activities can be described from first principles of thermodynamics. In this chapter, we consider the utility and limitations of thermodynamic extracellular enzyme activity models for understanding the dynamics of ecosystem processes, and we review our current understanding of the thermal ecology of extracellular enzymes in soils.

13.2 What Controls Enzyme Temperature Sensitivity?

13.2.1 Enzyme Conformation

Enzymes are proteins that catalyze reactions by lowering the activation energy of biochemical reactions. There are two aspects to the temperature sensitivity of enzymes. The first aspect is their thermal stability or their ability to maintain their structure across a range of temperatures. The structure of proteins is the main determinant of their thermal stability, and is defined by the terms primary, secondary, and tertiary elements. The primary structure of a protein or enzyme is its linear amino acid sequence in the secondary structure; protein folding conformations are created by interactions between amino acid side chains that are dispersed along the polypeptide; the tertiary or native structure is the fully folded state of the globular protein, which may include multiple secondary elements (Straub 1964). The folding of proteins is driven by differences in Gibbs free energy of various conformations between the unfolded and the folded protein. The term "macrostability" defines the energy released by the enzyme taking on its 3D tertiary structure.

The second aspect of enzyme temperature sensitivity is the temperature sensitivity of catalytic *activity*, which is determined primarily by the accessibility of the active site of the enzyme. "Microstability" refers to the energy associated with reversible, local changes in structure. The microstability is responsible for the flexibility or rigidity of the *active site* of an enzyme, which is the location of substrate or ligand binding that leads to catalysis (Privalov and Tsalkova 1979). The overall stability of an enzyme is the free energy difference between the macrostability and the microstability (Zavodszky et al. 1998). The active site of enzymes is more flexible than the whole enzyme due to weaker molecular interactions (Tsou 1993), and the active site of an enzyme loses its function (with the addition of chemical denaturants or heat) more quickly than the whole protein can be denatured (Tsou 1993), (Fig. 13.1).

The conformation of enzymes has been described by many models, the most influential of which are Koshland's "induced fit" model and Straub's "fluctuation fit" model. The "induced fit" model postulates that enzyme activation is induced by a change in the conformation of the active site due to interactions of the active site with the substrate. The "fluctuation fit" model of enzyme function says that the

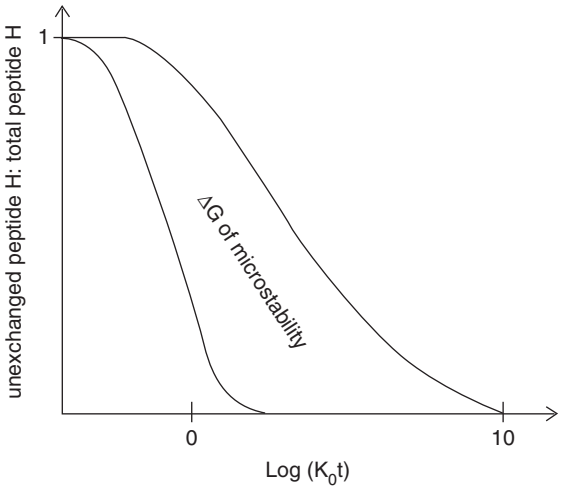

Fig. 13.1 The relationship between the relative proportion of unexchanged peptide hydrogen to the total number of hydrogen in the protein and the Log (K_0t). K_0 is the rate constant for the exchange of hydrogen from the primary structure of a protein. This is the tendency of a protein to undergo micro-unfolding, and the ΔG of microstability is the energy required for the exchange of one peptide hydrogen (adapted from Privalov and Tsalkova 1979)

native conformation of an enzyme can exist in many states in solution. These general principles of enzyme conformation apply across all thermal regimes, but specific adaptations are also made in order to maintain enzyme function across different temperature conditions.

Cold-adapted enzymes have more flexible active sites (Hochachka and Somero 1984) created by a weakening of the intramolecular forces in the active site of the enzyme (Gerday et al. 1997). In contrast, the active sites of heat-adapted enzymes are more rigid (Zavodszky et al. 1998). The changes in flexibility in cold-adapted and thermophilic enzymes are due to one or more changes in the amino acid structure of the active site (Zavodszky et al. 1998). Cold-adapted microorganisms may produce cold-adapted enzymes that catalyze reactions at lower temperatures than their mesophilic counterparts by adjusting their chemical structure (Gerday et al. 1997). The activation energy of these enzymes is lower than that of mesophilic enzymes. Cold-adapted enzymes may have limited range of thermal stability around their temperature optima due to the conformational changes that increase the activity of the enzyme at low temperatures, resulting in a loss of function at the active site before the 3D structure of the protein denatures (Gerday et al. 1997). Thermophilic enzymes have more rigid active sites below their temperature optimum. Zavodszky et al. (1998) found that 3-isopropylmalate dehydrogenase (IPMDH) isolated from the thermophilic bacteria *Thermus thermophilus* did not denature until 17°C higher than the mesophilic homolog, indicating that macrostructure plays an important role in thermal adaptation as well. At their optimum temperature, the flexibility of the active site of a thermophilic enzyme is equal to that of its mesophilic counterpart at its temperature optimum (Secundo et al. 2005; Zavodszky et al. 1998). Thus, it appears that the flexibility and activity of enzymes are closely related, and that organisms have evolved to create enzymes with thermal optima at their habitation temperature.

13.2.2 Modeling Enzyme Kinetics

Thermodynamic models of biochemical and ecological processes begin with the Arrhenius equation:

$$V(T) = V(T_0)\mathrm{e}^{-E_a/k(1/T_0 - 1/T)},$$

where $V(T)$ is the reaction rate at temperature T, $V(T_0)$ is the reaction rate at a reference temperature T_0, E_a is the activation energy in eV, and k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}). E_a is the energy differential between reactants and the transitional species that subsequently decay into products. This activation energy determines the rate of change in reaction rate with temperature. A reaction rate that doubles with a 10°C rise in temperature [$Q_{10} = 2$] has an E_a of 0.5 eV [48 kJ mol^{-1}]. Enzymes facilitate reactions by stabilizing transition states, thereby lowering E_a. Although the Arrhenius model was originally developed to describe simple reactions, it is used to describe the apparent temperature dependency of metabolic processes at every scale of biological organization. In metabolic scaling theory, the model is often considered in relation to area: volume ratios that limit rates of environmental exchange, e.g., oxygen or carbon dioxide in the case of respiration or photosynthesis. This normalization, usually represented as a fractal scalar with a value of 0.75 rather than a surface area to volume ratio, allows the temperature dependence of metabolic processes, e.g., growth and respiration, to be compared on common basis across organizational and body size scales (Gillooly et al. 2001; Allen et al. 2005). Meta-analyses of the temperature dependence of organismal metabolism yield a mean E_a of 0.62 eV (Gillooly et al. 2001).

When considering biochemical processes such as soil EEA that occur over large ranges of temperature, E_a is not the only parameter that influences responses. Enzymes have an effective range and an optimal temperature (T_{opt}) of operation that is determined by their size and composition. At low temperature, enzymes or the matrix with which they are associated freeze. At temperatures only a few degrees above their optimum, the tertiary structure of enzymes begins to denature, unless it is stabilized by interactions with particle surfaces or humic complexes. In ecological systems, enzyme classes, such as laccase or β-glucosidase, are populations of isoenzymes of diverse origin (Di Nardo et al. 2004) that differ in size, polypeptide sequence, and post-translational modifications. Consequently, estimates of E_a and optimal temperature become statistical distributions. These measures are further extenuated by environmental interactions with organic and inorganic particles that can substantially alter both E_a and T_{opt} values by compromising the conformational flexibility of the enzyme. Thus, at the ecosystem scale, organic matter abundance and composition, and soil texture and mineralogy also influence EEA response to temperature fluctuations. In this context, E_a is used as an empirical measurement of system response to temperature change and T_{opt}, which is usually much greater than in situ temperature, is a measure of the relative stability, i.e., turnover rate, of the soil enzyme pools.

These enzyme–environment interactions combined with succession or variation in the composition of the enzyme producer community introduces a scale dependence to apparent EEA temperature response. The T_{opt} and E_a estimates determined for a single sample will not be the same as those calculated by comparing samples collected over spatial or temporal gradients. As the scale of comparison expands, T_{opt} and apparent E_a are conflated and apparent temperature response becomes increasingly a function of resource inputs and community composition to an extent that rates may even be inversely related to temperature, i.e., have an apparent E_a that is negative.

The mean E_a for extracellular enzymes that are not conformationally constrained is about 0.3 eV. In contrast, the E_a for microbial [and plant] metabolism, and presumably extracellular enzyme production, is around 0.62 eV (Gillooly et al. 2001). Reported values of apparent E_a for the metabolism of bacterial communities range from 0.41 to 1.14 eV (Kristensen et al. 1992; Sagemann et al. 1998; Thamdrup et al. 1998; Price and Sowers 2004; Lopez-Urrutia and Moran 2007). The high value of 1.14 eV comes from an analysis by Price and Sowers (2004) that includes data from several types of ecosystems and spans a temperature range from $-20°C$ to $30°C$. Enzymes that are conformationally compromised by sorption to elements of the soil matrix may have E_a values similar to that of microbial growth. As a result, the E_a for a particular extracellular enzymatic activity, e.g., phosphatase, is broadly distributed within a soil or litter matrix and some fraction of activity may have an apparent $E_a > 1$ eV.

Superficially, thermal control of EEA seems like a simple thing to model. But temperature responses are conflated with myriad other ecological variables. As a result, estimates of apparent E_a are specific to the enzymes, systems, temperature range, and spatiotemporal scale under consideration.

13.2.3 Michaelis–Menten Model

The kinetics of simple enzymes (i.e., enzymes with one active site that interact with a single substrate) is described by the Michaelis–Menten model as a hyperbolic function:

$$V = \mathrm{Vmax}(S/(S + \mathrm{Km})),$$

where V is reaction rate, S is substrate concentration, Vmax is the rate of substrate conversion when all enzymes are operating at maximum capacity, and Km is a half-saturation constant (i.e., the substrate concentration at which the rate of substrate conversion is equal to Vmax/2). Km is also a measure of the binding affinity of substrate and enzyme.

When the Michaelis–Menten model is applied to ecological systems, its assumptions do not apply and Vmax and Km no longer reflect the biochemical attributes defined in its original context (Williams 1973). In such cases, these parameters are

more accurately described as apparent Vmax (^{App}Vmax) and apparent Km (^{App}Km) with ^{App}Vmax, a relative measure of enzyme abundance, and ^{App}Km, a relative measure of substrate concentration. Spatiotemporal variation in ^{App}Vmax most likely reflects differences in the concentration of rate-limiting enzyme, rather than the replacement of one enzyme by another of different structure, particularly when the rate-limiting "enzyme" in question is actually a population of enzymes of similar function produced in many versions, by multiple organisms, under different controls, and heterogeneously dispersed within the environment. Similarly, spatiotemporal variation in ^{App}Km most likely reflects differences in the concentration of the substrate pool because natural substrates act as competitive inhibitors for reactions measured by adding labeled or artificial substrates to environmental samples (Chrost 1990):

$$^{App}\mathrm{Km} = \mathrm{Km}(1 + I/\mathrm{Ki}),$$

where I is the concentration of inhibiter, in this case the background concentration of environmental substrate, and Ki is the half-saturation constant for the enzyme-inhibiter reaction.

In the Michaelis–Menten model, Vmax and Km are independent parameters. However, in ecological systems, ^{App}Vmax and ^{App}Km may be correlated because EEA is tightly controlled by a hierarchy of positive and negative feedback processes linked to substrate availability that operate at the molecular, cellular, and population levels (Chrost 1990; Chróst and Siuda 2002; Lugtenberg et al. 2002; Vial Ludovic et al. 2007). At the molecular level, the activity of individual enzymes is affected by competitive and non-competitive inhibition reactions as well as substrate concentration; at the cellular level, enzyme expression is controlled by induction and repression pathways linked to environmental cues; at the population level, enzyme expression may be coordinated by quorum signals. This correlation between environmental substrate concentration, estimated as ^{App}Km, and enzymatic capacity (^{App}Vmax) can be obscured, particularly at fine spatiotemporal scales, because the extracellular enzyme pool is, to varying extent, spatiotemporally decoupled from the organisms that produced them. Substantial fractions of the pool, particularly for soils, may be stabilized by sorption to humic or mineral colloids, or associated with cell fragments and extracellular polysaccharides creating lags in EEA response to changes in bacterial metabolism (Wilczek et al. 2005).

Activation energies are parameters that mechanistically link enzyme kinetics and temperature responses through the Arrhenius function. In the Michaelis–Menten function, the temperature sensitivity of Vmax is directly related to the activation energy for the enzyme reaction (Davidson and Janssens 2006). In addition, the Km parameter of the Michaelis–Menten function also increases with temperature, which reduces the substrate binding affinity of the enzymes. When substrate concentrations are near Km, this effect can offset the temperature effects on Vmax, resulting in little temperature dependence of the enzyme reaction (Davidson et al. 2006). A mechanistic model that includes temperature sensitivities of Vmax and Km would be superior to non-mechanistic empirical relationships, such as $Q10$.

Biological responses to temperature are often characterized in terms of the parameter Q_{10}, which is the factor by which a biological process changes in response to a 10°C temperature increase (Lloyd and Taylor 1994). Although many biological processes show a Q_{10} of approximately 2, this factor varies with temperature and is not based on a particular biological mechanism, in contrast to the Michaelis–Menten model. As a purely empirical parameter, Q_{10} values cannot be reliably extrapolated beyond measured response ranges or applied to novel systems.

13.2.4 Enzyme Binding to Soil Particles

Within the mineral matrix of the soil, organic matter–mineral binding and physical occlusion of organic matter within soil aggregates both act to limit the mixing of enzymes with otherwise decomposable OM (Tisdall and Oades 1982; Sollins et al. 1996; Jastrow and Miller 1997; Six et al. 2002). The turnover times of free, or bio-available, soil organic matter compounds can be orders of magnitude less than those for the same compounds found in association with soil minerals (Sørensen 1972). Such physical isolation of reactants violates a precept of kinetic theory (Davidson and Janssens 2006; Ågren and Wetterstedt 2007). But organic matter adsorption to mineral surfaces is a chemical process too. Organic matter binds with mineral particles *via* several types of non-covalent bonds (e.g., van der Waals forces, hydrogen bonding). Rates of formation (adsorption) and breakdown (desorption) of those bonds both tend to increase with increasing temperature. But because adsorption reactions are exergonic and have lower E_a's, the equilibrium between adsorption and desorption shifts toward desorption with increasing temperature – more compounds are in solution at warmer temperatures (ten Hulscher and Cornelissen 1996). Thus, increased desorption at higher temperatures could contribute to the temperature sensitivity of in situ enzyme activity. Since enzyme activities are typically measured in lab assays where substrate is non-limiting, temperature sensitivity of in situ enzymes may be under-predicted.

Davidson et al. (Davidson and Janssens 2006; Davidson et al. 2006) have argued that a conceptual framework based on activation energies and substrate availabilities would be a more useful alternative to Q_{10} models. Sinsabaugh and Shah (2010) developed a modeling approach that combines thermal scaling with resource availability. Using estimates of apparent Km, Vmax, and E_a for six extracellular enzymes that mediate nutrient acquisition from carbohydrate, protein, lipid, and organic phosphate pools, they were able to predict variation in bacterial production rates over an annual cycle in two rivers that experience seasonal changes in both temperature and the supply of multiple resources.

13.3 Indirect Effects of Temperature on Enzyme Activities

In general, the overall metabolic rate of enzyme-producing organisms increases with temperature with a mean E_a of 0.62 eV over the range 5–40°C. Thus, the rate of extracellular enzyme production is more responsive to temperature than the kinetics of the enzymes themselves. It is not currently possible to directly measure enzyme production rates in soils (Wallenstein and Weintraub 2008), and data from pure cultures are scarce and are likely to far exceed field rates where resources are limited.

Microorganisms utilize carbon for processes such as growth, maintenance, and enzyme production. Carbon allocation varies based on substrate availability, temperature, moisture, and other environmental factors. The type of carbon assimilated by microbes results in different amounts of energy available for growth and maintenance. Carbon utilization efficiency (CUE) is a measure of how efficiently microorganisms metabolize versus mineralize carbon. In aquatic systems, CUE has been shown to be relatively insensitive to changes in temperature (del Giorgio and Cole 1998; Seto and Misawa 1982); however, in soils it has been demonstrated that carbon utilization efficiency can be temperature dependent (Devevre and Horwat 2000; Steinweg et al. 2008). CUE is currently a fixed parameter in ecosystem models such as CENTURY (Parton et al. 1987); however, it has been demonstrated that CUE is lower at warmer temperatures regardless of the quality of soil organic matter (Steinweg et al. 2008). Low CUE results in more CO_2 produced per unit of substrate incorporated into biomass. Thus, temperature can affect the relative allocation of resources toward enzyme production.

Changes in temperature not only affect enzyme production rates by microorganisms but also affect enzyme degradation rates in the environment. Enzyme turnover is the result of proteolytic enzyme from activity as well as abiotic reactions. Both these processes should increase with temperature, but may show different temperature sensitivities due to differences in activation energy. Enzyme-catalyzed reactions generally show lower activation energies than uncatalyzed reactions, so the temperature sensitivity of the abiotic reactions may be higher (Tabatabai 1982). However, the rates of these reactions are also lower, so the net impact on enzyme activity may be small.

13.4 Temperature Sensitivity of Extracellular Enzymes under Field Conditions

Most contemporary studies of extracellular enzymes focus on spatial or temporal patterns in potential activities, which are typically measured at a single reference temperature in lab assays. This approach neglects the importance of temperature in controlling in situ activities (Wallenstein and Weintraub 2008). In most ecosystems, soil temperatures vary on diel to seasonal time scales, and change in response to long-term climate trends. If we assume that enzyme activity roughly doubles for

every 10°C increase in temperature, then the effect of temperature clearly may have a greater impact on in situ activity rates than seasonal fluctuations in enzyme potential at most sites. For example, Wallenstein et al. (2009) developed a quantitative model of in situ B-glucosidase activities based on seasonal lab-based measurements of B-glucosidase potential activities at two temperatures, and using daily soil temperature data from an Arctic tundra site. They found that temperature explained 72% of the variation in predicted in situ activities. Temperature had a larger influence on modeled in situ enzyme activity than seasonal changes in enzyme pools. Clearly, temperature controls on in situ enzyme activities needs to be further explored in other biomes.

The assumption that all enzymes are equally sensitive to temperature, or even that the same class of enzyme exhibits a consistent temperature sensitivity within a single site, has not been borne out in the literature. In fact, several studies have demonstrated that the temperature sensitivity of extracellular enzymes changes seasonally (Fenner et al. 2005; Koch et al. 2007; Trasar-Cepeda et al. 2007; Wallenstein et al. 2009). The most likely explanation is that the measured enzyme pool consists of different isoenzymes (enzymes with the same function, but different structure) through time, which may be produced by different organisms or by a single species capable of producing multiple isoenzymes (Loveland et al. 1994; Sanchez-Perez et al. 2008). Consistent with this hypothesis, Di Nardo et al. (2004) found temporal changes in laccase and peroxidase isoenzymes during leaf litter decomposition. There is also some evidence for biogeographical patterns in enzyme temperature sensitivity. For example, many studies have observed that enzymes from microbes inhabiting cold environments have unusually low temperature optima (Huston et al. 2000; Coker et al. 2003; Feller 2003). Nonetheless, these observations suggest that microbes producing enzymes that maintain optimal activity under native soil conditions are favored. Thus, soil microbial community composition is likely controlled to some extent through feedbacks with enzyme efficacy.

It is widely assumed that enzyme activity roughly doubles with a 10°C increase in temperature ($Q_{10} = 2$); however, the accumulated evidence of numerous studies suggests a wide range in temperature sensitivities for different enzymes, and measured Q_{10}'s are often <2 (McClaugherty and Linkins 1990; Frankenberger and Tabatabai 1991a, b; Lai and Tabatabai 1992; Wirth and Wolf 1992; Criquet et al. 1999; Parham and Deng 2000; Elsgaard and Vinther 2004; Trasar-Cepeda et al. 2007). For example, Trasar-Cepeda et al. (2007) measured the Q_{10} of nine different enzymes in three different soils and found that the Q_{10} at 20°C exceeded 2.0 only for B-glucosidase in one of the soils. Most of the enzymes in that study had a Q_{10} closer to 1.5, corresponding to an E_a of 0.3 eV. However, the apparent temperature sensitivity of enzymes in lab assays with unlimited amounts of substrate and without constraints to diffusion may differ markedly from in situ temperature sensitivities. Even despite methodological concerns, there are insufficient data to assess the degree to which enzyme temperature sensitivity varies across spatial gradients or in response to other environmental factors.

Although there are insufficient data to establish generalized patterns in the temperature sensitivity of specific classes of enzymes, it is clear that their

temperature sensitivities differ within a single environment (Koch et al. 2007; Wallenstein et al. 2009). For example, Koch et al. (2007) found that at low temperatures, the relative temperature sensitivity of C-degrading enzymes was greater than aminopeptidases (which degrade N-rich proteins), suggesting that relative N availability could be decreased directly by temperature. Similarly, in the study by Wallenstein et al. (2009), N-degrading enzymes tended to have a lower Q_{10} (overall mean of 1.59) than C-degrading enzymes (overall mean of 2.07). Thus, without any changes in enzyme pools, the relative in situ activity of these enzymes would change along with temperature, resulting in higher rates of C-mineralization relative to N-mineralization. Because different enzymes have different temperature sensitivities, changes in soil temperature may also alter the relative rates of decomposition of different components of soil organic matter. Therefore, seasonal changes in temperature can alter the balance of SOM (Soil Organic Matter) components contributing to soil respiration *without any changes in soil enzyme pools* (or measured enzyme potentials). Natural or human-driven changes in climate could also alter the relative rate of decomposition of SOM components, and ultimately, the quantity and composition of SOM.

One of the most important reasons for understanding the temperature sensitivity of soil enzymatic reactions is to improve predictions of soil respiration responses to temperature. Although there is considerable variability, about half of the CO_2 respired from soil can be attributed to heterotrophic respiration of soil organic matter (Ryan and Law 2005; Czimczik et al. 2006; Scott-Denton et al. 2006). A large fraction of this amount depends on the activity of extracellular enzymes, since much of the dead plant material entering soils are in polymeric form. However, the temperature sensitivity of heterotrophic soil respiration may be decoupled from enzyme temperature sensitivity because enzymatic products must undergo diffusion, uptake, and intracellular metabolism before CO_2 is produced (Ågren and Wetterstedt 2007). Only if the activity of extracellular enzymes is currently limiting the entire flux of carbon to a microbe would heterotrophic respiration rates reflect the temperature sensitivity of extracellular enzymes. This situation is unlikely to occur because microbes also respire labile compounds derived from non-polymeric sources, such as root exudates (Bader and Cheng 2007). Even polymer-derived metabolites may have been released by extracellular enzymes at considerable spatial and temporal distance from the respiring microbe. This decoupling along with the considerable influence of autotrophic respiration on total soil CO_2 efflux suggests that time-integrated models will be needed to link the temperature sensitivity of extracellular enzymes with the temperature response of soil respiration.

13.5 Conclusions

Given the critical roles of enzymes in ecosystem functioning, our limited understanding of enzyme activities under field conditions is an important limitation to our ability to model ecosystem processes under current and future climates. Temperature is among the most important drivers of enzyme activities, yet its role is often

neglected, in part due to the challenges involved in measuring or modeling temperature effects. However, there is no easy solution to this challenge. We do not presently have the ability to measure in situ enzyme activities directly and thus cannot simply develop empirical relationships between temperature and enzyme activity. Theoretical models of enzyme behavior under substrate limiting conditions provide a good starting point for modeling approaches. Yet, these simple models cannot account for other temperature-sensitive biological, chemical, and physical processes that affect enzyme activity. Biological responses include changes in enzyme production rates, shifts in isoenzyme production, which affect the statistical distribution of enzyme temperature sensitivities within a population, and changes in microbial community composition which could affect the resource efficiency of enzyme production. Physiochemical processes that affect enzyme activities include absorption–desorption reactions, substrate diffusion rates, and enzyme degradation rates. Clearly, the range and complexity of factors that affect in situ enzyme temperature sensitivity make for a most challenging problem.

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