Inactivation of enzymes challenges the current view of temperature response of enzymatic reactions

Sébastien Fontaine, Gaël Alvarez

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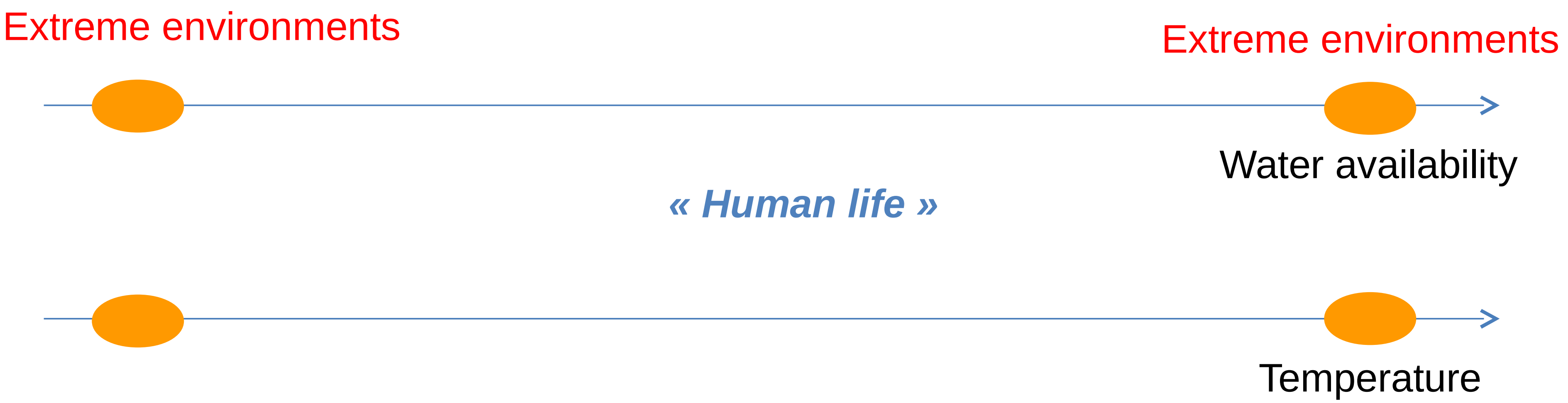
Extreme environments

- What does it mean extreme environments?

A concept a bit anthropocentric .



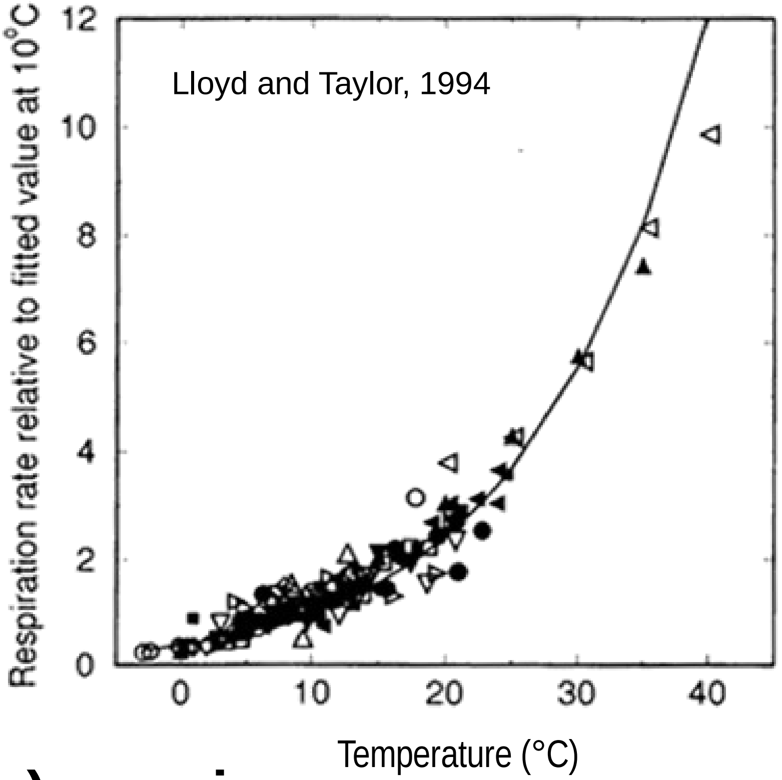
Extreme environments are not exceptions but their understanding requires a fine knowledge of the response of biological processes to environmental factors (T°C…)

Impact of temperature on microbial degradation: current theory

- Describes decomposition as a substrate-limited process

- Thermo-dependence is embedded in the rate constant k

(Arrhenius equation)



In the short-term (hours to days) warming accelerates C decomposition

Attenuation of warming effect with time

- Harvard Forest, soil warming +5°C (10 years)

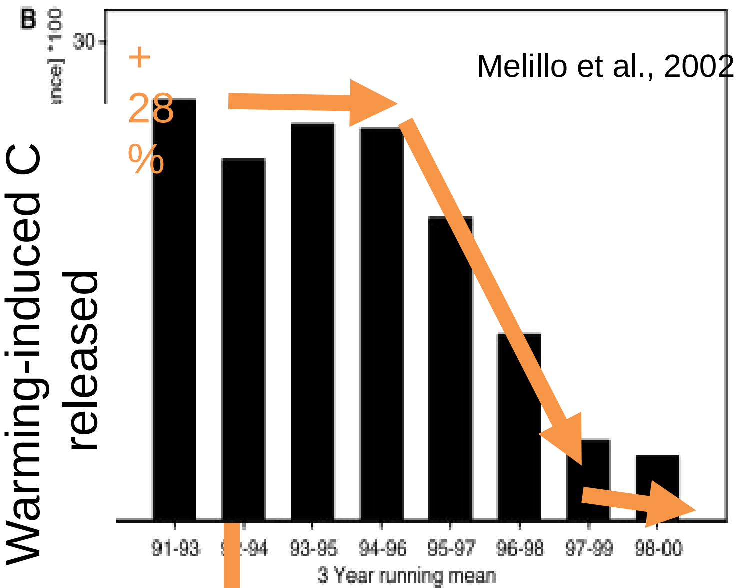
Stimulation

of soil respiration

is transitory

(6-7 years)

% of control



of the same treatment. (B) Percentage increase in the amount of carbon released from the heated plots relative to the disturbance control plots. The data are presented as 3-year running means for the period from 1991 through 2000.

- Discrepancy **short vs. long term response**

Re-analysis of thermo-dependence of enzymatic reactions

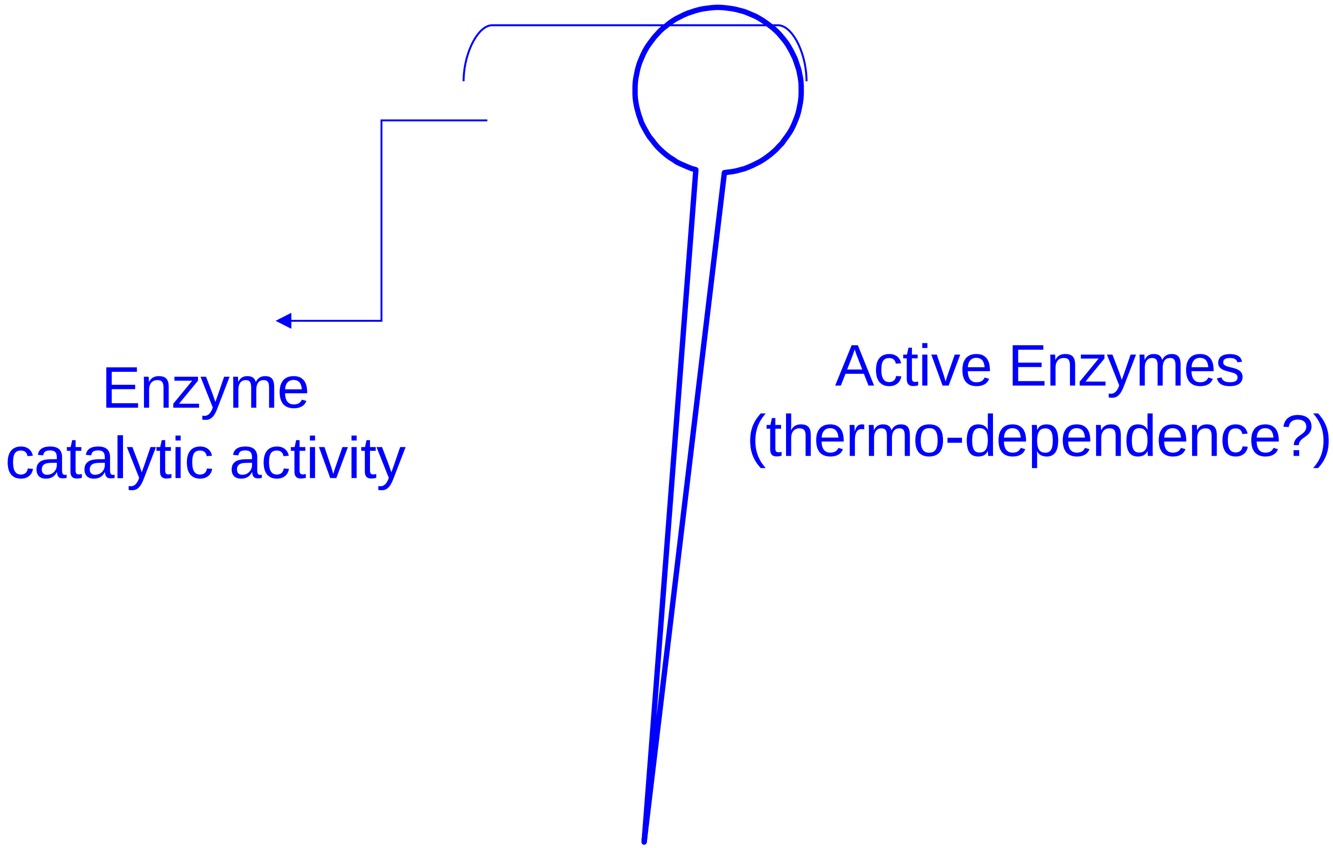
Velocity of enzyme reactions is co-limited by substrate concentration and enzyme pool

- Michaelis-Menten equation

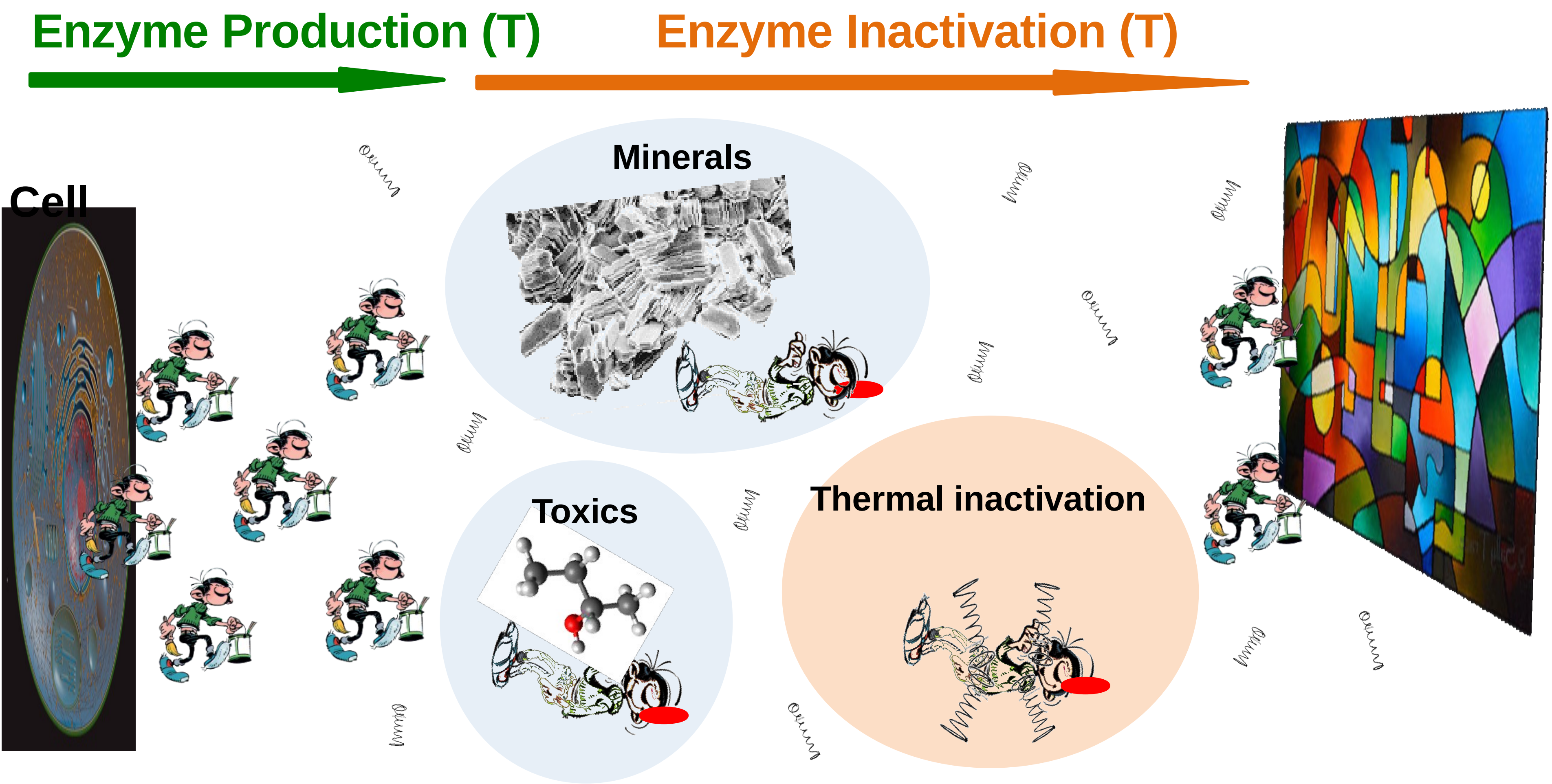
Maximum velocity at saturating substrate

Substrate limitation

Let us to consider the case of excess of substrate



Active enzyme pool controlled by microbial production and enzyme inactivation



Thermal inactivation of enzymes

A well-known process by enzymologists

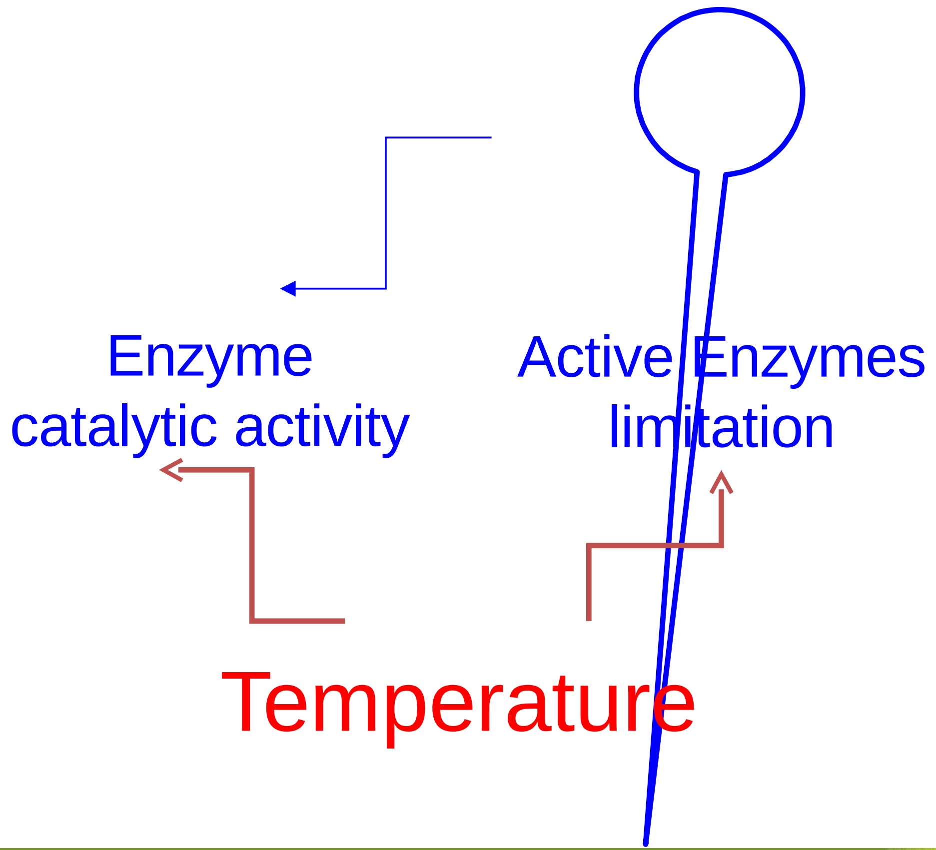
- Induced by the Brownian movement and collisions between molecules

=> loss of protein conformational structure and active sites

- Continuous process acting at all temperature (+/- process of ageing)

- Intensifying with temperature

Describing the temperature response of enzymatic activity by accounting for the thermo-dependence of enzyme pool



Modelling the enzymatic system

- **Substrate degradation**

- **Enzyme pool dynamics**

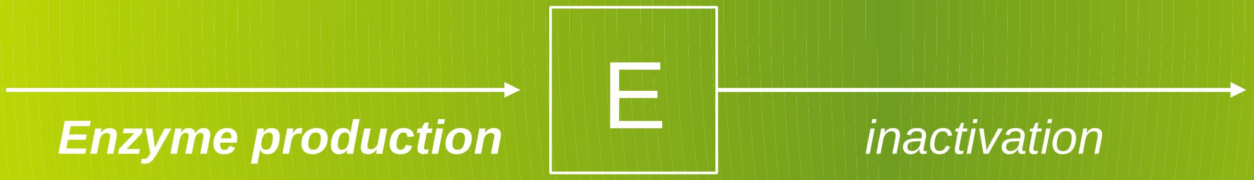
Constant flux of enzyme (might depend on T)

continuous enzyme inactivation

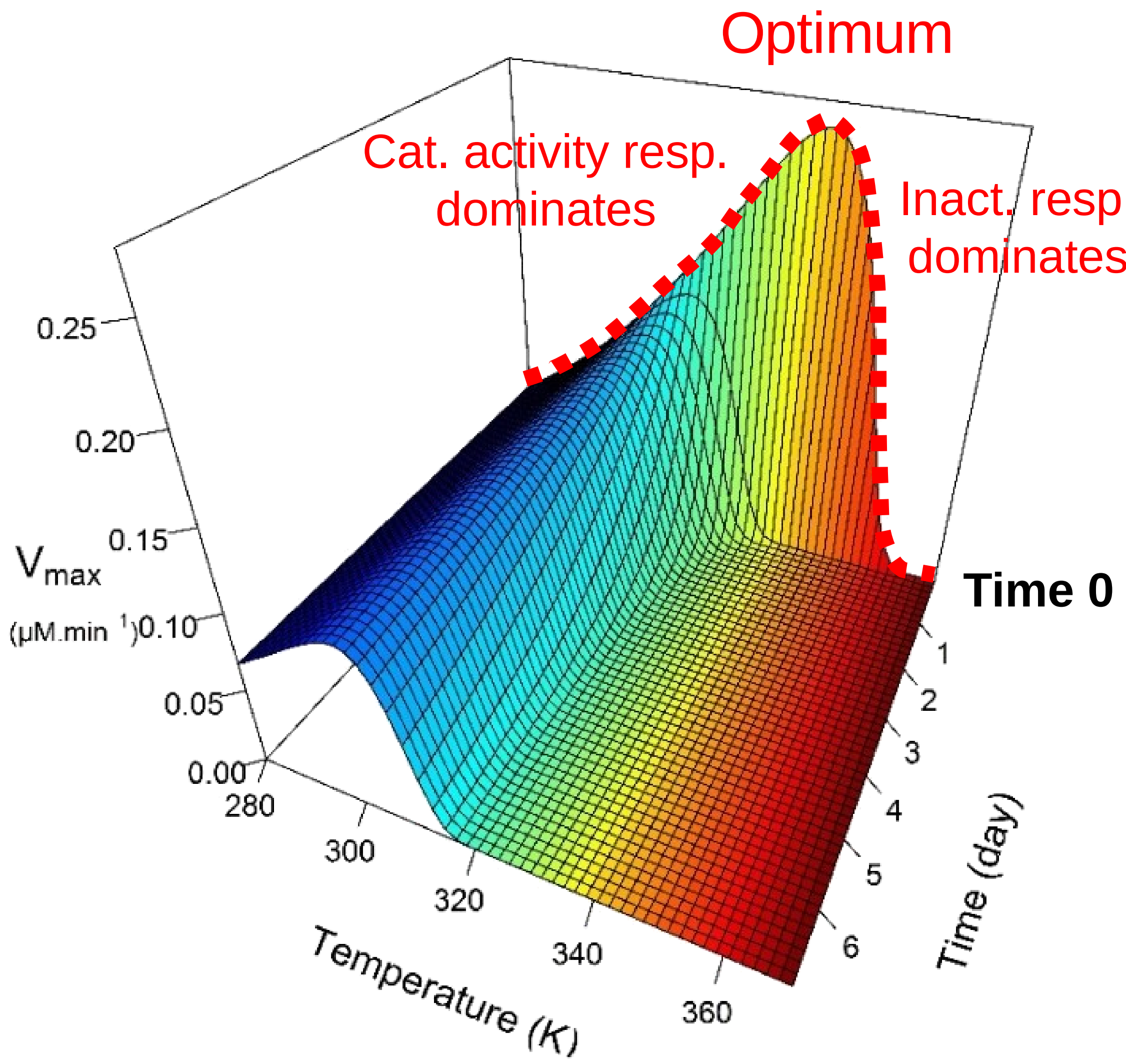
Analysis of the theory

1. **Batch reactor (□ = 0) : effect of a pool of enzyme**

2. **Enzyme flow reactor : with enzyme production**



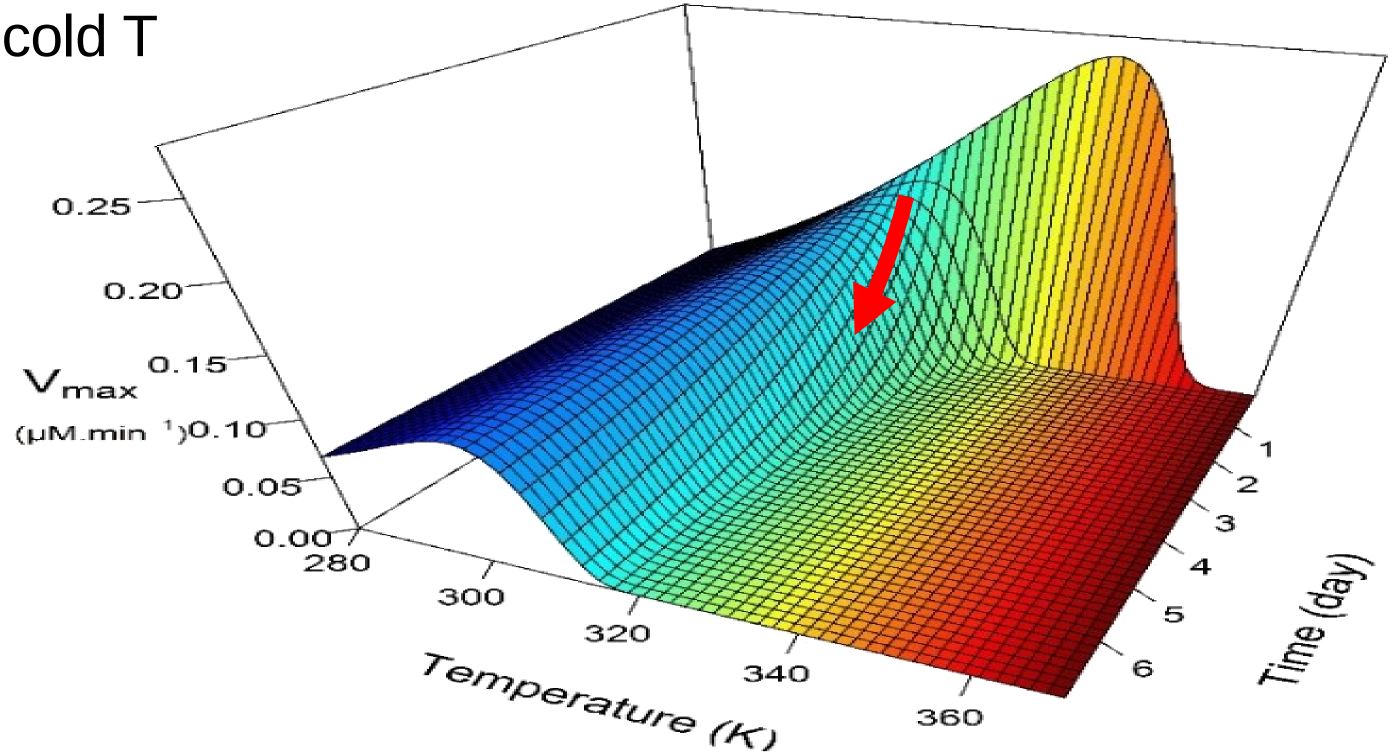
The reaction velocity (Vmax) mediated by a pool of enzyme is given by:



For a given time, Vmax shows an optimum

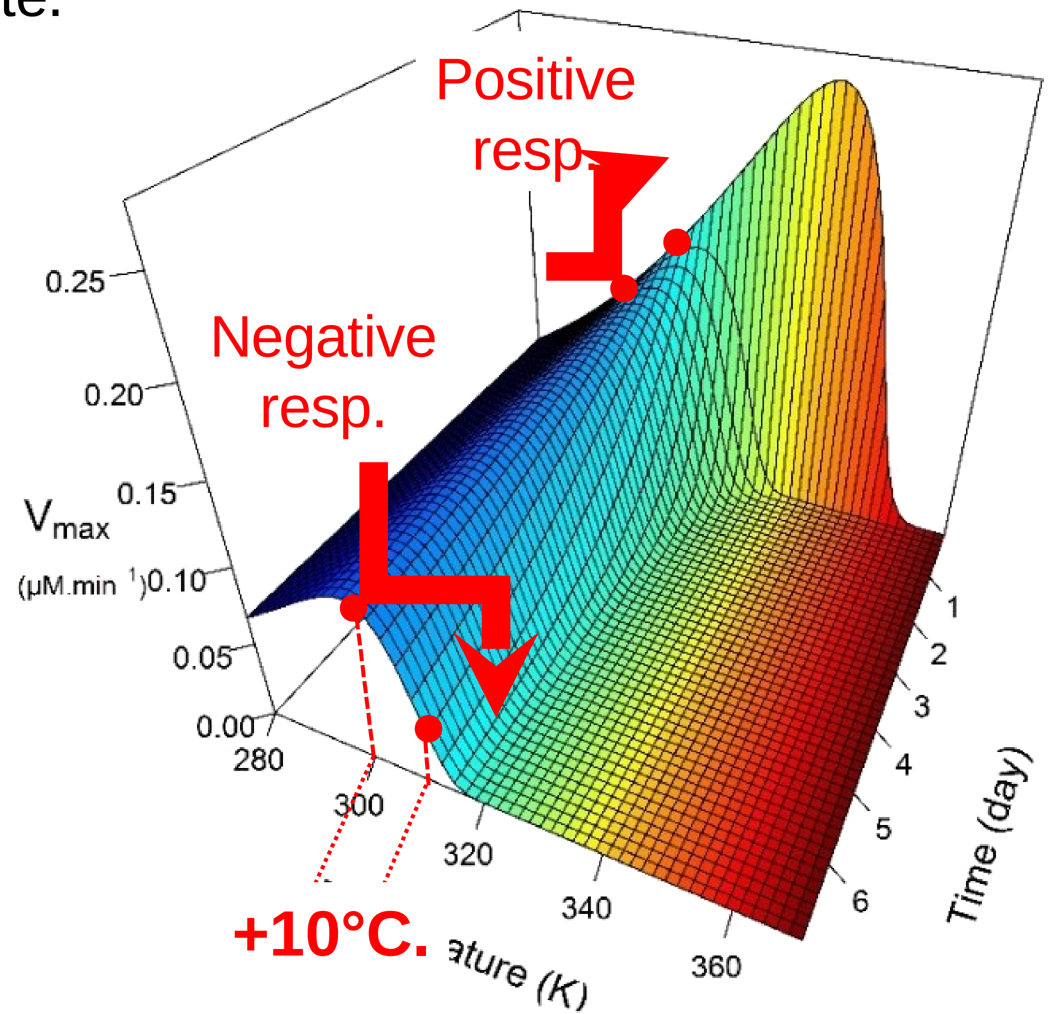
T optimums shift with time towards lower values

due to slower enzyme inactivation at cold T



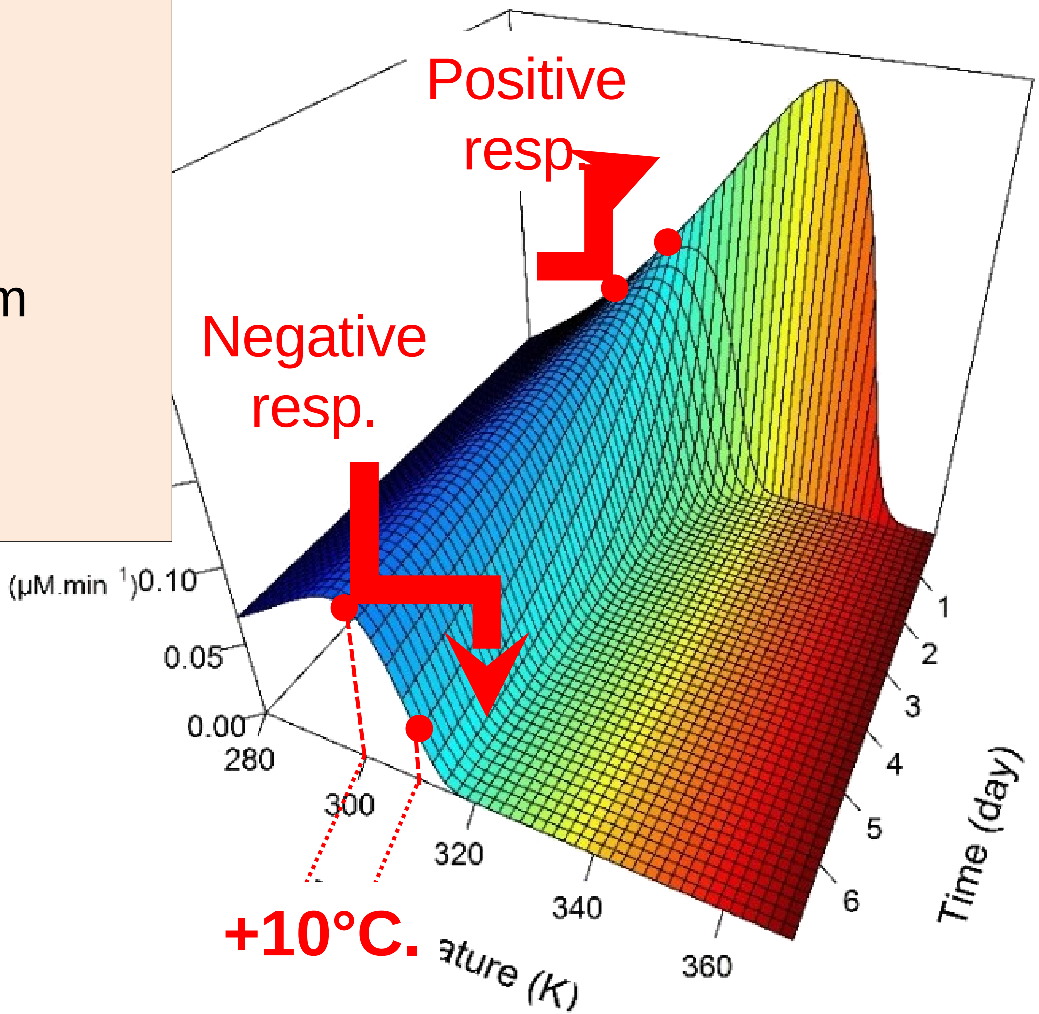
Batch reactor – Vmax

=> Temperature response of *Vmax* changes with time and can be opposite.



Temperature optimum is not a relevant trait

- depends on assay duration
- does not inform on the long-term effect of temperature



Batch reactor – Reaction yield

To capture the long-term temperature response of enzymatic activities we need to consider the cumulative enzyme activity:

- Reaction Yield
- **Total amount of substrates degraded by the enzyme pool** until its complete inactivation (Integration of RY with ***t* → +∞)**

Epower

catalytic power of enzyme

RY∞ per unit of E0

Batch reactor – Reaction yield

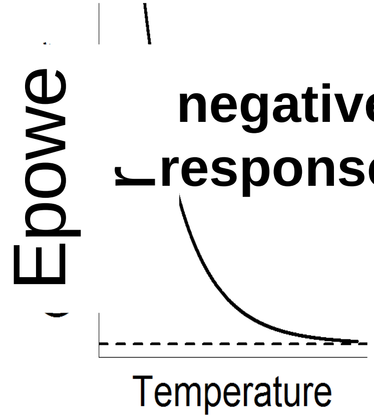
Epower

catalytic power of enzyme

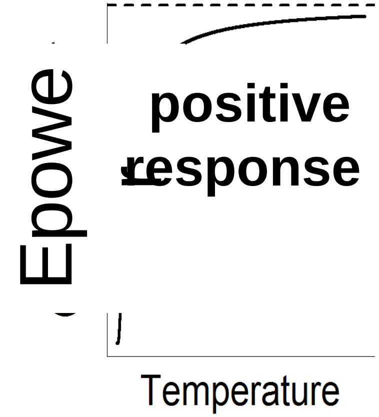
RY∞ per unit of E0

- **Temperature response of Epower**

EAinact > EAcat

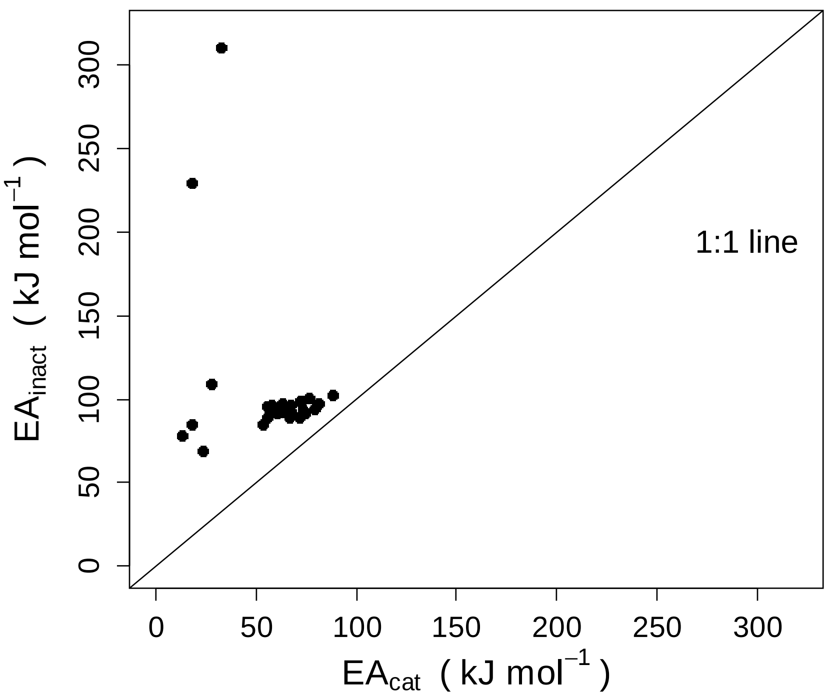


EAinact < EAcat



Meta-analysis of EAs for catalysis and thermal inactivation

Enzyme	Catalysis *EAcat* (kJ.mol-1)	Inact. *EAinact* (kJ.mol-1)	References
Dihydrofolate reductase	**67**	**93**	*Daniel et al., 2008*
Alkaline phosphatase	**55**	**90**	*Daniel et al., 2008*
Acid Phosphatase	**79**	**95**	*Daniel et al., 2008*
β-Glucosidase	**63**	**95**	*Daniel et al., 2008*
3-isopropylmalate dehydrogenase	**72**	**100**	*Daniel et al., 2008*
Ammonia-Lyases	**80**	**97**	*Daniel et al., 2008*
ATPase	**74**	**92**	*Daniel et al., 2008*
α-Glucosidase	**71**	**0**	*Daniel et al., 2008*
3-isopropylmalate dehydrogenase	**73**	**94**	*Daniel et al., 2008*
Dihydrofolate reductase	**66**	**90**	*Daniel et al., 2008*
Glutamate dehydrogenase	**57**	**94**	*Daniel et al., 2008*
Malate dehydrogenase	**53**	**85**	*Daniel et al., 2008*
Alkaline phosphatase	**57**	**97**	*Daniel et al., 2008*
Fumarase	**60**	**92**	*Daniel et al., 2008*
γ-Glutamyl transferase	**63**	**98**	*Daniel et al., 2008*
Glutamate dehydrogenase	**63**	**93**	*Daniel and Danson, 2013*
Malate dehydrogenase	**55**	**96**	*Daniel et al., 2008*
α-Glucosidase	**64**	**96**	*Daniel et al., 2008*
Dihydrofolate reductase	**67**	**97**	*Daniel et al., 2008*
α-Glucosidase	**88**	**103**	*Daniel et al., 2008*
3-isopropylmalate dehydrogenase	**76**	**101**	*Daniel et al., 2008*
β-Glucosidase	**81**	**98**	*Daniel et al., 2008*
Citrate synthetase	**88**	**103**	*Daniel and Danson, 2013*
Alkaline phosphatase	**72**	**99**	*Daniel et al., 2008*
Catalase	**18**	**230**	*Sizer, 1944*
Tannase	**17**	**85**	*Nie et al., 2012*
Tannase	**23**	**69**	*Yu and Li, 2006*
Xylanase	**32**	**311**	*Cobos and Estrada, 2003*
5-lipoxygenase	**28**	**110**	*Lagutina et al., 1990*
Lipase	**13**	**79**	*Bhatti and Amin, 2013*



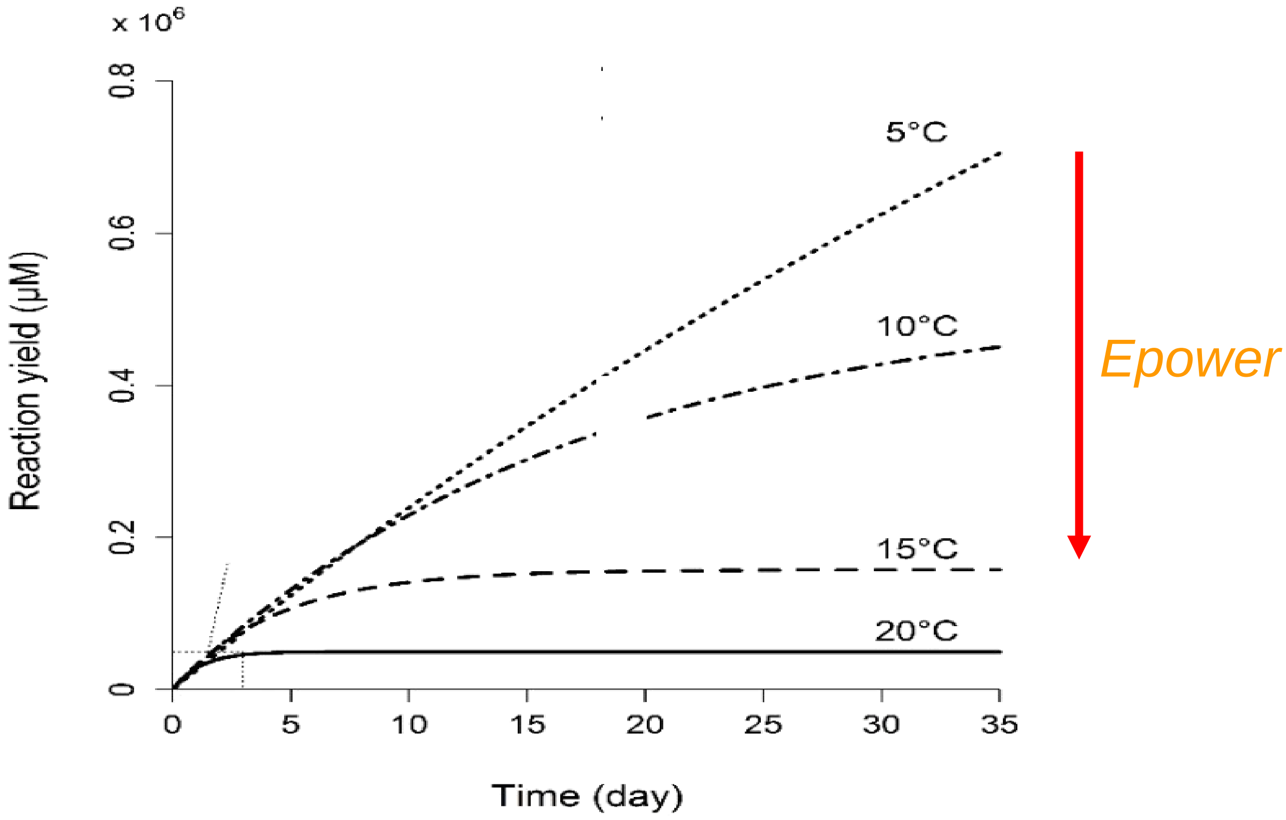
Universal pattern of enzyme

thermodynamic properties

- Enzyme inactivation is more sensitive to T than enzyme activity

EAinact > EAcat

The cumulated amount of degraded substrate decreases with temperature



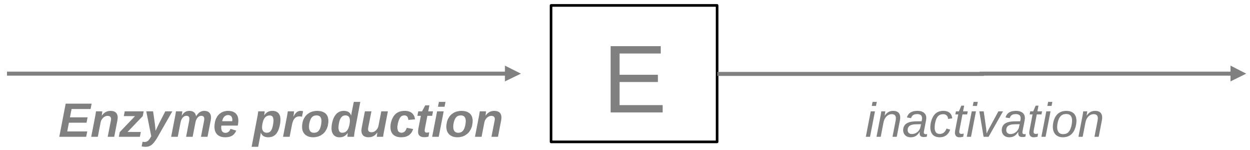
- ✓ Over the long-term, T has negative effect on enzymatic activities

Enzyme flow reactor - Steady-state analysis

Consider a pool of enzymes replenished by an enzyme production

Enzyme flow reactor - Steady-state analysis

Consider a pool of enzymes replenished by an enzyme production



When enzyme production compensates for enzyme inactivation,

the pool of active enzymes is at steady-state

- Reaction velocity
  at steady-state

Enzyme flow reactor - Steady-state analysis

- Reaction velocity at steady-state

Epower

Similar to batch reactor,

Epower decreases with T

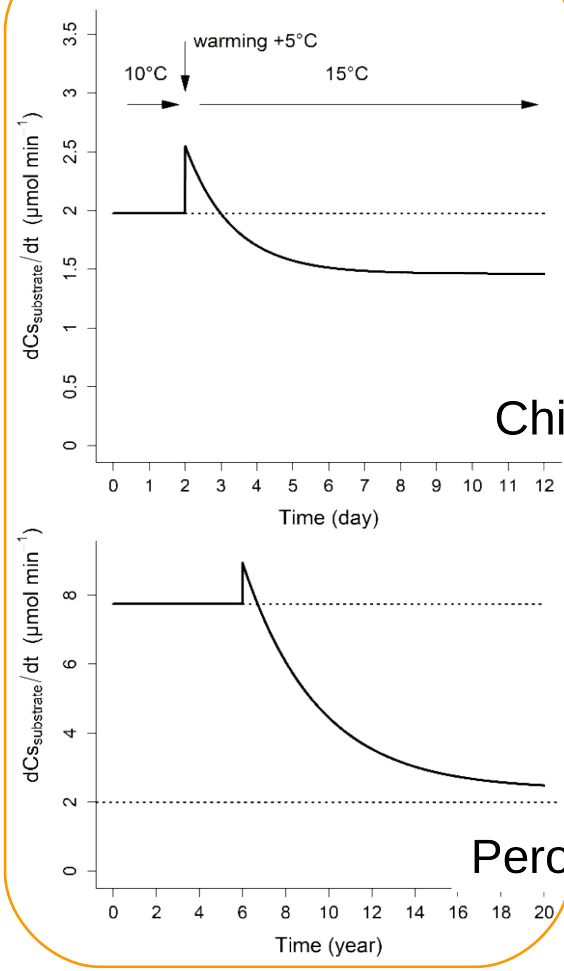
Dynamic response of reaction velocity - 5°C warming

If enzyme production remains constant …

1. Instantaneous positive response of *kcat* after warming

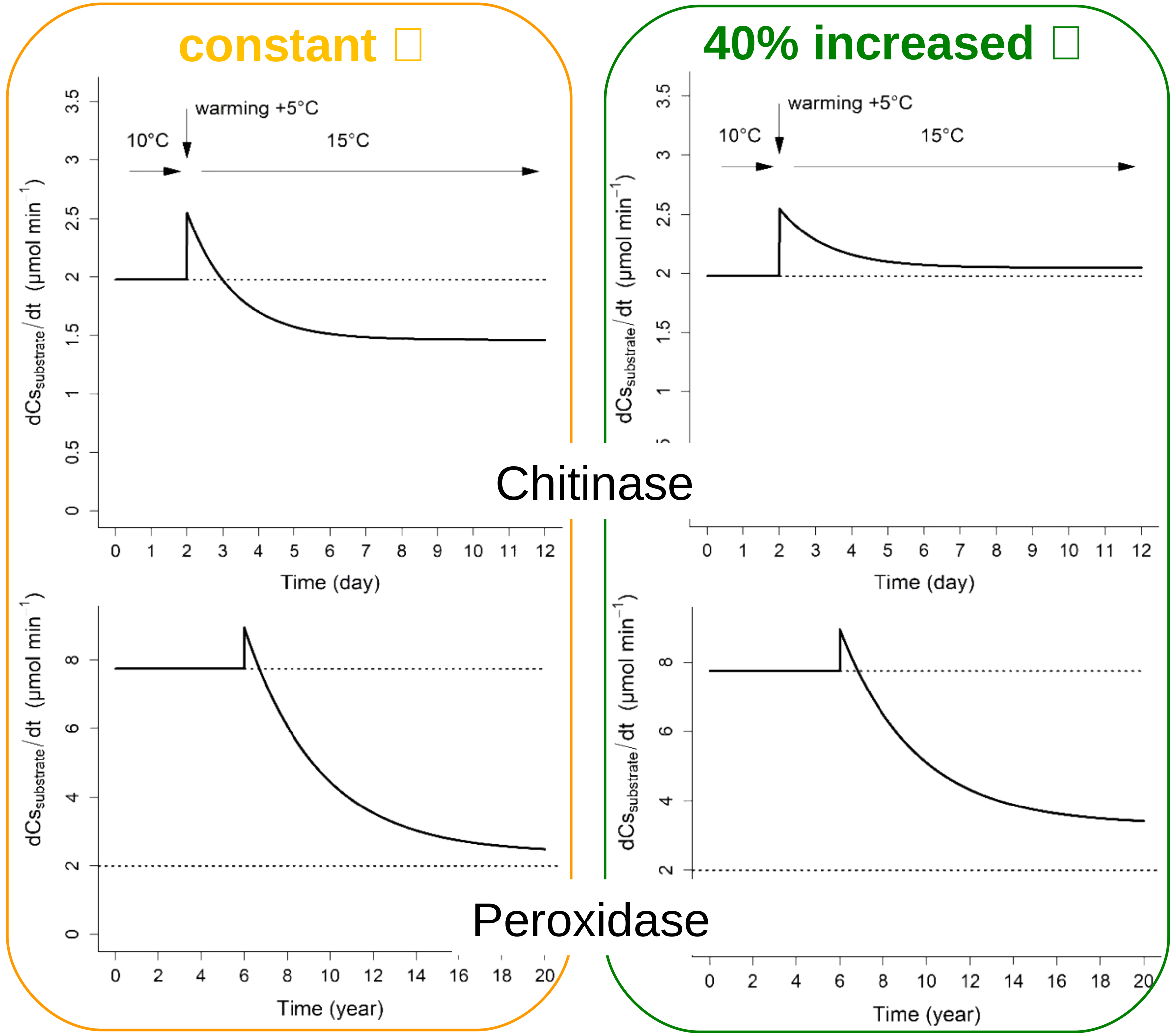
2. Thermal inactivation decreases *Vmax* with time

constant



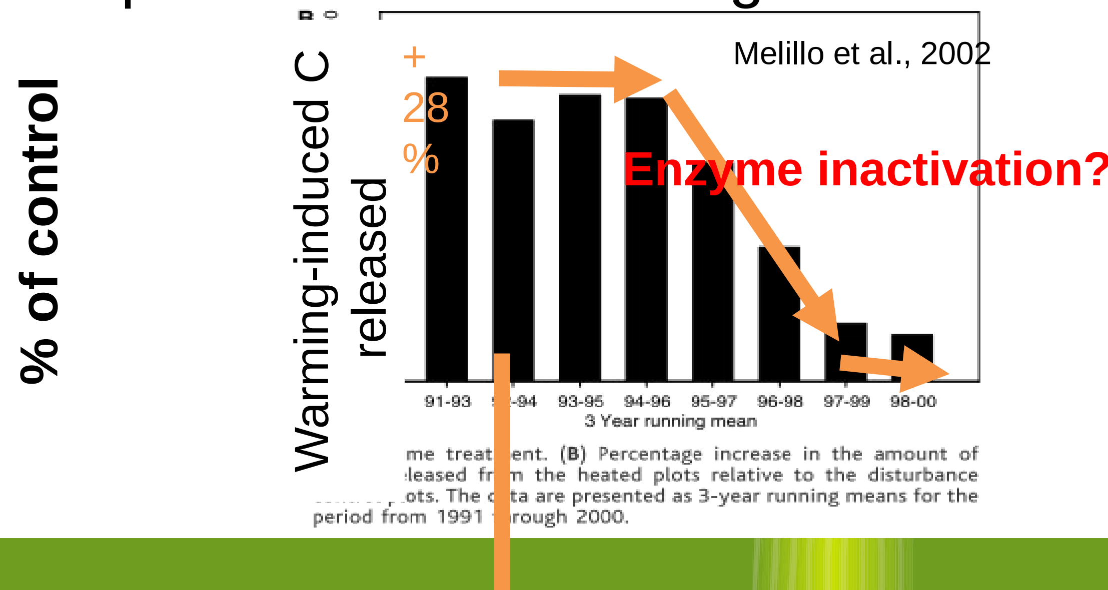
Dynamic response of decay rate (5°C warming)

40% increase of enzyme production with warming …



Conclusions & Implications

- Enzymes do not have optimum temperature.
- *Epower* is a relevant enzyme trait to characterize T effect on enzyme activities
- Cold temperature can promote enzyme activities
- The enzyme inactivation may explain the temporal change in T response of microbial degradation of soil C

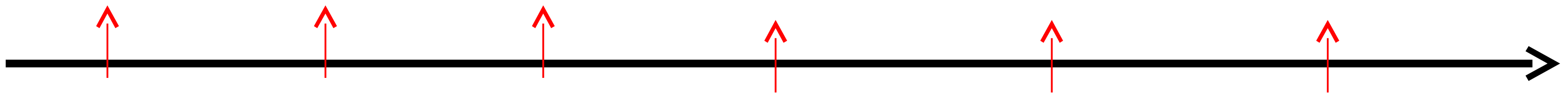


Conclusions & implications

- The universal pattern *EAinact* > *EAcat* indicates that the Epower systematically decrease with T
  - ✓ What evolutionary process can explain this pattern?
  - ✓ What are the consequences for cell physiology and flux of matter?
  - ✓ How can microorganisms adapt to the lower Epower of their enzymes in warm environment ?

These questions could be assessed in NEXER of Franciso Matus :

Characterisation of soil enzymes activities & their thermodynamics properties
Extraction of microorganisms & characterisation of their physiological and enzymes traits



Latitudinal gradient of T

Thank you for your attention