

Supplementary material

Type of contribution: Article

Temperature sensitivity and enzymatic mechanisms of soil organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro

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Supplementary table S1. Characteristics of sampling sites and soils from altitudinal transect on Mt. Kilimanjaro, MAT – mean annual temperature.

Altitude, m a.s.l.	Altitudinal zone	Vegetation type	C _{org}	N	C/N	pH	MAT °C
			%	%			
950	colline zone	low mountain tropical forest	12.8	0.9	14.2	4.5	22
2010	middle mountane zone	<i>Ocotea-Podocarpus</i> forest	15.2	0.87	17.5	4.0	17.5
2435	upper mountane zone	<i>Podocarpus</i> forest	10.7	0.62	17.3	4.5	12.5
2780	lower subalpine zone	<i>Erica excelsa</i> / <i>Podocarpus</i> forest	20.9	1.2	17.4	3.7	8.5
3020	middle subalpine zone	<i>Erica excelsa</i> / <i>Erica</i> shrubland	11.6	0.7	16.6	4.3	7.2

Supplement 2. Derivation of $Q_{10\text{total}}$ and S_{crit} .

Both V_{max} and K_m are responsible for the reaction rate (v) in Michaelis–Menten kinetics. As V_{max} increases with increasing temperature, the reaction rate can potentially increase, but as K_m also increases with increasing temperature, the affinity for the substrate decreases, so the reaction is slowed.

If a canceling effect occurs, the temperature increase does not cause the reaction rate to increase.

Let the 10 °C shift in temperature cause $Q_{10}^{V_{\text{max}}}$ -fold increase in V_{max} and $Q_{10}^{K_m}$ -fold increase in K_m . Then the reaction rates at 10 °C: $v^{10\text{°C}} = V_{\text{max}} * S / (K_m + S)$

and at 20 °C: $v^{20\text{°C}} = Q_{10}^{V_{\text{max}}} * V_{\text{max}} * S / (Q_{10}^{K_m} * K_m + S)$

Accordingly, the Q_{10} of reaction rate (Q_{10}^{total}) is derived as:

$$Q_{10}^{\text{total}} = v^{20\text{°C}} / v^{10\text{°C}} = Q_{10}^{V_{\text{max}}} * V_{\text{max}} * S * (K_m + S) / (Q_{10}^{K_m} * K_m + S) * V_{\text{max}} * S,$$

$$\text{Finally, } Q_{10}^{\text{total}} = (Q_{10}^{V_{\text{max}}} * (K_m + S)) / (Q_{10}^{K_m} * K_m + S) \quad (1S)$$

Canceling means: $v^{10\text{°C}} = v^{20\text{°C}}$, i.e.

$$V_{\text{max}} * S / (K_m + S) = Q_{10}^{V_{\text{max}}} * V_{\text{max}} * S / (Q_{10}^{K_m} * K_m + S) \quad (2S)$$

We transformed Eq. 1S to analyze how high the $Q_{10}^{K_m}$ (i.e. the K_m increase) should be to counterbalance a V_{max} increase by the factor of $Q_{10}^{V_{max}}$:

$$Q_{10}^{K_m} = (Q_{10}^{V_{max}} K_m) / K_m + (Q_{10}^{V_{max}} - 1) * S / K_m, \text{ or}$$

$$Q_{10}^{K_m} = Q_{10}^{V_{max}} + (Q_{10}^{V_{max}} - 1) * S / K_m. \quad (3S)$$

The substrate concentration in Equation 2S equals the critical substrate concentration (S_{crit}) below which the canceling effect occurs. Theoretically, three situations can occur as a response of Michaelis-Menten parameters to temperature increase:

- 1) both K_m and V_{max} increase equally: $S_{crit} = (Q_{10}^{K_m} - Q_{10}^{V_{max}}) * K_m / (Q_{10}^{V_{max}} - 1)$. If $Q_{10}^{K_m} = Q_{10}^{V_{max}}$, canceling effect can occur only at zero substrate concentrations;
- 2) K_m increase is weaker than V_{max} : $Q_{10}^{K_m} < Q_{10}^{V_{max}}$. Canceling effect can occur only if $Q_{10}^{V_{max}} < 1$, i.e. both V_{max} and K_m decrease with temperature increase;
- 3) K_m increase is greater than V_{max} : $Q_{10}^{K_m} > Q_{10}^{V_{max}}$. Canceling effect can occur at $S_{crit} = [(Q_{10}^{K_m} - Q_{10}^{V_{max}}) / (Q_{10}^{V_{max}} - 1)] * K_m$

Theoretically it is possible **that a temperature increase even results in slower reaction rates** for enzyme kinetics at low substrate concentrations:

$$v^{20oC} / v^{10oC} = Q_{10}^{V_{max}} (K_m + S) / (Q_{10}^{K_m} K + S) < 1,$$

$$S < [(Q_{10}^{K_m} - Q_{10}^{V_{max}}) / (Q_{10}^{V_{max}} - 1)] * K_m$$

This relationship is valid only for greater temperature sensitivity of K_m versus V_{max} . ($Q_{10}^{K_m} > Q_{10}^{V_{max}}$).

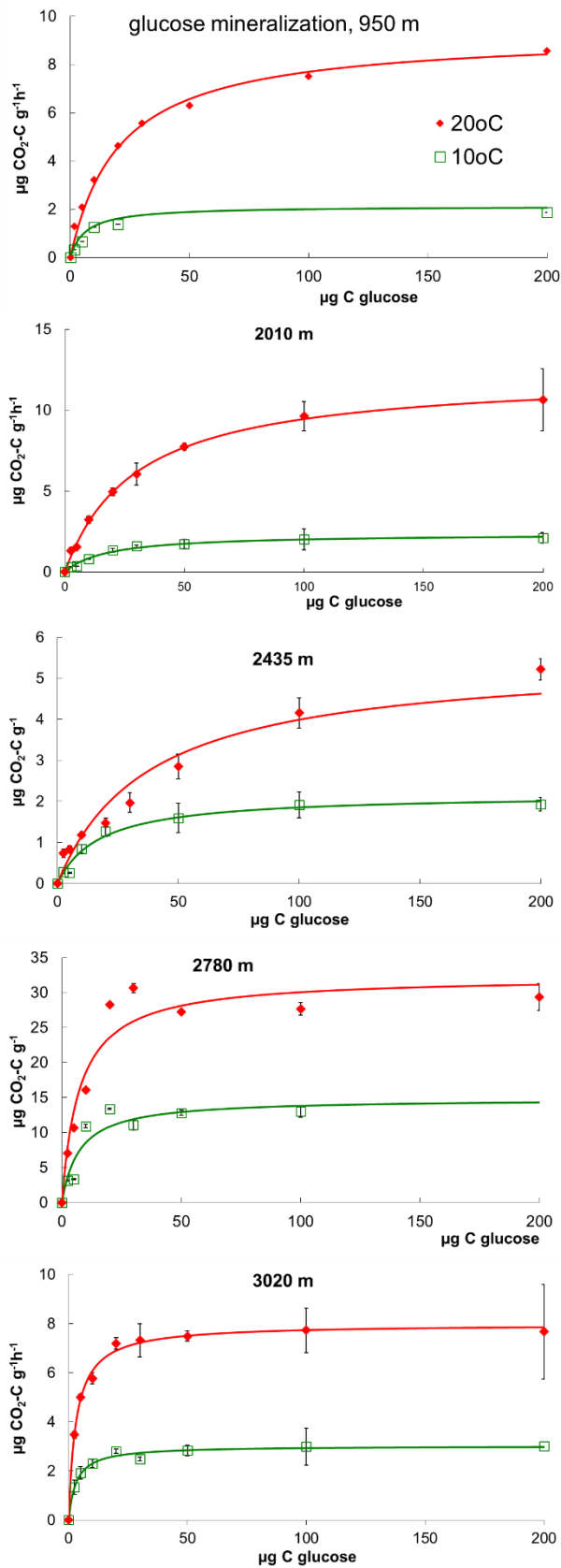
At $S > [(Q_{10}^{K_m} - Q_{10}^{V_{max}}) / (Q_{10}^{V_{max}} - 1)] * K_m$ the positive temperature response, i.e. increase in mineralization rates with the temperature can be predicted based on Michaelis–Menten kinetics.

Supplementary table S3. Substrate amounts below which a canceling effect occurs calculated by Eq.2 for the activity of hydrolytic enzymes in soils from 2010 and 3020 m a.s.l. on Mt. Kilimanjaro

Altitude	S_{crit}		
	Substrate, $\mu\text{mol g}^{-1}$		
	Glucosidase	Chitinase	Phosphatase
2010 m	1.68	2.15	0.46
3020 m	2.39	2.90	5.71

Supplementary table S4. Temperature-induced changes in substrate-to-enzyme affinity (K_m), in maximal rate (V_{max}) and activation energy (E_a) of glucose oxidation in soils from an altitudinal transect on Mt. Kilimanjaro. The data marked by same letters (case-specific) are not significantly different.

Altitude, ma.s.l.	$K_m, \mu\text{g C g}^{-1}$		$V_{max}, \mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$		$E_a, \text{kJ mol}^{-1}$
	10°C	20°C	10°C	20°C	
950	$6.38^d \pm 0.53$	$20.80^c \pm 1.36$	$2.13^J \pm 0.01$	$9.30^D \pm 0.21$	100.4
2010	$17.67^c \pm 2.18$	$29.39^b \pm 1.48$	$2.37^H \pm 0.09$	$12.24^C \pm 0.21$	111.9
2435	$17.49^c \pm 3.1$	$34.05^a \pm 2.84$	$2.18^{HJ} \pm 0.11$	$5.49^F \pm 0.13$	62.9
2780	$7.34^d \pm 1.64$	$7.31^d \pm 0.49$	$14.84^B \pm 1.39$	$32.29^A \pm 2.24$	53.0
3020	$3.12^e \pm 0.43$	$3.13^e \pm 0.25$	$3.02^G \pm 0.07$	$7.98^E \pm 0.12$	66.1



Supplementary Figure S5. Rate of $\text{CO}_2\text{-C}$ production during glucose oxidation as dependent on substrate concentration at 10 and 20 °C for different altitudes. Symbols – experimental data, lines – approximation by Michaelis–Menten kinetics. Bars show standard deviations of the means ($n=3$).