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1 **Gibbs energy or enthalpy - What is relevant for microbial C-turnover in soils?**

2 A letter to Wang & Kuzyakov, GCB, 2023

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28 The review by Wang and Kuzyakov (2023) addresses the link between microbial carbon utilization  
29 and energy transformation in soil and claimed to explore the relationship between microbial carbon  
30 use efficiency (CUE) and energy use efficiency (EUE) by analyzing published experimental theory and  
31 evidence. The authors expanded recent efforts to consolidate the largely fragmentary  
32 conceptualization of thermodynamic controls of coupled matter and energy flows in soil – a timely  
33 and relevant topic for understanding soil organic matter cycling under global change.

34 A robust consolidated concept for coupled CUE and EUE in soil systems requires applying  
35 thermodynamic state variables correctly for enabling energy balances. However, we found  
36 oversimplifications and severe inconsistencies in the application of thermodynamic principles and  
37 state variables in their review:

- 38 1. The authors state ‘that microorganisms need mainly energy but not C per se’. This statement  
39 is wrong, since organisms use the organic compounds generally as source for both energy  
40 and C, depending on the molecular composition (Lengler et al., 1999).

- 41 2. They did not mention that Gibbs energy depends to a minor extent on the oxidation half-  
42 reaction but dominantly on the reduction half-reaction, e.g. the formation of water, and the  
43 breakdown of C compounds. Microorganisms 'harvest' the highest energy from these  
44 reduction processes.
- 45 3. They declare their own understanding of the Gibbs energy "as the standard molar enthalpy  
46 of combustion of organic compounds ( $\Delta H$ )". However, such unfounded simplification  
47 increases the chance for misinterpretation of all following considerations and if used it  
48 should be justified carefully. They ignored the entropy ( $S$ ) impact on energy balancing  
49 according to the Gibbs-Helmholtz equation ( $\Delta G = \Delta H - T\Delta S$ ) (Assael et al., 2022). The text  
50 further completely lacks to point out that enthalpy changes can be measured by calorimetry,  
51 even though calorimetry has been applied in soil research.
- 52 4. LaRowe and van Capellen (2011) introduced the NOSC approach to correlate the substrate  
53 elemental composition to the Gibbs energy of the compound oxidation half-reaction  
54 ( $\Delta G_{Cox}^0 = 60.3 - 28.5 \cdot NOSC \text{ kJ/mol}$ ). In contrast, the authors empirically correlated the  
55 NOSC approach to the combustion enthalpy ( $\Delta_c H = -454 + 108 \cdot NOSC \left(\frac{\text{kJ}}{\text{mol C}}\right)$ ). This is  
56 confusing because the original paper states that the oxidation half-reaction needs to be  
57 combined with different electron acceptors (see 2, nitrate, sulfate etc.) whereas in the  
58 review NOSC alone should provide information on the full energy content of a compound as  
59 combustion enthalpy. This modified approach was not properly explained. In addition, the  
60 presentation of this concept in Fig 6 is at least confusing, as  $\Delta_c H$  does not increase with  
61 increasing NOSC.
- 62 5. Proceeding from eq. 6 to eq. 7 is not derived. Moreover, the enthalpy of formation of  $\text{CO}_2$  (-  
63 393.51 kJ/mol) and water (- 285.83 kJ/mol) is introduced but for the energy content of  
64 biomass ( $108 \cdot NOSC_{MB} - 454 \text{ kJ/mol}$ ) the combustion enthalpy is used. Due to this mixing of  
65 energy values related to different reference states, eq. 7 is presumably not correct. As a  
66 consequence, the presented linear regressions between CUE and EUE in Fig. 4 and the other  
67 related conclusions may be invalid. This even does not match the concepts of EUE presented  
68 by the authors.
- 69 6. The activation energy is compared with the reaction enthalpy in Fig. 6. Again, this is a  
70 mixture of two different concepts. The chemical activation energy controls process kinetics  
71 and the reaction enthalpies relate to the thermodynamics of the process. The 'real'  
72 activation energy of bioprocesses depends strongly on the available enzymes, whereas the  
73 enthalpy change of reaction ultimately depends only on all the reaction species involved and  
74 their stoichiometry.

- 75 7. If CUE and EUE are suggested to be compared, the comparison should be performed on the  
76 same basis. That is 'used C/energy to converted C/energy'. There is also a discrepancy of  
77 definitions between box 1 and eq. 6.
- 78 8. The authors postulated that based on their calculation approach the EUE must always be  
79 smaller than the CUE. However, these efficiencies do not reflect the ongoing microbial  
80 processes. The energy is transferred in the cell in the form of ATP or reduction equivalents  
81 and both are generated by the catabolic (combustion) reaction of substrates and thus reduce  
82 CUE. Consequently, the energy loss correlates with C loss, even if there is no C-fixing (bio-  
83 )reaction.
- 84 In general, it is our impression that the authors set up and postulate a number of 'general rules'  
85 without substantial support from their own or literature data. Hence, it appears that all these  
86 statements and equations are not valid.

87

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