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Bioenergetics of microbial maintenance metabolism in soil

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ABSTRACT

Both active and dormant soil microorganisms spend a substantial fraction of their carbon and energy resources to maintain themselves, yet microbial maintenance metabolism remains inadequately represented in models of soil carbon cycling. The calorespirometric ratio (CR) of heat to CO_2 release has been established as a powerful tool to investigate the bioenergetics of microbial growth in soil, but its application to non-growth metabolism has not been systematically explored. Here, we use dynamic modeling to assess how maintenance processes influence the coupling between microbial carbon and energy use as reflected by the CR and how maintenance alters the relationship between the CR and microbial carbon use efficiency (CUE). We find that maintenance metabolism reduces apparent CUE, while its effects on the CR depend on the energy content of the soil organic matter (SOM) or biomass compounds consumed to fuel the maintenance reaction. A compilation of literature data on the CR in different soils reveals highly variable values, indicating that soil microbes utilize a wide range of substrates and metabolic pathways to meet their maintenance demands. In arable soils, we find a close linear relationship between maintenance CR and average SOM energy content, while there is no clear pattern in forest soils. Compiled CR observations after glucose addition display a pronounced drop in CR at the onset of the retardation phase, suggesting a shift towards the use of energy poor substrates. We present a bioenergetic framework to incorporate maintenance metabolism in process-based models of soil microbial carbon use, and our compiled data show how maintenance processes affect the coupling between carbon and energy cycling both in unamended soils as well as after the addition of labile substrates.

1. Introduction

Soil organic matter (SOM) represents the largest reservoir of terrestrial organic carbon (C) and plays an important role for the future of the global C cycle and climate change (Jobbágy and Jackson, 2000; Jackson et al., 2017). Although microbial biomass constitutes only a small portion of this vast C pool, microbial activity is critical in determining the fate of SOM and has become a focus of global change studies (Liang et al., 2017; Tao et al., 2023). Soil microorganisms determine the balance between processes that produce and stabilize new SOM, such as the formation of biomass, metabolic products and necromass, and those that lead to the mineralization of fresh plant residues and existing SOM, resulting in C losses to the atmosphere as CO_2 (Schimel and Schaeffer, 2012; Lehmann and Kleber, 2015).

In the context of the delicate balance between C gains and losses, microbial carbon use efficiency (CUE) has emerged as a key concept (Geyer et al., 2016; Manzoni et al., 2018). CUE is broadly defined as the fraction of C consumed by microbes that is converted into new biomass during growth. This efficiency varies widely depending on environmental factors (Manzoni et al., 2012; Sinsabaugh et al., 2016), the quality and availability of substrates (Sinsabaugh et al., 2013; Blagodatskaya et al., 2014), and the composition of the microbial community (Geyer et al., 2016; Maynard et al., 2017). Recent advances have integrated this carbon-centered view in a bioenergetic framework that also considers microbial energy use efficiency (EUE, Gunina and Kuzyakov, 2022; Wang and Kuzyakov, 2023; Kästner et al., 2024). This approach builds on a thermodynamic understanding of microbial growth (von Stockar and Liu, 1999; Heijnen and Kleerebezem, 2010) that recognizes the fundamental links between the flows of matter and energy during metabolism as well as its thermodynamic constraints (Calabrese et al., 2021). While this framework has been successfully applied under highly controlled conditions, e.g., in biotechnology (von Stockar and Marison,

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1993), its application to the soil environment is still in an early stage (Herrmann and Bölscher, 2015; Barros, 2021; Kästner et al., 2024).

The simultaneous measurement of heat and CO_2 release from soil via calorespirometry is a simple yet powerful method for investigating the coupling between C and energy during microbial growth in the complex soil system (Barros et al., 2011, 2016). A key metric derived from this approach is the calorespirometric ratio (CR), which is defined as the ratio of heat to CO_2 released from the soil (Box 1, Fig. 1). It is closely linked to the efficiency and stoichiometry of microbial metabolism and can be used to estimate CUE and EUE in substrate amendment experiments (Hansen et al., 2004; Chakrawal et al., 2021; Yang et al., 2024) or to distinguish between aerobic and anaerobic metabolic pathways (Chakrawal et al., 2020; Endress et al., 2024a). Additionally, when combined with estimates of SOM energy content, the CR can provide insights into the quality of SOM and its susceptibility to mineralization. For example, it can reveal SOM utilization patterns in unamended soils depending on depth or management (Barros et al., 2020; Lestido-Cardama et al., 2024) or the increased mineralization of SOM after the addition of labile C sources to soil, also known as the priming effect (Blagodatskaya and Kuzyakov, 2008; Chakrawal et al., 2020; Wirsching et al., 2024). Therefore, it offers a promising avenue to investigate SOM stability and the characteristics of microbial SOM consumption that ultimately drive soil C stock dynamics (Gunina and Kuzyakov, 2022; Kästner et al., 2024).

Efforts to develop a process-based understanding of the CR have focused on simple growth reactions fueled by well-defined substrates or on the dynamics during the exponential growth phase after substrate amendment, when CO_2 and heat contributions from other processes are negligible (Hansen et al., 2004; Chakrawal et al., 2021; Endress et al., 2024a, 2024b). Under these conditions, a direct theoretical relationship between CUE and CR can be established (Chakrawal et al., 2020). However, even during phases of active microbial growth, maintenance and non-growth metabolism may represent a substantial fraction of microbial C use (e.g., Bölscher et al., 2024), resulting in potential deviations from this simple theoretical relationship. Moreover, in most soils and for most of the time, microbial growth is limited by low substrate availability. Under these conditions, the majority of microbial cells are non-growing or persist in a dormant state, with periods of activity triggered by spatially and temporally constrained pulses of substrate supply (Blagodatsky et al., 2000; Kuzyakov and Blagodatskaya, 2015). Due to the long periods of microbial inactivity on the one hand and the significant C and energy flows associated with non-growth metabolism during times of activity on the other hand, maintenance metabolism and the energetic costs of transitioning into and out of dormancy are likely to have a strong impact on soil C dynamics (Joergensen and Wichern, 2018; Dijkstra et al., 2022; Bölscher et al., 2024). Microbial maintenance processes, however, are defined ambiguously in literature. They encompass diverse processes such as physiological maintenance (e.g., cell motility, osmoregulation, and internal turnover of macromolecules), the formation and consumption of storage compounds, and the secretion of extracellular polymeric substances (EPS) and extracellular enzymes (van Bodegom, 2007; Kempes et al., 2017; see Box 1 for definitions). The ambiguity in defining microbial maintenance and activity is also reflected in mechanistic model representations (van Bodegom, 2007; Wang and Post, 2012), contributing to the considerable uncertainties associated with microbial C turnover in earth system models (Wieder et al., 2013, 2015; Wang et al., 2015). While incorporating bioenergetic principles offers a promising way to alleviate these limitations, a systematic evaluation of non-growth processes within the CR framework is still lacking.

In this study, we employ a process-based modeling approach to examine the information contained in joint observations of heat and CO_2 release as obtained in soil incubation experiments. Our analysis focuses specifically on microbial processes beyond growth that have not been

Box 1
The calorespirometric ratio and microbial maintenance

In biochemical reactions, the flows of matter and energy are tightly coupled. For an aerobic microbial growth reaction fueled by a substrate *S*,

$$\mathrm{S} + n_{\mathrm{O_2}}\mathrm{O_2} \rightarrow \mathrm{YB} + (1-\mathrm{Y})\,\mathrm{CO_2} + \Delta_r\mathrm{H} \tag{1}$$

the CO_2 release per mol C of substrate is determined by the yield Y, which corresponds to the stoichiometric coefficient of biomass B formation. Similarly, the heat produced per mol C of substrate is given by the reaction enthalpy $\Delta_r H$,

$$\Delta_r\mathrm{H} = \left(1 - \mathrm{Y}\frac{\gamma_B}{\gamma_S}\right)\Delta_c\mathrm{H_S} \tag{2}$$

which depends on the relative degrees of reduction of biomass γ_B and substrate γ_S as well as the combustion enthalpy of the substrate $\Delta_c H_S$. Equation (2) results from the application of the law of Hess to the growth reaction (1) (Chakrawal et al., 2020; Kästner et al., 2024). In this simple scenario, the reaction will produce a calorespirometric ratio (CR) of heat to CO_2 given by

$$\mathrm{CR} = \frac{\Delta_r\mathrm{H}}{(1-\mathrm{Y})} \tag{3}$$

which can be observed experimentally. However, if processes other than reaction (1) like maintenance metabolism also produce heat and CO_2, the observed CR will systematically deviate from Eqn. (3). In this article, we consider two types of maintenance (Fig. 1):

(i) exogenous maintenance, in which an external substrate is catabolized to fuel energy requirements and no biomass is consumed. The substrate may be identical to the one fueling the growth reaction (1), or it may be a different chemical compound, e.g., a part of SOM.
(ii) endogenous maintenance, in which internal compounds are consumed to meet maintenance requirements and thus a net biomass decrease occurs. The consumed biomass compounds may be identical to the average bulk biomass, or they may be more specific, such as storage compounds.

Note that both types of maintenance may also yield additional products other than CO_2 (e.g., EPS, extracellular enzymes, metabolic byproducts), and endogenous maintenance may be coupled with the formation of necromass. In any case, the CR of each individual reaction may be estimated in a manner similar to equation (3) (A detailed derivation is presented in sections 2.1 – 2.3).

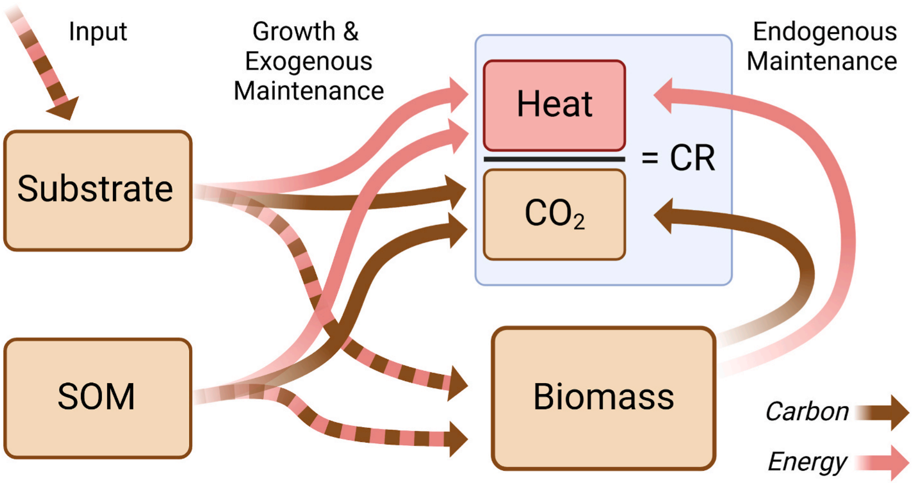

Fig. 1. Illustration of major C and energy fluxes in typical incubation experiments. Substrates added to soil as well as native SOM are utilized by microbes to fuel both their growth and maintenance requirements (here termed exogenous maintenance). Alternatively, microbes may consume their own biomass to sustain themselves if insufficient resources are available (here termed endogenous maintenance). In an aerobic setting, all these processes release heat and CO_2 at specific rates. Thus, their ratio (CR) can be leveraged to study the coupling between C and energy dynamics in the soil system over time.

described in detail within the CR framework, such as exogenous and endogenous maintenance (Box 1). For the purposes of our modeling investigation, we use the term "maintenance" to summarize all microbial C and energy flows that do not result in a net formation of new microbial biomass. We explore the quantitative implications of such processes for the theoretical relationship between CUE and CR. Finally, we compile and interpret CR values and SOM energy contents from unamended soils as well as dynamic CR measurements for the retardation phase after microbial growth on glucose, thus providing experimental insights into the energetics of microbial non-growth metabolism and SOM mineralization.

2. Materials and methods

2.1. Growth and non-growth reactions and their energetics

The derivation of the microbial growth reaction (Eqn. (1)) and its energetics (Eqns. (2) and (3)) has been presented in detail before (Chakrawal et al., 2020; Endress et al., 2024a). In brief, we consider an aerobic catabolic reaction fueled by external substrate S,

$$S + n_{cat,O_2} O_2 \rightarrow CO_2 + \Delta_{cat} H_S, \tag{4}$$

expressed for the consumption of 1 mol C of S, with a catabolic reaction enthalpy $\Delta_{cat} H_S$ equal to the combustion enthalpy $\Delta_c H_S$ of the substrate and the stoichiometric coefficient of catabolic O_2 consumption n_{cat,O_2}. If $\Delta_c H_S$ is unknown, it may be estimated using Thornton's rule (Thornton, 1917) via the relative degree of reduction γ_S of the substrate:

$$\Delta_c H_S = \frac{\gamma_S}{4} \cdot 455 \pm 15 \text{ kJ mol}^{-1} \text{ C} \tag{5}$$

where

$$\gamma_S = \frac{4n_C + n_H - 2n_O - 3n_N}{n_C} \tag{6}$$

is calculated using the number n_X of atoms of element X in the compound. This formulation assumes that CO_2, NH_3 and H_2O are the reference compounds with zero degree of reduction (Chakrawal et al., 2020; Kästner et al., 2024). For biomass, we consider the empirical formula $CH_{1.571}O_{0.429}N_{0.143}$ with $\gamma_B \approx 4.284$. Note that Eqn. (5) can also be used to estimate the reaction enthalpy of an aerobic endogenous maintenance reaction converting biomass to CO_2. In the case of anaerobic catabolic reactions, the reaction enthalpy has to be estimated from the enthalpies of formation of the involved compounds using the law of Hess or obtained from the literature (for examples, see Chakrawal et al., 2020; Endress et al., 2024a; Kästner et al., 2024).

For the anabolic reaction, we use the electron balance approach for the formation of 1 mol C of biomass B (Battley, 2009):

$$\frac{\gamma_B}{\gamma_S} S \rightarrow B + \left(\frac{\gamma_B}{\gamma_S} - 1 \right) CO_2 + \Delta_r H_{ana} \tag{7}$$

which yields $\Delta_r H_{ana} = 0$ based on Thornton's rule. To obtain the overall growth reaction expressed per mol C of substrate consumed and with a fixed yield coefficient Y, the catabolic and anabolic reactions are multiplied by $\left(1 - Y\frac{\gamma_B}{\gamma_S}\right)$ and Y, respectively. Their sum then yields Eqn. (1), and the sum of their reaction enthalpies yields the heat production per mol of C consumed (Eqn. (2)). Note that this approach can also be used to estimate the reaction enthalpy of other processes, such as the formation of necromass from biomass during turnover (see also below). In this case, biomass serves as a substrate for the formation of necromass and CO_2, and the reaction enthalpy depends on their relative degrees of reduction as well as the (necromass) yield coefficient of the process according to Eqn. (2).

Finally, the CR value associated with any individual reaction is obtained as the ratio of its reaction enthalpy to its stoichiometric coefficient of CO_2 production (Eqn. (3)). If several processes $i = 1, 2, \ldots$ occur simultaneously, the overall CR is given by the ratio of the sum of all reaction enthalpies $\Delta_r H_i$ to that of all coefficients of CO_2 production $n_{CO_2,i}$, each weighted by the respective reaction rate r_i:

$$CR = \frac{\sum_i \Delta_r H_i \cdot r_i}{\sum_i n_{CO_2,i} \cdot r_i} \tag*{(Eqn. 8)}$$

2.2. Dynamic modeling

A wide variety of systems of ordinary differential equations (ODEs) can be constructed based on the flows of C and energy outlined above to explore, e.g., the effects of maintenance processes, microbial dormancy or SOM utilization and their interactions (Chakrawal et al., 2020, 2021; Endress et al., 2024a, 2024b; Wirsching et al., 2024). A simple ODE system to simulate a typical soil incubation experiment with substrate amendment is given by

$$\frac{dS}{dt} = -G - M_{exo} \tag{Eqn.9}$$

$$\frac{dB}{dt} = YG + Y_{som}U_{som} - M_{endo} \tag{Eqn.10}$$

$$\frac{dCO_2}{dt} = (1-Y)G + (1-Y_{som})U_{som} + M_{exo} + M_{endo} \tag{Eqn.11}$$

$$\frac{dQ}{dt} = \Delta_{\mathrm{r}}H_G G + \Delta_{\mathrm{r}}H_{som}U_{som} + \Delta_{\mathrm{cat}}H_S M_{exo} + \Delta_{\mathrm{r}}H_{endo}M_{endo} \tag{Eqn.12}$$

where B and S denote the concentrations of biomass and added substrate, respectively, and CO_2 and Q denote the $\mathrm{CO_2}$ and heat released by microbial metabolism, which may be measured via calorespirometry. The flux G represents the microbial growth reaction on added substrate (e.g., glucose) and takes the form $G = \nu\phi B$, where ν denotes the maximum specific growth rate and $\phi = \phi(S) \in [0,1]$ represents the effect of substrate saturation/availability on growth (for example, $\phi(S) = \frac{S}{K+S}$ yields typical Michaelis-Menten or Monod kinetics). Similarly, the flux M_{exo} represents exogenous maintenance utilizing the same added substrate S and can thus be written as $M_{exo} = m_{exo}\phi B$ with a specific exogenous maintenance rate m_{exo}. In addition, $M_{endo} = m_{endo}B$ represents endogenous maintenance with specific rate m_{endo}. Note that this simple formulation assumes $\mathrm{CO_2}$ as the only product of maintenance fluxes. Finally, growth on native SOM is represented by the flux U_{som} (e.g. first order kinetics) with efficiency Y_{som}. Setting $U_{som} \equiv 0$ gives a model without priming, while $Y_{som} \equiv 0$ can be chosen to study exogenous maintenance fueled by SOM instead of added substrate S.

For all reactions, the reaction enthalpies are calculated as outlined above and in Box 1, and the corresponding rates of heat release are given by the product of these reaction enthalpies and the reaction rates (Eqn. (8)).

Our simulations were obtained using a model that also considers the activity state of the microbial population via the index of physiological state $r \in [0,1]$, which denotes the active fraction of biomass (Panikov, 1995; Blagodatsky et al., 1998; Endress et al., 2024b). The dynamics of r are described by

$$\frac{dr}{dt} = Y\nu r\left(\frac{S}{S+K_r} - r\right) \tag{Eqn. 13}$$

with half-saturation constant K_r, such that microbes become active in the presence of sufficient substrate S and turn inactive as substrate gets depleted. Since only the active fraction performs the growth reaction, the corresponding grow fluxes G and U_{som} of the extended model read $G = \nu\phi B{\cdot}r$ and $U_{som} = \nu_{som}B{\cdot}r$. In addition, the model considers a dynamic switching from exogenous to endogenous maintenance as substrate availability becomes insufficient to fuel maintenance requirements (Wang and Post, 2012). Therefore, we set $M_{endo} = (1-\phi)m_{exo}B$ for a total maintenance rate of $m_{exo}B$ independent of substrate concentration.

For simplicity of notation and conceptual analysis, we assume that the specific maintenance coefficients are the same for the active and inactive biomass fractions, but differences between active and inactive maintenance requirements can easily be incorporated via additional parameters (Endress et al., 2024b) to reflect the lower energy demand of inactive cells (Dijkstra et al., 2022).

A summary of all model variables and parameters including values and units is provided in the SI (Table S1). Parameter values were chosen to represent typical dynamics after glucose addition as obtained via calibration, e.g., in (Chakrawal et al., 2021; Endress et al., 2024a, 2024b). Simulations were performed in Python (version 3.9.18) using numerical integration of the ODE system via the *radau* method of the *solve_ivp* function in the *scipy.optimize* package (version 1.11.4, Virtanen et al., 2020).

2.3. Theoretical relationship between CR and CUE in the presence of maintenance

The theoretical relationship between the CR and CUE for simple growth reactions with a constant biomass yield coefficient Y (i.e., the case when CUE equals the coefficient Y) is given by Eqn. (3) and has been examined in detail before (Hansen et al., 2004; Chakrawal et al., 2020; Yang et al., 2024). To derive the relationship in the presence of maintenance, it is helpful to introduce an auxiliary variable θ denoting the fraction of the total consumed carbon that is partitioned to the growth reaction. For the case of exclusively exogenous maintenance fueled by the same substrate as growth, this fraction

$$\theta = \frac{G}{G+M_{exo}} = \frac{\nu}{\nu + m_{exo}} \tag{Eq.14}$$

is constant. To calculate the CUE at each point in time, we divide the net change in biomass by the amount of total consumed C to get

$$CUE = \frac{\frac{dB}{dt}}{\frac{dS}{dt}} = \theta Y \tag{Eqn. 15}$$

Similarly,

$$\begin{aligned} CR = \frac{\frac{dQ}{dt}}{\frac{dCO_2}{dt}} &= \frac{\Delta_{\mathrm{r}}H_G G + \Delta_{\mathrm{cat}}H_S M_{exo}}{(1-Y)G + M_{exo}} = \frac{\left(1 - \mathrm{Y}\frac{\gamma_{\mathrm{B}}}{\gamma_S}\right)\Delta_{\mathrm{cat}}H_S\nu + \Delta_{\mathrm{cat}}H_S m_{exo}}{(1-Y)\nu + m_{exo}} \\ &= \frac{\left(1 - \theta Y\frac{\gamma_{\mathrm{B}}}{\gamma_S}\right)\Delta_{\mathrm{cat}}H_S}{1-\theta Y} \end{aligned} \tag{Eqn. 16}$$

so that the relationship between CUE and CR is equivalent to that of a pure growth reaction with a reduced yield coefficient $\theta Y \leq Y$. Therefore, the presence of exogenous maintenance does not cause deviations from the relationship of a pure growth reaction, but merely results in shifts along the curve.

In the case of simultaneous growth and endogenous maintenance, the fraction

$$\theta = \frac{G}{G + M_{endo}} = \frac{v\phi}{v\phi + m_{endo}} \quad \text{(Eq.17)}$$

is not constant but instead attains a maximum of $\frac{v}{v+m_{endo}}$ under substrate saturation. This maximum will approach a value of 1 if the maximum growth rate far exceeds the maintenance rate (i.e., $v \gg m_{endo}$). Under such conditions, almost all carbon flow occurs through the growth reaction. As the substrate saturation ϕ declines over the course of an incubation, θ decreases towards its minimum value of 0, indicating that all carbon flow is due to endogenous maintenance.

For CUE, we have

$$CUE = \frac{Yv\phi - m}{v\phi + m} \quad \text{(Eq.18)}$$

which simplifies to

$$CUE = (1 + Y)\theta - 1 \quad \text{(Eq.19)}$$

Thus, CUE varies between the value of the yield coefficient Y if all consumed carbon is derived from substrate and fuels the growth reaction (i.e., $\theta = 1$) and the value of -1 if all consumed carbon is derived from biomass and fuels endogenous maintenance (i.e., $\theta = 0$).

For CR, we obtain

$$CR = \frac{v\phi\Delta_r H_G + m\Delta_r H_{endo}}{(1 - Y)v\phi + m} \quad \text{(Eq.20)}$$

which simplifies to

$$CR = \frac{\theta \cdot \Delta_r H_G + (1 - \theta)\Delta_r H_{endo}}{1 - \theta Y} \quad \text{(Eq.21)}$$

Therefore, the system yields a CR value equal to that of the growth reaction if endogenous maintenance is negligible and a value equal to that of the endogenous maintenance reaction if growth is negligible. Varying θ from 0 to 1 yields the full range of the theoretical CUE-CR relationship. The true value of $\Delta_r H_{endo}$ will depend on the composition and thus the energy content of the biomass being consumed. For example, the aerobic decomposition of bulk biomass with an average degree of reduction of $\gamma_B \approx 4.284$ (Yang et al., 2024) yields $\Delta_r H_{endo} \approx 487$ kJ mol^{-1} C according to Thornton's rule (Thornton, 1917).

2.4. Unamended soil data compilation

To study the bioenergetics of soil microbial maintenance in the absence of labile substrate, we compiled CR values measured in unamended soils from the literature. If available, we also analyzed measurements of average SOM energy content determined via thermogravimetric-differential scanning calorimetry (TG-DSC, Kucerik et al., 2016) in those soils to study the connection between maintenance metabolism and SOM quality. Only soils classified as arable, grassland or forest soils were included in the analysis. We used the mean values reported in the studies for each soil, i.e., each presented value represents the mean of several replicates (typically 2 or 3). All values were converted to a unit of kJ mol^{-1} C CO_2 or kJ mol^{-1} C SOM for CR and SOM energy content, respectively. The compiled data are provided as supporting material (S1 Data).

2.5. Compilation of CR data after glucose addition

To study the bioenergetics of maintenance after the depletion of a substrate pulse, we also compiled dynamic CR curves measured after glucose addition to arable soils. In the two studies that only featured a single treatment (Endress et al., 2024a; Yang et al., 2024), we considered the mean value of the CR across all replicates at each timepoint. For the three studies that measured CR in several treatments, e.g., using multiple soils (Harris et al., 2012), manipulation of spatial heterogeneity (Endress et al., 2024b), or different temperatures and degrees of salinization (Wang et al., 2025), we show the mean across all replicates of all treatments as well as the mean of each individual treatment. For all studies, the time of maximum activity was estimated as the mean time of maximum heat release rate across all replicates.

2.6. Statistics

To assess patterns in the compiled CR and average SOM energy content data as well as the relationship between the two, we used linear mixed models with additional fixed effects of land use type (arable, grassland, forest) and soil layer (0-5 cm, 5-10 cm, LF horizon, H horizon) as well as a random effect of site to account for measurements obtained from multiple plots within the same study area (see S1 Data). The analysis was performed using the *mixedlm* function of the *statsmodels* package (version 0.14.0, Seabold and Perktold, 2010) in Python (version 3.9.18).

3. Results

3.1. The joint dynamics of CO_2 and heat release after substrate addition reveal contributions of processes beyond microbial growth

Plotting the cumulative heat produced by a soil sample against the cumulative CO_2 emissions over the course of an incubation experiment after batch substrate addition offers a simple connection to the net C and energy balance of the system (Fig. 2a). In such a plot, the observations trace out a trajectory that begins at the origin when the substrate is first added, moving upward and to the right as heat and CO_2 are continuously released. When expressed as a percentage of the initial C and energy added as substrate, the endpoint of the trajectory reflects the overall C and energy balance of the soil system after the incubation, indicating net losses if CO_2 and heat emissions exceed the amount of initially added substrate and a net storage of C and energy otherwise. While this measure does not reveal anything about the nature of the retained C (e.g., biomass, necromass, or residual substrate) or the origin of the evolved CO_2 (e.g., substrate or SOM), it indicates the net storage or loss of C and energy due to the substrate amendment. In our model simulations with and without a priming effect, the additional release of C and energy per added substrate due to SOM decomposition is immediately discernible (Fig. 2a, red and black lines).

The diagonal in this plot corresponds to the complete aerobic oxidation of the added substrate, i.e., the catabolic reaction that fuels both growth and exogenous maintenance demands. Deviations from this diagonal direction reveal C and energy flows resulting from biochemical reactions other than growth or from physical processes (Fig. 2a). For instance, trajectories curving to the right of the diagonal suggest processes that release less heat per CO_2 compared to the aerobic catabolism of the substrate. Such processes might include the catabolism of energy poor compounds (e.g., oxidized SOM), or an efficient growth reaction if the substrate is more oxidized than microbial biomass ($\gamma_S < \gamma_B$). Conversely, trajectories curving upward indicate processes with a higher relative heat release, such as catabolism or growth fueled by an energy

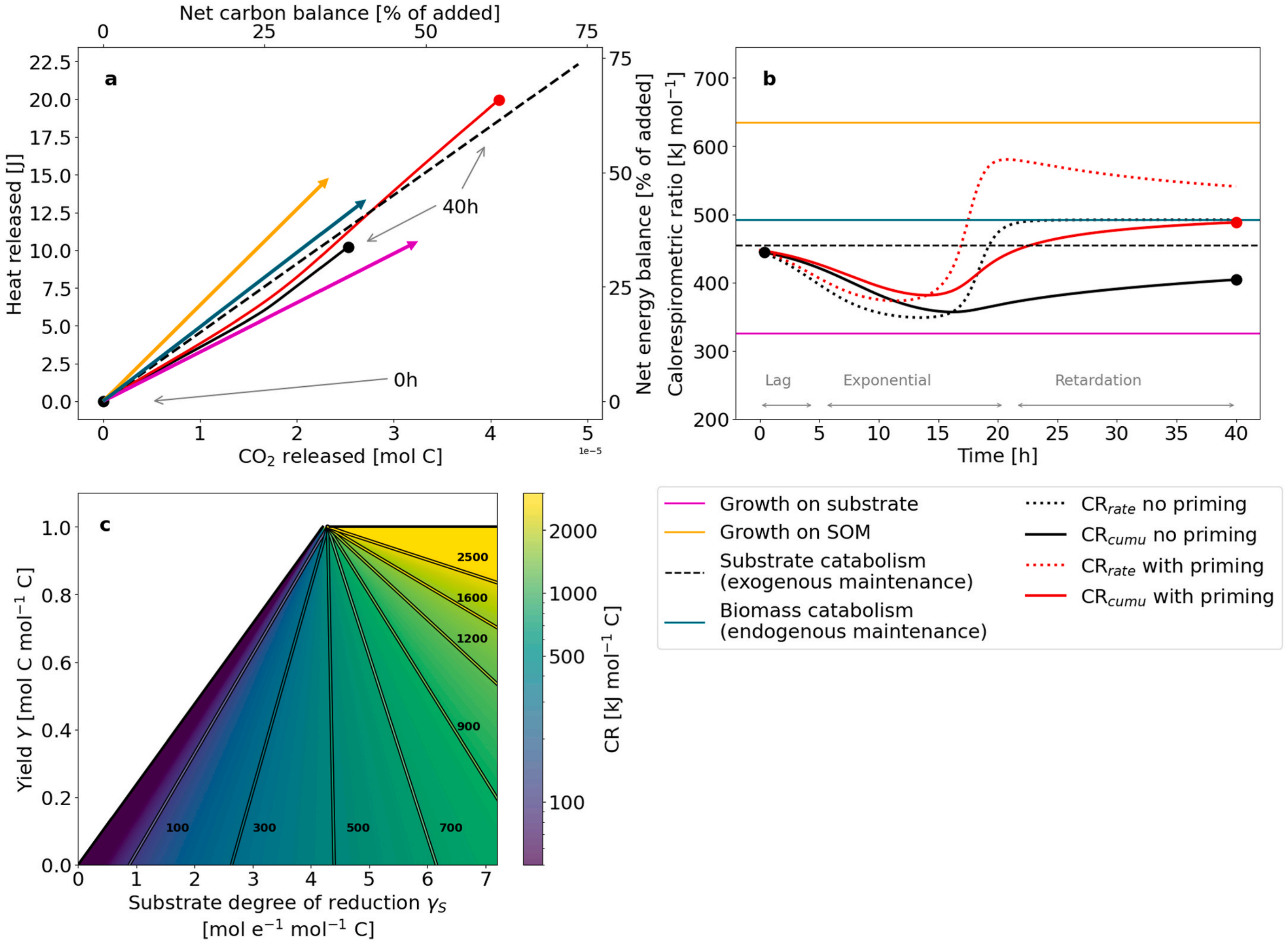

Fig. 2. Simulated patterns of heat and CO$_2$ release and CR after substrate addition. a In a plot of cumulative heat release against cumulative CO$_2$ release, the diagonal corresponds to complete oxidation of the added substrate. Deviations reveal the presence of other processes, such as efficient aerobic growth fueled by added substrate (magenta, relative degree of reduction $\gamma_S = 4$), growth fueled by highly reduced, energy rich SOM (orange, $\gamma_{SOM} = 5$), or bulk biomass consumption for endogenous maintenance (teal, $\gamma_B = 4.284$). Model simulations are performed without (black) and with (red) additional consumption of SOM (positive priming) and assume glucose as the added substrate. **b** The CR calculated from cumulative values (solid black and red) resembles the cumulative curves shown in panel a. In contrast, the CR calculated from the corresponding rates shows much more pronounced variations and reflects shifts in the dominant processes over time. **c** Identical CR values can result from different combinations of substrate energy content (measured as relative degree of reduction, γ_S) and growth yield (Y) in a simple growth reaction. Thus, the underlying process cannot be inferred from CR alone. The black line indicates the maximum theoretic yield based on simple thermodynamic considerations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rich compound (e.g., reduced SOM). For example, the positive priming of highly reduced, energy rich SOM ($\gamma_{SOM} = 5$) in our model simulation causes such a deviation relative to the trajectory without priming (Fig. 2a).

Therefore, the trajectory records shifts in dominant processes and their cumulative contributions over the course of an experiment or a simulation. During exponential growth following the addition of a labile substrate, the trajectory is primarily driven by the microbial growth reaction, which accounts for most of the C and energy flow. However, deviations before and after this phase indicate contributions from other sources of heat and CO$_2$. These variations can be discerned in more detail through an analysis of the temporal dynamics of the CR (Fig. 2b).

When the CR is calculated from cumulative heat and CO$_2$ release (CR$_{\text{cumu}}$), its dynamics mirror the trajectories observed in the cumulative state space (compare the solid black and red lines Fig. 2a and b). By contrast, the CR calculated from the instantaneous rates of heat and CO$_2$ release (CR$_{\text{rate}}$) reflects the dominant processes producing heat and CO$_2$ at each specific time point. As a result, CR$_{\text{rate}}$ is far more sensitive to changes in the dominant processes (dashed lines in Fig. 2b). Its value determines the direction of the trajectory at each time point in the cumulative plot, i.e., it corresponds to the tangent to the trajectory at that point.

In our simulations, CR$_{\text{rate}}$ clearly delineates distinct phases of microbial activity after substrate addition (Fig. 2b). During the initial lag phase, there is a strong contribution from exogenous maintenance with a CR of 469 kJ mol^{-1} C, corresponding to the combustion enthalpy of glucose as the added substrate in the model. During the exponential growth phase, the CR drops to a value characteristic of the macrochemical growth reaction. Finally, during the retardation phase as substrate becomes depleted, endogenous maintenance fueled by biomass consumption with a CR of ~490 kJ mol^{-1} C dominates.

The exact quantitative values of the CR depend on the characteristics of the underlying processes, such as the energy contents of the compounds involved and the yield coefficients of the reactions. Notably, any given CR value can arise from multiple processes (Fig. 2c). For example, a CR of 300 kJ mol^{-1} C could result from the oxidation of a low-energy substrate with a degree of reduction (γ_S) of approximately 2.64 and no growth, i.e., a yield coefficient of 0. Alternatively, the same value might be produced by a growth reaction fueled by a more energy-rich, more reduced substrate with $\gamma_S = 3.42$ and a yield of 0.5. In fact, numerous such combinations of energy contents and yield coefficients can result in the same CR value (Fig. 2c). Consequently, the specific parameter combination responsible for an observed CR value in an experiment cannot be identified based solely on the CR, especially if the dominant substrate is unknown or its properties poorly characterized. Nonetheless, the observed value provides information about the set of feasible parameter combinations (e.g., lines in Fig. 2c) and can thus be combined with additional measurements, e.g., quantification of yield coefficients or energy contents of consumed substrates, to unravel the quantitative details of the process.

3.2. *Maintenance metabolism alters the relationship between CUE and CR*

Both exogenous and endogenous maintenance reactions systematically alter the relationship between CUE and CR, depending on the energy content of the external (exogenous) or microbial (endogenous) compounds consumed in these reactions and their relative contributions to overall CO_2 and heat flow (Fig. 3). In a simple growth reaction, the apparent CUE equals the true growth yield (Y) of that reaction, defined as the stoichiometric coefficient of biomass formation. However, the inclusion of maintenance reactions reduces the apparent CUE below this value of Y. For exogenous maintenance, the CUE may decline to zero if all the C and energy are channeled through the (catabolic) maintenance reaction. In the case of endogenous maintenance, the apparent CUE can even become negative if the rate of biomass decomposition exceeds the gains from growth, resulting in a net loss of biomass.

The effect of maintenance on the CR is more complex and depends on the energy content of the maintenance substrate. When the same substrate fuels both growth and exogenous maintenance, the reaction enthalpy of the maintenance reaction equals the reaction enthalpy of substrate catabolism (under aerobic conditions, this is the combustion enthalpy of the substrate, i.e., $\Delta H_{exo} = \Delta_C H_S$, see Fig. 3). In this scenario, exogenous maintenance shifts the CR-CUE relationship along the original curve of the pure growth reaction, toward lower CUE values. In principle, different substrates may fuel the growth and maintenance reactions in different proportions, in which case the enthalpy of the maintenance reaction will differ from that of the growth reaction's catabolic component, shifting the CR-CUE relationship both toward lower CUE values and toward a distinct ΔH_{exo} (not shown). Similarly, endogenous maintenance shifts the relationship toward lower CUE values and toward the enthalpy of the endogenous maintenance reaction consuming microbial biomass (i.e., ΔH_{endo}), which varies depending on the characteristics of biomass being consumed (e.g., bulk biomass, intermediate metabolites or storage compounds).

During an incubation experiment or a simulation, estimates of CUE and CR trace out a trajectory on this plot (Fig. 3). For example, a model simulation incorporating a concentration-dependent transition from exogenous to endogenous maintenance again reveals distinct phases. In the initial lag phase, microbial growth is minimal, and the abundant substrate primarily fuels exogenous maintenance. During exponential growth, the trajectory converges toward the theoretical prediction for the growth reaction, as most C is allocated to biomass formation. Once the substrate is depleted, microbes switch to consuming their own biomass during the retardation phase, resulting in a net biomass loss. Consequently, the relationship between CUE and CR deviates most markedly from that of a pure growth reaction during the early and late stages of incubation, when the relative contributions of maintenance reactions to total C and energy flows are largest.

3.3. *Unamended soils display a wide range of CR values and SOM energy contents*

We collected a total of 74 experimental CR values derived from heat and CO_2 measurements of unamended soils reported by eight studies

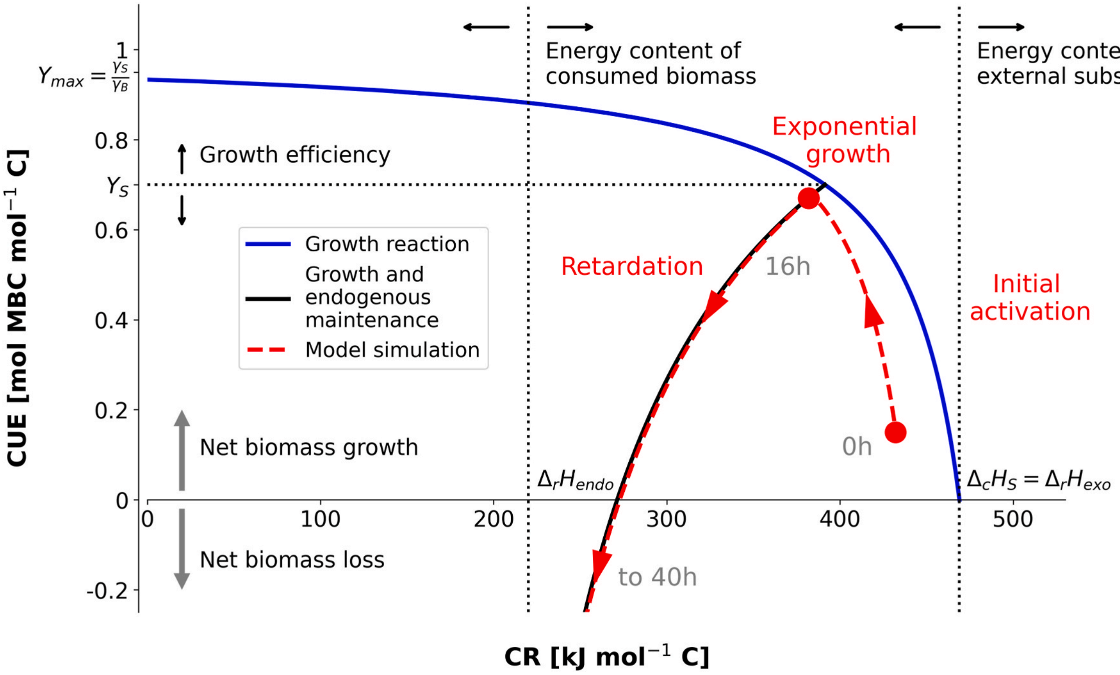

Fig. 3. Theoretical and simulated relationship between CUE and CR in the presence of endogenous and exogenous maintenance. The presence of endogenous maintenance modifies the relation between CUE and CR predicted for the pure growth reaction on glucose (blue) in the direction of lower apparent CUE (black). In a model simulation (dashed red), glucose initially fuels microbial exogenous maintenance during the initial lag phase (with CR equal to $\Delta H_{exo} = \Delta_C H_S$). During the subsequent phase of efficient exponential growth, most C is channeled through the growth reaction. After substrate depletion, microbes switch to endogenous maintenance (with a CR of ΔH_{endo}), resulting in an eventual decline of microbial biomass during the retardation phase by the end of the incubation. Details are presented in the methods section. Note that ΔH_{endo} was set to a low value of 220 kJ mol^{-1} C for visualization purposes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Barros et al., 2011, 2017, 2020; Harris et al., 2012; Herrmann and Bölscher, 2015; Barros, 2021; Lestido-Cardama et al., 2024; Wirsching et al., 2024) to investigate the bioenergetics of soil microorganisms under substrate-limited conditions. These values were obtained from forest, grassland, and arable soils, with a strong bias towards Spanish forest soils. The average CR across the three land types was 388 ± 150 kJ mol^{-1} C and there was no significant effect of land type, but observed values spanned a wide range from below 200 kJ mol^{-1} C to more than 800 kJ mol^{-1} C (Fig. 4a). While all measurements from arable and grassland soils were obtained from the top 5 cm of the mineral layer, some forest samples were also collected from top organic horizons (LF, H) and the 5-10 cm mineral layer. Across the forest soils, CR values obtained from the organic layers (445 ± 86 kJ mol^{-1} C) were significantly higher than those obtained from the mineral layer (336 ± 140 kJ mol^{-1} C).

In addition, three of the studies also provided a measure of SOM energy content ($\Delta_C H_{SOM}$) in a total of 47 soils via thermogravimetry-differential scanning calorimetry (TG-DSC). The average $\Delta_C H_{SOM}$ across all samples was 466 ± 49 kJ mol^{-1} C, with most values falling between 360 and 700 kJ mol^{-1} C and no significant effect of land use type. In forest soils, SOM energy content in the LF layer (i.e., largely unprocessed OM) was significantly lower than in both mineral layers.

In those 47 soils for which both CR and $\Delta_C H_{SOM}$ estimates are available, we find a strong impact of land use type on the correlation between the two quantities (Fig. 4b). In the eight arable soils, the CR of unamended samples and the energy content of SOM follow a close linear relationship with a slope that is indistinguishable from one (0.94, CI [0.76,1.13]) and a slight offset of −80 kJ mol^{-1} C (CI [−179, 18]). In contrast, we observe an inverse relationship with a negative slope in the forest data, although this relationship is not significant ($n = 38$, $p = 0.176$). It can be primarily attributed to samples from the LF layer, and the slope is less negative if the data is restricted to the mineral layers of the forest soils ($n = 21$, p = 0.51).

3.4. *CR values drop after the exponential growth phase in glucose addition experiments*

We compiled dynamic CR curves from five published studies (Harris et al., 2012; Endress et al., 2024a, 2024b; Yang et al., 2024; Wang et al., 2025) that monitored heat and CO_2 release from arable soils over the course of incubation experiments after glucose addition. In all these experiments, the measured CR falls in a broad range between 400 and 750 kJ mol^{-1} C during the lag and exponential phases of microbial growth but drops significantly to values well below 400 kJ mol^{-1} C during the retardation phase (Fig. 5). Notably, this CR decrease of a few hundred kJ mol^{-1} C is observed across all the different soils, experimental setups and treatments employed in these studies and despite large differences in the absolute CR values and the kinetics of heat and CO_2 release in the experiments. Specifically, we find that the drop in CR occurs around the time of maximum microbial activity as revealed by the rates of CO_2 and heat release (star markers in Fig. 5), which corresponds to the time of glucose depletion and thus the onset of the retardation phase.

4. Discussion

Our results highlight the strengths and the limitations of the joint analysis of C and energy flows in soils via calorespirometry. The trajectory of cumulative heat and CO_2 emissions provides a succinct overview of the coupled net C and energy balances during incubations (Fig. 2a), and the sensitive CR_{rate} can reveal changes in microbial metabolism and substrate use over time (Fig. 2b; Endress et al., 2024a; Wirsching et al., 2024). When combined, these cumulative and rate-based assessments disclose both which processes contributed most to the total C and energy flux and which processes dominated at different times. The connection between the CR and the efficiency of microbial growth in particular has long been recognized (Hansen et al., 2004) and it has been extended to metabolic pathways other than aerobic growth (Chakrawal et al., 2020). However, the simple inverse relation (Fig. 3) between CR and CUE only pertains to pure growth reactions on well-characterized substrates. Therefore, it is only applicable if other sources of CO_2 and heat are negligible, and our results corroborate the idea that this is only justified during the exponential growth phase following amendment with labile substrates (Figs. 2 and 3) (Hansen et al., 2004; Barros et al., 2016; Wirsching et al., 2024). Beyond this special case, the interpretation of measured CR values requires a quantitative framework with specific assumptions about dominant processes, including maintenance metabolism. Such a process-based approach faces two main challenges. First, the CR integrates the heat and CO_2 release of many chemical and physical sources into a single metric, and these individual process contributions must be disentangled to obtain a correct interpretation of the CRs numerical value (Barros et al., 2016). This quantification can be achieved via targeted measurements of variables that complement the information contained in the CR. These may include microbial biomass to obtain independent estimates of growth yield and turnover, ^{13}C labeling to detect the priming of SOM (Kästner et al., 2024; Wirsching et al., 2024), or O_2 consumption and anaerobic soil volume to quantify anaerobic contributions (Endress et al., 2024b; Schlüter et al., 2024). Second, many processes may produce a wide range of CR values depending on quantitative details such as the involved energy contents or stoichiometry and thus cannot be associated with a fixed CR value *a priori* (Fig. 2c). To

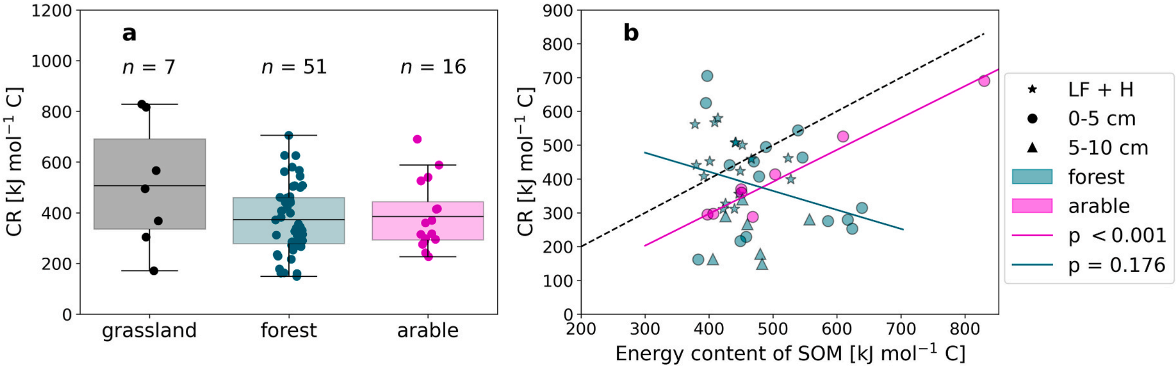

Fig. 4. Compilation of CR vales for unamended soils from the literature as well as their relation to SOM energy content. a Estimates of CR for unamended soils grouped by land use. Each point represents a mean value of replicates reported by the respective study (total of 8 studies). **b** Relationship between CR and SOM energy content in studies that measured the average energy content of SOM via thermogravimetry-differential scanning calorimetry (TG-DSC) in addition to CR. In the eight arable soils with available estimates, there is a close linear relationship between the two quantities, with CR values of ~80 kJ mol^{-1} lower than SOM energy content.

address this challenge, the detailed properties of dominant processes need to be quantified. For example, this may include the estimation of yield coefficients from the formation of products, such as biomass, storage compounds (Mason-Jones et al., 2023) or fermentation and anaerobic respiration products (Zheng et al., 2024), the composition and energy content of SOM (Kästner et al., 2024) as well as biomass and necromass (Chakrawal et al., 2022; Camenzind et al., 2023).

In fact, the potential of the CR to suggest such targeted measurements represents much of its utility. When interpreted using a quantitative modeling framework, the CR enables the identification of plausible process combinations underlying the observations, thereby generating falsifiable hypotheses. This process relies on quantitatively accurate CR measurements due to the sensitivity of the CR that results from its definition as a quotient, and the improvement of experimental setups to achieve the required accuracy is an ongoing endeavor (Barros et al., 2011; Endress et al., 2024; Fricke et al., 2024; Yang et al., 2024). The quantitative analysis also presents special opportunities and challenges from a modeling point of view. Biogeochemical models of soil C cycling are frequently characterized by substantial equifinality, resulting in parameters that are poorly identifiable (Marschmann et al., 2019). Heat measurements provide an additional variable for calibration and validation (Chakrawal et al., 2021), and thermodynamic considerations can help to constrain certain parameters such as yield coefficients and growth rates (Desmond-Le Quéméner and Bouchez, 2014; Trapp et al., 2018; Zheng et al., 2024). Nonetheless, the wide range of parameter values that yield similar CR values complicate the inference process (Fig. 2c). While this issue can to some extent be alleviated by the measurement of key variables as suggested above, parameter values calibrated primarily using calorespirometric data are likely to be poorly constrained and must be interpreted with caution (a more detailed exploration of equifinality is presented in the SI).

Our theoretical examination of exogenous and endogenous maintenance illustrates how the complexity of microbial metabolism may cause deviations from predictions that are generated based on the thermodynamics of simple growth reactions (Fig. 3). While it remains difficult to determine the type of substrate fueling maintenance in practice and microbes likely utilize both external substrates and internal reserves continuously, such deviations offer an opportunity to tackle some of the ambiguity of non-growth processes in soil, both experimentally and in mechanistic models. In terms of modeling, the need to assign energy flows (i.e., heat release) in addition to the various carbon flows that are typically used to describe maintenance (van Bodegom, 2007; Wang and Post, 2012) forces us to clarify the biochemical details of these processes, or at least to make specific assumptions about them. For example, our results can be used to generate testable predictions regarding the expected temporal patterns of the CR and CUE in the presence of maintenance (Figs. 2 and 3), depending on the added substrate (i.e., ΔH_{exo}) and the presumed mode of endogenous maintenance, e.g., bulk biomass consumption with $\Delta H_{endo} \approx 490$ kJ mol^{-1} C based on the biomass stoichiometry $CH_{1.571}O_{0.429}N_{0.143}$ (see also Fig. 2b and section 2.1). Notably, while we expect both the growth and (exogenous) maintenance reactions to be primarily fueled by the added C source in typical experiments with labile substrate amendment, the framework can easily be extended to account for several substrates or several metabolic pathways.

While only a limited number of studies have evaluated CUE and CR dynamically and with sufficient accuracy so far, the few existing results after glucose addition display a typical temporal pattern showing a marked decrease of CR with time (Fig. 5) that is in line with the theoretical prediction of CR change with growth retardation and larger contribution of maintenance to the produced heat and CO_2 (cf. Fig. 3). This consistent shift towards lower CR values after glucose depletion across different soils, incubation setups and treatments points toward the widespread utilization of a substrate with lower energy content for maintenance. Such a substrate might consist of more oxidized SOM (exogenous maintenance) or oxidized metabolic intermediates and other cellular compounds (endogenous maintenance). Notably, these lower CR values during the retardation phase suggest that consumption of bulk biomass is not the dominant process fueling maintenance in these studies (i.e., CR < 490 kJ mol^{-1} C, see Fig. 2), which is also consistent with substantial compositional changes occurring in microbial cells under starvation (Lever et al., 2015). This observation thus challenges the common modeling representation of (endogenous) maintenance as a direct C flux from an undifferentiated microbial biomass pool to CO_2. To address this shortcoming, more explicit model representations of microbial biomass and non-growth processes that provide a mechanistic interpretation of the observed CR pattern could be leveraged, for example based on the formation and consumption of storage compounds

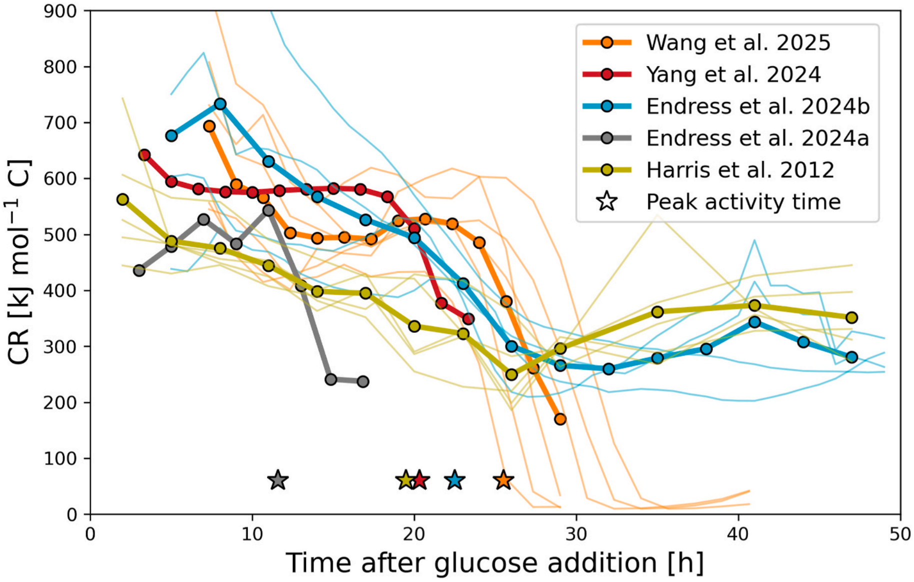

Fig. 5. Dynamics of the CR after glucose addition compiled from five studies show a drop at the onset of retardation after glucose depletion. Bold lines and markers indicate mean values across all treatments analyzed in a study, thin lines indicate mean values of replicates for each treatment (studies of Harris et al., 2012; Endress et al., 2024b; Wang et al., 2025, details in method section). Star markers show the average time of maximum microbial activity as indicated by heat release rate.

(Manzoni et al., 2021), variable C:N ratios (Chakrawal et al., 2022) or other distinct biomass pools as in dynamic energy budget models (e.g., Marschmann et al., 2024).

While differences in the timing of the maximum activity and the CR drop can be explained by the differing temperatures and other experimental conditions across studies, the large spread in absolute CR values during the lag and growth phase also warrants further investigation (Barros et al., 2010; Endress et al., 2024a, 2024b; Wirsching et al., 2024; Yang et al., 2024). In particular, we note that our compilation revealed CR values that fall well above the theoretical prediction for aerobic growth on glucose in several experiments (i.e., CR > 469 kJ mol^{-1} C). Such elevated values may be caused by the additional use of energy rich compounds like reduced SOM or storage compounds as well as the contribution of anaerobic pathways (e.g., Endress et al., 2024a), but the deviations in specific experiments cannot be investigated based on the CR alone.

Together, the high CR values during the lag phase and the CR drop during the retardation phase suggest a possible interpretation in terms of changes in maintenance metabolism related to the activity state of the microbial community: Initially, dormant microbes appear to catabolize relatively energy rich compounds (e.g., reduced storage compounds) to fuel their reactivation and to build the cellular machinery required for growth in response to a substrate pulse. During the exponential growth phase, the active microorganisms utilize the abundant added substrate to fuel their maintenance needs in addition to growth. Later, as the substrate is depleted, microorganisms transition back to a dormant state. This transition appears to be fueled by relatively energy-poor compounds (e.g., metabolic intermediates) and may involve forming energy-rich internal reserves (i.e., a change in overall biomass composition characterized by low CR). A combination of calorespirometric measurements with a quantification of typical microbial storage compounds (e.g., Mason-Jones et al., 2023) could clarify this interpretation.

The wide range of CR values in unamended soils revealed by our literature compilation also highlights the need for a broad exploration of the bioenergetics of soil microbial metabolism under substrate limitation. To interpret these values, we assume that no net formation of biomass took place in the compiled experiments, which are typically performed on short timescales (hours to days) under stable conditions. While microbes certainly still synthesized biomass compounds in these samples, this does not impact the observed CR if a comparable amount of biomass is turned over to CO_2 over the course of the considered time span (i.e., no net growth). In such systems with negligible (net) growth, the CR may reflect (i) the net consumption of SOM without significant decline in biomass (exogenous maintenance) or (ii) the net consumption of biomass or specific biomass components like storage compounds (endogenous maintenance). While the CR alone cannot disclose which of these is prominent, it nonetheless provides insights into the balance of C and energy limitation of microorganisms when little substrate is available. While the data compilation covers only a limited number of soils (and even fewer research groups) with a bias towards forest soils, the results span much of the range of physiologically plausible CR values. This high variability likely reflects the vast chemical diversity of SOM and its bioavailable fractions as well as the metabolic capabilities of communities in natural (unamended) soils (Ahamed et al., 2023; Zheng et al., 2024). Furthermore, we found a striking linear correspondence between the CR and the energy content of SOM in arable soils (Fig. 4b). This finding suggests that microbes utilize SOM with an energy content similar to that of the average SOM in these soils. Specifically, microbes appear to utilize more energy rich compounds for maintenance if available, instead of a strong preference for, e.g., more easily decomposable but energy poor SOM (Gunina and Kuzyakov, 2022; Wang and Kuzyakov, 2023). Within our modeling framework, this pattern could be interpreted as microbes primarily relying on the average SOM around them to persist via exogenous maintenance.

We find a different pattern in forest soils, where microbes tend to metabolize compounds of higher energy content if the average energy content of SOM is low, particularly in the LF layers. We speculate that the high availability of organic C in the LF layer may be the primary cause of this relationship, allowing microbes to selectively use substrates with high energy. In contrast, microbes utilized low energy substrates in the mineral layers of forest soils despite higher average SOM energy content, indicating a preference for easily decomposable compounds and selective stabilization of energy-rich reduced substances (Keiluweit et al., 2017; Kästner et al., 2024). More studies measuring the CR of unamended soils, especially of arable and grassland soils, are required to corroborate and extend these findings. Finally, while such CR values cannot be directly used to explain those obtained during the lag or retardation phases after substrate amendments (Fig. 5), a systematic analysis of the relationship between the two offers another promising next step to illuminate the bioenergetics of non-growth processes in soils, both in the absence of labile substrates and after the depletion of a substrate pulse.

5. Conclusions

Our theoretical results highlight how the joint analysis of carbon and energy flows in the soil system via the CR can be used to identify relevant microbial processes and to formulate falsifiable hypotheses. In particular, we show that maintenance metabolism significantly alters the relationship between the CR and microbial CUE, implying that CUE cannot be inferred from CR measurements under conditions other than ideal exponential growth.

Instead, we suggest that such measurements can be leveraged to illuminate microbial metabolism under carbon limitation. Compiled CR values from unamended soils span much of the range expected for aerobic metabolism, indicating the use of chemically diverse SOM compounds in these soils for microbial maintenance. Future studies should investigate the close connection between the CR and SOM energy content we found in arable soils, as well as we the lack of such a connection in forest soils.

The universal drop in the CR after glucose depletion across studies and experimental conditions also warrants further investigation. We propose the microbial use of relatively energy poor, oxidized compounds for maintenance during the onset of the retardation phase as a potential explanation of this pattern, but the nature and source of these compounds remains to be explored.

CRediT authorship contribution statement

Martin-Georg Endress: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Sergey Blagodatsky:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability statement

All data analyzed in this study is provided as supplementary material. All code required for modeling and figure generation is available at 10.6084/m9.figshare.30000955.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Martin-Georg Endress reports financial support was provided by German Research Foundation. Sergey Blagodatsky reports financial support was provided by German Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2026.110124.

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