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Leveraging energy flows to quantify microbial traits in soils

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ABSTRACT

Heat dissipation from organic matter decomposition is a well-recognized proxy for microbial activity in soils, but only a few modeling studies have used heat signals to quantify microbial traits such as maximum substrate uptake rate, specific growth rate, mortality rate, and growth efficiency. In this contribution, a hierarchy of coupled mass-energy balance models is proposed to estimate microbial traits encoded in model parameters using heat dissipation and respiration data from glucose induced microbial activity. Moreover, the models are used to explain the observed variability in calorespirometric ratios (CR)—the ratio of heat dissipation to respiration rate. We parametrized four model variants using heat dissipation and respiration rates measured in an isothermal calorimeter during the lag-phase only or during the whole growth-phase. The four variants are referred to as: (i) complex physiological model, (ii) simplified physiological model, (iii) lag-phase model, and (iv) growth-phase model. Model parameters were determined using three combinations of data: A) only the heat dissipation rate, B) only the respiration rate, and C) both heat dissipation and respiration rates. We assumed that the 'best' parameter estimates were those obtained when using all the data (i.e., option C). All model variants were able to fit the observed heat dissipation and respiration rates. The parameters estimated using only heat dissipation data were similar to the 'best' estimates compared to using only respiration rate data, suggesting that the observed heat dissipation rate can be used to constrain microbial models and estimate microbial traits. However, the observed variability in CR was not well captured by some model variants such as the simplified physiological model, in contrast to the lag- and growth-phase model that predicted CR well. This suggests that CR can be used to scrutinize how well metabolic processes are represented in decomposition models.

1. Introduction

The activity of microorganisms in soils—a crucial driver of carbon (C) cycling—is mediated by functional traits that determine microbial responses to environmental conditions. To assess these traits, measurements of microbial respiration rate are often combined with stable isotope tracing. This combination allows estimating traits such as microbial C use efficiency (CUE: ratio of microbial growth over substrate consumption) (Manzoni et al., 2018), maximum substrate uptake rate, specific growth rate (Blagodatskaya et al., 2014), and the tradeoffs between growth rate and yield (Lipson et al., 2009; Blagodatskaya et al., 2014). Using respiration rate and other types of C-based data, trait-based models are being developed to identify microbial physiological properties that affect heterotrophic respiration and more in general C cycling in soils (Allison, 2012; Manzoni et al., 2014; Malik et al., 2020). However, estimating microbial traits as encoded in model parameters from respiration data is challenging—even simple models are typically overparameterized (Marschmann et al., 2019). Furthermore, respiration data only provide limited information of decomposition processes. For example, the oxidation state of organic matter cannot be obtained only from CO_2 data but the combination of CO_2 with oxygen uptake rate (Maskow et al., 2010). This leaves a potential gap between theory development and testing, which can be partly filled by leveraging complementary information, such as that provided by heat exchange measurements (Braissant et al., 2013; Maskow and Paufler, 2015; Chakrawal et al., 2020). Here, we explore the potential of heat dissipation measurements for estimating microbial traits encoded in model parameters.

Recently, with the increasing use of calorimetry and calorespirometry in soil science, there has been renewed interest in the energetic aspects of organic matter decomposition in soils (Maskow and Babel, 1998; Harris et al., 2012; Bölscher et al., 2016; Amenabar et al.,

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2017; Arcand et al., 2017; Herrmann and Colombi, 2019). Concurrent measurements of heat dissipation and respiration rates from the same soils offer new insights on the metabolic pathways of substrate utilization, providing information to build and constrain physiologically detailed soil C cycling models (Hansen et al., 2004; Chakrawal et al., 2020). Following this rationale, here we develop a hierarchy of coupled C and energy balance models sufficiently detailed to allow estimating microbial traits as encoded in model parameters.

In a macrochemical view, microorganisms oxidize a substrate via catabolic processes and perform biosynthesis via anabolic processes during the decomposition of organic matter in soils. The Gibbs energy dissipated from catabolic processes drives the biosynthesis and heat is exchanged with the environment (Von Stockar et al., 2006). This heat dissipation rate can be measured using isothermal calorimeters in real-time (Barros Pena, 2018). Moreover, the ratio of heat dissipation to respiration rate—referred to as the calorespirometric ratio (CR)—integrates the energetics and the kinetics of microbial growth (Hansen et al., 2004; Wadsö and Hansen, 2015; Chakrawal et al., 2020). CR is useful to identify the quality of organic matter (in terms of its degree of reduction) and the metabolic pathways of microbial growth (Hansen et al., 2004; Chakrawal et al., 2020). For example, under aerobic conditions, deviations from CR of 469 kJ mol^{-1} CO_2 indicate the presence of anaerobic pathways. Along these lines, here we study the causes of variability in observed CR using our coupled C and energy balance models and assess whether CR can be used to test model predictions of microbial metabolism.

Despite the extensive use of heat exchanges from soils to understand microbial activity (Barros Pena, 2018; Herrmann et al., 2014), only a few attempts have been made to estimate microbial traits from such data (Barja and Núñez, 1999; Nuñez et al., 1994; Wadsö and Hansen, 2015). Nuñez et al. (1994) and Barja and Núñez (1999) estimated apparent yield, half saturation constant, and maximum specific growth rate from heat dissipation data; however, their approach focused mainly on the exponential growth-phase. Wadsö and Hansen (2015) used CR to estimate growth yield assuming aerobic conditions. However, they neglected the dynamic nature of growth and decay of microbial metabolic rates in response to substrate addition, which can provide information on several additional functional traits. In fact, the dynamics of microbial decomposition of a single substrate can be described by Monod kinetics, which contains two parameters; i.e., the maximum substrate uptake rate and the half-saturation constant. The microbial biomass balance contains two additional parameters; i.e., growth efficiency and microbial mortality constant. In this study, these four model parameters are treated as microbial functional traits.

In calorimetric experiments, microorganisms are often activated by adding an external substrate (e.g., glucose), and the heat response is measured against a control without the added substrate. Since microorganisms are inactive before substrate addition, often a lag is observed before the exponential growth-phase. This distinction allows separating calorimetric incubation experiments into two groups, 1) lag-phase and 2) growth-phase (including the decline in activity following substrate depletion). In our experiments, we added glucose, which generates lag- and growth-phases lasting approximately 4–6 h and 48–120 h, respectively. The lag-phase experiments focus mainly on microbial maintenance activities, while the growth-phase experiments provide information also on microbial growth kinetics (Harris et al., 2012; Herrmann et al., 2014; Herrmann and Bölscher, 2015). Therefore, leveraging experimental observations at different time scales, it is possible to focus on specific aspects of microbial responses (maintenance vs. growth). In addition, this allows simplifying models by removing processes that occur at a much longer (or shorter) scales, thus limiting the number of parameters to estimate. Building on this idea, we select models with a suitable level of complexity for each type of experiment and test whether the model parameters estimated using only respiration or only heat dissipation data are comparable to those estimated using both data sources.

Specifically, we ask three questions, using glucose addition experiments as a case study: 1) Do heat dissipation and respiration rates from microbial decomposition of soil organic matter contain complementary information, or is one of these rates alone sufficient to estimate microbial functional traits such as the maximum rate of substrate uptake, half-saturation constants, microbial mortality constant, and growth efficiency? 2) Can we reliably estimate at least some microbial traits from the exponential growth-phase data, and are these estimates comparable to those obtained using data from longer incubation studies? 3) Can a simple model help to interpret the observed variability in CR across different soil treatments and time scales?

2. Materials and methods

2.1. Data

Two incubation experiments with concurrent measurements of heat dissipation and respiration rates were carried out. The two experiments differed in incubation length: one focusing on the lag-phase and the other including the growth-phase as well.

2.1.1. Soil

Soil samples for both incubations were taken from the Ultuna Long-Term Soil Organic Matter experiment (Uppsala, Sweden; 60°N, 17°E; Herrmann and Witter, 2008). The experiment was started in 1956 on a postglacial clay loam (36.5% clay, 41% silt, and 22.5% sand) classified as a Eutric Cambisol (Fao, 1998). Since then, soils (2 × 2 m blocks) have been treated with different nitrogen fertilizers or organic amendments, and all treatments are replicated in four blocks (Table S1 in the Supplementary Information; see (Herrmann and Witter, 2008) and references therein for further details). Nitrogen was applied at sowing at a rate of 80 kg N ha^{-1} $year^{-1}$, and the organic amendments every other year in the autumn at a rate of 8 Mg ha^{-1} ash-free organic matter. In June 2010, eight sub-samples to a depth of 7 cm were taken from each replicate block, sieved through a 2-mm sieve, composited and mixed per replicate block, and stored frozen at −18 °C.

2.1.2. Lag-phase incubation (LPI) experiment

The following soil treatments were selected for the lag-phase incubation experiment: (i) Peat, (ii) Peat + N, (iii) Sawdust (SD), (iv) Sawdust + N (SD + N), (v) Farmyard Manure (FYM), (vi) Green Manure (GM), (vii) Straw and (viii) Straw + N. The selected soil treatments received a similar amount of organic matter, but of different composition. In June 2017, soil samples were thawed and wetted to 45% of their water holding capacity (WHC) and pre-incubated for 8 day at 20 °C to allow the microbial respiration flush from fresh organic matter released due to the sampling and freezing procedure to subside (Herrmann and Witter, 2008; Coucheney et al., 2013).

Six aliquots of each replicate soil (4 g) were amended with either Milli-Q water or glucose at five concentration levels (0.417, 4.17, 41.7, 166.7, and 333.3 μmol glucose-C g^{-1} soil; 60 μL per gram of soil bringing the water content to 55% of WHC) and incubated for 5.5 h at 20 °C in a TAM Air isothermal calorimeter (TA Instruments, Sollentuna, Sweden). The rate of heat dissipation was recorded at an interval of 15 min, and CO_2 was measured using bicarbonate traps simultaneously in the same vessel (Herrmann and Bölscher, 2015). A disadvantage of using bicarbonate traps is that it provides only the total amount of CO_2 released at the end of the experiment and not the time evolution. However, for a short incubation during the lag-phase, the respiration rate is assumed to be constant and can be approximated by the total CO_2 produced divided by the incubation time. Microbial biomass C (Table S1) was determined by fumigation extraction (Vance et al., 1987) using a k_{ec} factor of 0.45 for conversion to microbial biomass-C (Wu et al., 1990).

2.1.3. Growth-phase incubation (GPI) experiment

The growth-phase data (48 h) on heat dissipation and respiration

rates were derived from a published data set (Harris et al., 2012). Five soil treatments were selected: (i) $Ca(NO_3)_2$, (ii) $(NH_4)_2SO_4$, (iii) Straw + N, (iv) farmyard manure, and (v) sewage sludge. Briefly, duplicate samples (either 5 or 20 g) were amended with either DI-water or with 4.17 μmol glucose-C per gram of soil. The 5 g soil samples were inserted into a TAM Air isothermal calorimeter, and the rate of heat dissipation was measured continuously over a 48 h incubation period at 25 °C. In a parallel set of samples (20 g soil samples), CO_2 production rate was measured using a portable infrared gas analyzer (EGM-4, Environmental Gas Monitor, PP systems, U.K.) at different time intervals over a 48 h incubation period at 25 °C.

2.2. Mass and energy balances

A hierarchy of mass and energy balance models is proposed to simulate the heat and C fluxes from the substrate-induced response of microbial biomass in soils (Fig. 1A). In the experiments we aim to model, a known amount of external substrate (in our case glucose) is added to soils, and the microbial response is measured as respiration and/or heat dissipation rates. Since these rates are measured against a control that had not been amended, the observed microbial activity can be assumed to be caused only by the external substrate. Priming of the native organic matter could also occur, but was neglected here.

2.2.1. Complex physiological model

We considered a two pool (substrate and microbial biomass) model to simulate substrate decomposition similar to Panikov (1996) and Blagodatsky and Richter (1998) (Fig. 1A). Microbial cell constituents can be divided into two categories; i.e., P- and U-components (Panikov, 1996). The P-components (e.g., ribosomes or rRNA and ribosomal proteins, etc.) are necessary for growth, and the U-components (e.g., secondary metabolism, protective pigments, reserved substances, transport systems of high affinity, etc.) are needed for survival. In the starvation state, microorganisms maintain the U-components, but as soon as a substrate is added, P-components increase exponentially, giving rise to microbial growth. Therefore, P- and U-components can be interpreted as proxies of the growing and non-growing microbial populations, respectively (following the notation in Stenström et al. (1998)), and we use this terminology thereafter. The index of physiological state, r, defines the proportion of P-components in the total microbial C and thus represents the active fraction of microbial biomass.

The substrate is taken up by microorganisms following Monod kinetics with an apparent growth efficiency Y (van Bodegom, 2007). Microbial mortality (turnover) is assumed to follow a first-order function of microbial C and to recycle necromass in the substrate compartment. The mass balances of substrate C (C_S), microbial C (C_B), and CO_2 can be written as (expressed in C-mol g^{-1} soil h^{-1}),

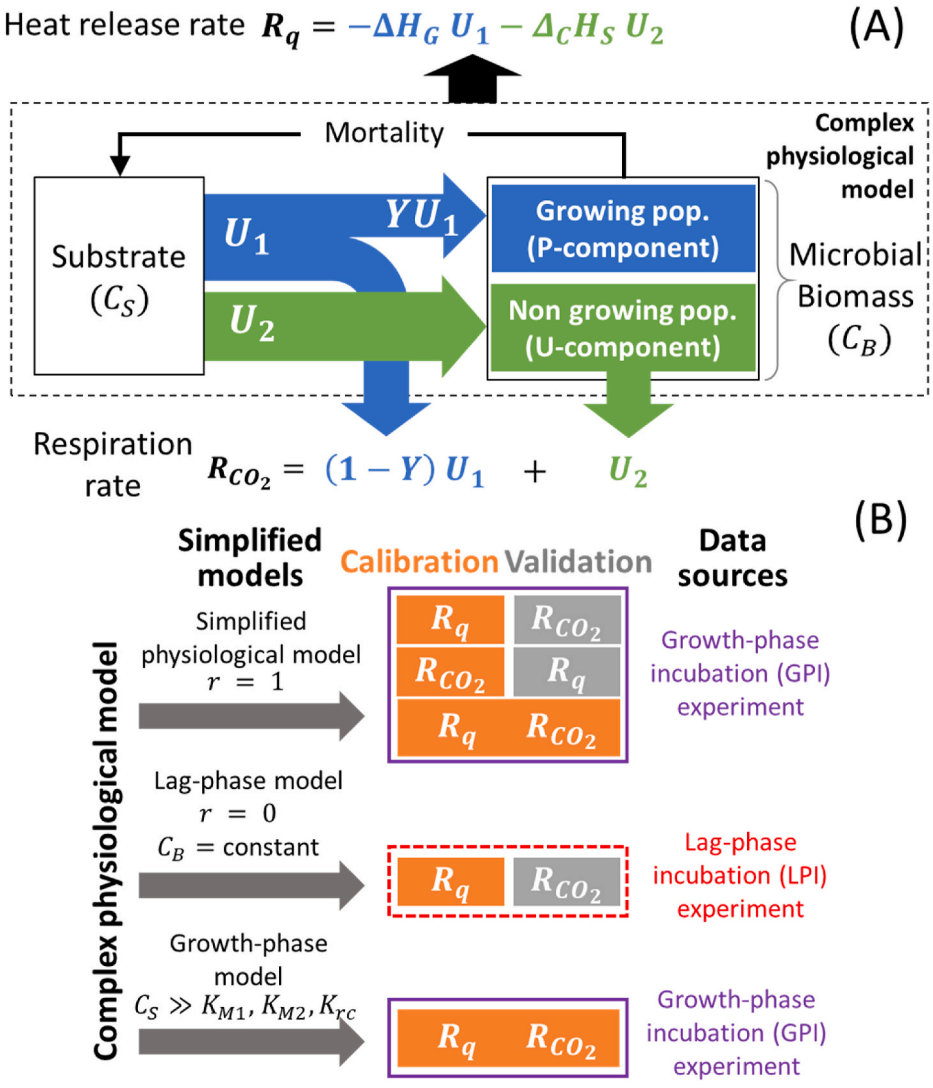

Fig. 1. (A) Schematic of the two-pool (substrate C_S, and microbial biomass C_B) complex physiological model. The substrate is taken up at rates U_1 and U_2 by the growing and the non-growing populations of microorganisms. The growing population utilizes a substrate with growth efficiency Y (thus growth rate = YU_1); in contrast, the non-growing population uses substrate only to sustain its maintenance costs (thus maintenance rate = U_2). The total CO_2 production rate is given by $R_{CO_2} = (1-Y)U_1 + U_2$, and the total rate of heat dissipation as $R_q = -\Delta H_G U_1 - \Delta_C H_S U_2$, where ΔH_G and $\Delta_C H_S$ are the enthalpy changes (kJ C-mol^{-1} substrate) of the metabolic reactions of the growing and non-growing populations, respectively. (B) The complex physiological model is simplified to the 'simplified physiological model' when the growing population is the dominant form of microbial biomass, the 'lag-phase model' when the non-growing population is the dominant form, and the 'growth-phase model' when the substrate is not limiting. The index of physiological state r controls the proportion of total microbial C into the growing and the non-growing populations. These three models were calibrated and validated using observed datasets from growth-phase and lag-phase incubation experiments, except the growth-phase model that was only calibrated (see Table 1).

$$\frac{dC_S}{dt} = -U_1 - U_2 + k_d C_B, \quad (1)$$

$$\frac{dC_B}{dt} = YU_1 - k_d C_B, \quad (2)$$

$$\frac{dCO_2}{dt} = (1-Y)U_1 + U_2. \quad (3)$$

The substrate uptake rates U_1 and U_2 fuel microbial growth and maintenance, respectively, and are defined as follows,

$$U_1 = \frac{k_s C_S C_B}{C_S + K_{M1}} r, \quad (4)$$

$$U_2 = \frac{m_s C_S C_B}{C_S + K_{M2}} (1-r), \quad (5)$$

where k_s and m_s are the maximum substrate uptake rates; and K_{M1}, and K_{M2} are the half-saturation constants for the growing and non-growing populations, respectively, and r is the index of physiological state. The quantity $Y \times k_s$ is the maximum specific growth rate (μ_{max}).

The governing equation for r is based on Panikov (1996) and given as follows,

$$\frac{dr}{dt} = \frac{Yk_s C_S}{C_S + K_{M1}} r \left(\frac{C_S}{C_S + K_{rc}} - r \right), \quad (6)$$

where K_{rc} is the half-saturation constant for the rate of physiological state increase. Equation (4) describes changes in r that are driven by substrate availability: as C_S increases when r is low, the active fraction of the microbial population increases, but as r nears the substrate-dependent value of $\frac{C_S}{C_S+K_{rc}}$, its growth stabilizes. When C_S decreases, the term $\frac{C_S}{C_S+K_{rc}}$ also decreases, causing r to be reduced.

We then coupled the C balance equations to heat dissipation. In an isothermal, closed, and constant volume system with no external source of energy, the only source of heat is derived from the chemical reactions within the system. Thus, the rate of heat production can be expressed as the rate of reaction (i.e., the rate of substrate uptake) multiplied by the enthalpy change of the reaction (i.e., the amount of heat dissipated per unit substrate utilized). Heat is released only from the metabolism of microorganism; therefore, the heat dissipation rate is written as,

$$R_q = -\Delta H_G U_1 - \Delta_C H_S U_2, \quad (7)$$

where R_q is the rate of heat dissipated in kJ g^{-1} soil h^{-1}, and ΔH_G and $\Delta_C H_S$ are the enthalpy changes (kJ C-mol^{-1} substrate) of the metabolic reactions of the growing (substrate 'G') and non-growing populations, respectively. In the latter, the substrate is mineralized without growth, so $\Delta_C H_S$ is simply the enthalpy of combustion of the substrate (subscript 'C' for 'combustion' and 'S' for 'substrate').

Generally, calorimetric experiments run for a few days, and the oxygen concentration in the reaction vials is not limiting the reaction. Therefore, when a substrate (glucose) is metabolized aerobically, the microbial growth reaction can be written as (Kleerebezem and Van Loosdrecht, 2010),

$$C_S + \left(\frac{\gamma_S - Y\gamma_B}{4} \right) O_2 \rightarrow YC_B + (1-Y)CO_2 + \Delta H_G \quad (8)$$

where γ_S (for glucose, $\gamma_S = 4$) and $\gamma_B = 4.2$ are the degrees of reduction of the substrate and microbial C, respectively. It is worth noting that Eq. (8) is only a macrochemical representation of complex biochemical reactions resulting in microbial growth. By writing the enthalpy balance of Eq. (8) and using the enthalpy of combustion as the reference state, we obtain ΔH_G as follows,

$$\Delta H_G = \Delta_C H_S - Y \Delta_C H_B \quad (9)$$

where $\Delta_C H_S$ and $\Delta_C H_B$ are the enthalpies of combustion of the substrate and microbial biomass ($\Delta_C H_B = -492$ kJ C-mol^{-1} biomass is the average standard enthalpy of combustion of bacteria (Popovic, 2019); $\Delta_C H_S = -469$ kJ C-mol^{-1} glucose is the standard enthalpy of combustion of glucose (Wagman et al., 1982)).

In the following sections, we present a set of simplifications to the complex physiological model (Fig. 1B). These simplified models were used to describe the observed heat dissipation and respiration datasets. Afterward, a three-pronged approach is introduced to estimate microbial traits.

2.2.2. Simplified physiological model

Assuming that all microorganisms belong to the growing population, the complex physiological model was simplified by fixing $r = 1$ (Fig. 1B). Therefore, Eq. (6) becomes redundant, and the resulting mass and energy balances are given as follows,

$$\frac{dC_S}{dt} = -\frac{k_s C_S C_B}{C_S + K_{M1}} + k_d C_B \quad (10)$$

$$\frac{dC_B}{dt} = Y \frac{k_s C_S C_B}{C_S + K_{M1}} - k_d C_B \quad (11)$$

$$R_{CO_2} = (1-Y) \frac{k_s C_S C_B}{C_S + K_{M1}} \quad (12)$$

$$R_q = -\Delta H_G \frac{k_s C_S C_B}{C_S + K_{M1}} \quad (13)$$

Equation (10)–(13) are hereafter referred to as the 'simplified physiological model'. The unknown model parameters encoding microbial traits are k_s, K_M, k_d, and Y. The initial microbial C (C_{B0}) is also regarded as a fitting parameter, because the concentration of active biomass is not known; the initial substrate concentration is instead assumed to be known. Equations (10) and (11) are also equivalent to those used in earlier soil microbial models (Manzoni and Porporato, 2007; German et al., 2012), which however did not consider the energy balance.

2.2.3. Lag-phase model

The complex physiological model was reduced to represent lag-phase activity after the substrate addition and before microbial growth occurs by setting $r = 0$ (i.e., only the non-growing population is modeled; Fig. 1B). Under these conditions, the substrate is used to maintain stable microbial biomass (i.e., growth uncoupled microbial activity, Reischke et al., 2014). Thus, the respiration (Eq. (3)) and the heat dissipation rates (Eq. (7)) become functions of substrate concentration only, because C_B is constant:

$$\frac{dC_S}{dt} = -\frac{k_s' C_S}{C_S + K_{M2}} \quad (14)$$

$$R_{CO_2} = \frac{k_s' C_S}{C_S + K_{M2}}, \quad (15)$$

$$R_q = -\Delta_C H_S \frac{k_s' C_S}{C_S + K_{M2}}, \quad (16)$$

where k_s' is the maximum rate of substrate utilization with units C-mol substrate g^{-1} soil h^{-1} ($k_s' = m_s C_B$). Furthermore, the turnover of microbial C was neglected because of the longer turnover times compared to the lag-phase duration (Spohn et al., 2016). We refer to Eqs. (15) and (16) as the 'lag-phase model'.

2.2.4. Growth-phase model

Previous modeling efforts have shown that Eqs. (10)–(13) cannot capture the details of the exponential growth-phase that follows the lag-phase (Blagodatsky et al., 2000; Blagodatsky and Richter, 1998; Panikov, 1996). Therefore, we considered an additional model—referred to

as the 'growth-phase' model—designed to infer microbial traits using only data from the initial exponential growth-phase, when substrate availability is unlimited (Fig. 1B). Under substrate saturation conditions, K_{M1}, K_{M2} and K_{rc} are negligible compared to C_S, and Eqs. (2)–(7) are simplified as follows,

$$\frac{dC_S}{dt} = -k_s C_B r - m_s C_B (1-r) \quad (17)$$

$$\frac{dC_B}{dt} = Y k_s C_B\, r, \quad (18)$$

$$R_{CO_2} = (1-Y) k_s C_B r + m_s C_B (1-r), \quad (19)$$

$$R_q = -\Delta H_G\, k_s C_B r - \Delta_C H_S\, m_s C_B (1-r), \quad (20)$$

$$\frac{dr}{dt} = Y k_s\, r\,(1-r). \quad (21)$$

The analytical solutions of Eqs. (18) and (21) are,

$$C_B(t) = C_{B0}\left(1 - r_0 + r_0 e^{Y k_s t}\right), \quad (22)$$

$$r(t) = \frac{r_0 e^{Y k_s t}}{1 - r_0 + r_0 e^{Y k_s t}}. \quad (23)$$

Inserting Eqs. (22) and (23) in Eqs. (19) and (20) gives analytical expressions for the respiration and heat dissipation rates,

$$R_{CO_2}(t) = \beta_{C0} + \beta_{C1} e^{\beta_{C2} t}, \quad (24)$$

$$R_q(t) = \beta_{H0} + \beta_{H1} e^{\beta_{H2} t}, \quad (25)$$

where, β_{Ci} and β_{Hi} (with $i = 0, 1,$ and 2) are defined as

$$\beta_{C0} = m_s C_{B0}(1-r_0), \quad (26)$$

$$\beta_{C1} = (1-Y) k_s C_{B0} r_0, \quad (27)$$

$$\beta_{C2} = Y k_s, \quad (28)$$

$$\beta_{H0} = -\Delta_C H_S\, m_s C_{B0}(1-r_0), \quad (29)$$

$$\beta_{H1} = -\Delta H_G\, k_s C_{B0} r_0, \quad (30)$$

$$\beta_{H2} = Y k_s. \quad (31)$$

In Eqs. (24) and (25), $\beta_{C0} + \beta_{C1}$ and $\beta_{H0} + \beta_{H1}$ are time-invariant quantities representing respiration and heat dissipation rates during the lag-phase, respectively (i.e., when $t \approx 0$); and $\beta_{C2} = \beta_{H2}$ is the maximum specific growth rate. Equations (24) and (25) are collectively referred to as the 'growth-phase model'. The six unknown parameters are β_{Ci} and β_{Hi} (with $i = 0, 1,$ and 2, Eqs. (26)-(31)), which were estimated separately by nonlinear least-square fitting of Eqs. (24) and (25) to observed respiration and heat dissipation rates during the growth-phase, respectively. Because these parameters groups are not shared between the mass and energy balance equations, the growth-phase model was only used for the calibration of heat and respiration data, but not for the validation.

2.3. Microbial trait estimation

Microbial traits encoded in model parameters were estimated separately from the two datasets (Fig. 1). Datasets from the GPI experiment were used to parametrize the simplified physiological (Eq. (10)–(13)) and the growth-phase model (Eqs. (24)–(25)), whereas datasets from the LPI experiment were used for the lag-phase model (Eqs. (15)–(16)).

2.3.1. Parameterization of the simplified physiological model using GPI data

For the simplified physiological model, we used a three-pronged approach for parameter estimation—using only heat dissipation rate, only respiration rate, or both, using five treatments from the GPI dataset. These three approaches were identified based on which dataset was used for calibration or validation: approach A) when only heat dissipation rate was used for calibration and respiration rate for validation, B) when only respiration rate was used for calibration and heat dissipation rate for validation, or C) when both heat and respiration rates were used for calibration (see Table 1). The parameters estimated from approach C were considered as the 'best' estimates since they were obtained by using all available information, and were therefore used as references for the parameters from approaches A and B.

Following approaches A, B, or C, we estimated the maximum rate of substrate uptake (k_s), half-saturation constant for Monod kinetics (K_{M1}), growth efficiency (Y), microbial mortality constant (k_d) and initial (active) microbial C (C_{B0}). The simplified physiological model was solved numerically in Matlab (2020a) using the ordinary differential equation solver *ode45,* a non-linear least square (*lsqcurvefit*) algorithm was used to find the best-fit parameters, and the *nlparci* and *nlpredci* functions to find the 95% confidence intervals of estimated parameters and simulations. To avoid overfitting and to ensure consistent temporal resolution of heat dissipation and respiration rate data, we binned the observed R_q in a 75 min interval time series (original data was at 15 min interval) and took the mean of observed R_q from all the replicates in each bin. A similar pre-processing was performed on respiration rate data as well.

2.3.2. Parameterization of the lag-phase model using LPI data

For the lag-phase model, we followed only approach A and estimated the two parameters k_s' and K_{M2} using the LPI data. These two parameters were fitted to the heat dissipation rates averaged across replicates, separately for each of the eight soil treatments.

2.3.3. Parameterization of the growth-phase model using GPI data

For the growth-phase model, parameters β_{Ci} or β_{Hi} were estimated individually from the fitting of Eq. (24) or (25) to observed heat dissipation and respiration rates from the GPI dataset. However, there are five unknowns (m_s, k_s, Y, C_{B0}, r_0) in the expressions of β_{Ci} or β_{Hi}. Therefore, it was not possible to estimate all these five parameters from estimated β_{Hi} without knowing the value of any two physiological parameters. Some authors have fixed the values of the ratio of maintenance and growth specific uptake rate (m_s/k_s) and the growth efficiency to estimate active and total microbial biomass (Blagodatsky et al., 2000; Wutzler et al., 2012). Here, we took a different approach and estimated Y from separately estimated values of β_{C1} and β_{H1} (see Section 2.4). Having calculated Y, we were still left with three equations (either Eqs. (26)–(28) or Eqs. (29)–(31)) and four unknowns (m_s, k_s, C_{B0}, r_0). Therefore, it was not possible to estimate all five parameters, and we used the growth-phase model only to estimate k_s and Y.

2.3.4. Performance metrics

The obtained model parameters and goodness of the fit were assessed using root mean squared error (RMSE) and coefficient of determination (r^2). The RMSE units are the same as those of the variable being modeled but are not reported in the figures due to lack of space.

2.4. Calorespirometric ratios (CR)

Calorespirometric ratio (CR) is the ratio of heat dissipation and respiration rate. CR has been related to the enthalpic content of the organic substrate being decomposed and has been used to diagnose the pathways of microbial metabolism (Hansen et al., 2004; Herrmann et al., 2014; Wadsö and Hansen, 2015; Chakrawal et al., 2020). In the simplified physiological model, CR (CR_P) was calculated by taking the ratio of Eq. (13) to Eq. (12),

Table 1
Calibration and validation approach for different model variants, data sources, and lists of estimated parameters (see also Fig. 1B).

Parameterization approach	Calibration	Validation	Data	Model	Estimated parameters*
A	Heat dissipation rates	Respiration rates	LPI	Lag-phase model (Eq. (15) and (16))	$k_s^{'}$ and K_{M2}
			GPI	Simplified physiological model (Eq. (10)–(13))	k_s, K_{M1}, Y, k_d, and C_{B0}
B	Respiration rates	Heat dissipation rates	GPI	Simplified physiological model	k_s, K_{M1}, Y, k_d, and C_{B0}
C	Heat dissipation & respiration rates	Assumed to be 'best' estimates; not validated	GPI	Simplified physiological model	k_s, K_{M1}, Y, k_d, and C_{B0}
				Growth-phase model (Eq. (24) and (25))	k_s and Y

*maximum rate of substrate uptake (k_s or $k_s^{'}$), half-saturation constants (K_{M1} and K_{M2}), microbial mortality constant (k_d), growth efficiency (Y), and initial (active) microbial biomass C (C_{B0}).

$$CR_P = \frac{R_q}{R_{CO_2}} = -\frac{\Delta H_G}{1-Y} = -\frac{\Delta_C H_S - Y\Delta_C H_B}{1-Y}. \quad (32)$$

Similarly, CR in the lag-phase and the growth-phase models were denoted by CR_L and CR_G and were obtained by taking the ratios of Eqs. (15) and (16), and Eqs. (24) and (25), respectively,

$$CR_L = -\Delta_C H_S = 469 \text{ kJ C} - \text{mol}^{-1} \text{ glucose}, \quad (33)$$

$$CR_G = \frac{\beta_{H0} + \beta_{H1} e^{\beta_{H2} t}}{\beta_{C0} + \beta_{C1} e^{\beta_{C2} t}}. \quad (34)$$

Further, the ratio of β_{H1} (Eq. (26)) to β_{C1} (Eq. (23)) can be considered as the calorespirometric ratio $\left(CR_{EG} = \frac{\beta_{H1}}{\beta_{C1}}\right)$ in the exponential growth-phase and can thus be used to estimate Y. The relationship between CR_{EG} and the Y is exactly the same as in Eq. (32). By inserting the expression of ΔH_G from (9) into Eq. (32), and simplifying, we obtained Y as a function of CR_{EG},

$$Y = \frac{CR_{EG} + \Delta_C H_S}{CR_{EG} + \Delta_C H_B}. \quad (35)$$

2.5. Statistical analyses

We used two-way ANOVA to test if differences in the observed CR among different soil treatments and glucose levels in the LPI experiment were significant. Further, a *t*-test was used to test for the significance of differences between the model and experimentally estimated CR values for each soil treatment and at each glucose level individually.

3. Results

We compared the simulated and observed heat dissipation rates in the LPI experiment for all soil treatments (Fig. 2A and B, different colors). The parameters, $k_s^{'}$ and K_{M2}, estimated by fitting the observed heat dissipation rates to the lag-phase model (Eq. (16)) were used to validate the model using observed respiration rates, as shown in Fig. 2C and D. Overall, the lag-phase model was able to explain the observed

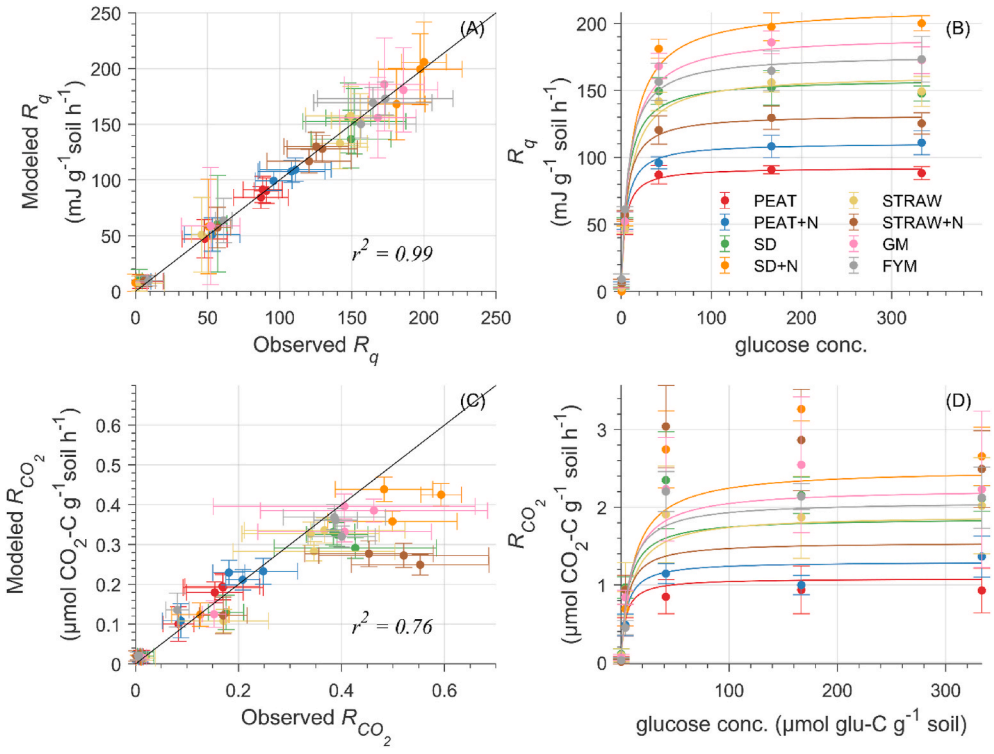

Fig. 2. Calibration of the lag-phase model using observed heat dissipation rates from the LPI dataset (panels A and B). Modeled and observed heat dissipation rates (R_q) are shown for eight different soil treatments (panel A) and four different glucose levels, in addition to the un-amended control (panel B). Validation of the lag-phase model using the observed respiration rates (R_{CO_2}) from the same LPI dataset (panels C and D). The error bars represent the 95% confidence intervals for the observed or modeled heat dissipation and respiration rates.

heat dissipation rates across different soil treatments with high accuracy ($r^2 = 0.99$) and also predicted respiration rates ($r^2 = 0.82$). However, at higher glucose concentrations, the model underestimated the observed respiration rates in some soil treatments (Fig. 2D).

The results from fitting the simplified physiological model to the GPI experiment using our three parameterization approaches are presented in Fig. 3. Further, the estimated parameters k_S, K_M, k_d, Y, and $C_{B,0}$ are summarized in Tables S3, S4, and S5 for approaches A, B, and C, respectively. The simplified physiological model fitted the observed R_q with a high degree of accuracy (i.e., $r^2 > 0.95$ in all soils, Fig. 3A1–A5); however, the heat dissipation rates during the initial lag-phase (first few hours) were not well captured by the model (Table S3). The predicted values of R_{CO_2} during the initial lag-phase (Fig. 3A6–A10) matched better the observed values compared to the heat dissipation rates.

With approach B, the calibration of the simplified physiological model using R_{CO_2} (Fig. 3B1–B5) predicted the overall shape of the heat dissipation rates (Fig. 3B6–B10), but with higher uncertainty (lower r^2) compared to approach A (Table S4). Including observed respiration rates with approach C (i.e., the 'best' calibration approach using both respiration and heat dissipation data) did not improve the model fit, since the RMSE and r^2 values were similar in approaches A and C (Fig. 3C1-C10; Table S5).

The parameters estimated from approaches A and C were nearly identical (Fig. 4)—i.e., approach A provided estimates close to the 'best' values obtained using all available data. In contrast, the mean values of the parameters estimated with approach B were different (but still of a similar order of magnitude) from those from approach A and C, and had much larger uncertainties (Fig. 4). Therefore, heat dissipation rates alone can be used to estimate microbial traits.

Results based on fitting of the growth-phase model in the exponential

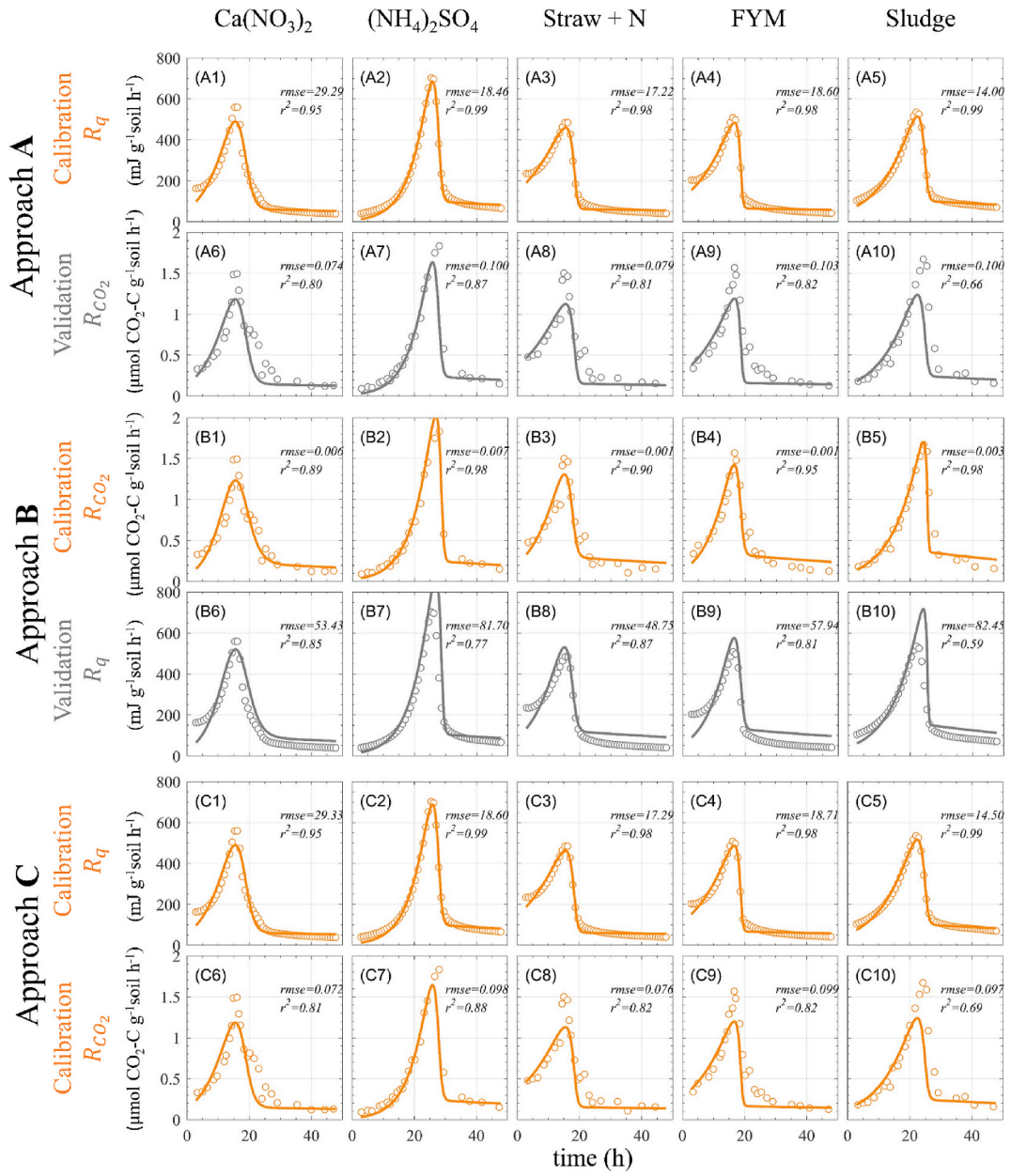

Fig. 3. Calibration and validation of the simplified physiological model using approach A (panels A1–A10) and B (panels B1–B10), and only calibration using approach C (panels C1–C10). Measured heat dissipation rates (R_q) and respiration rates (R_{CO_2}) are from the GPI dataset. Modeled and observed heat (and respiration) rates are shown as solid lines and open circles, respectively. The root mean square errors (RMSE) and coefficients of determination (r^2) are shown for each model fit. The three approaches are described in Table 1.

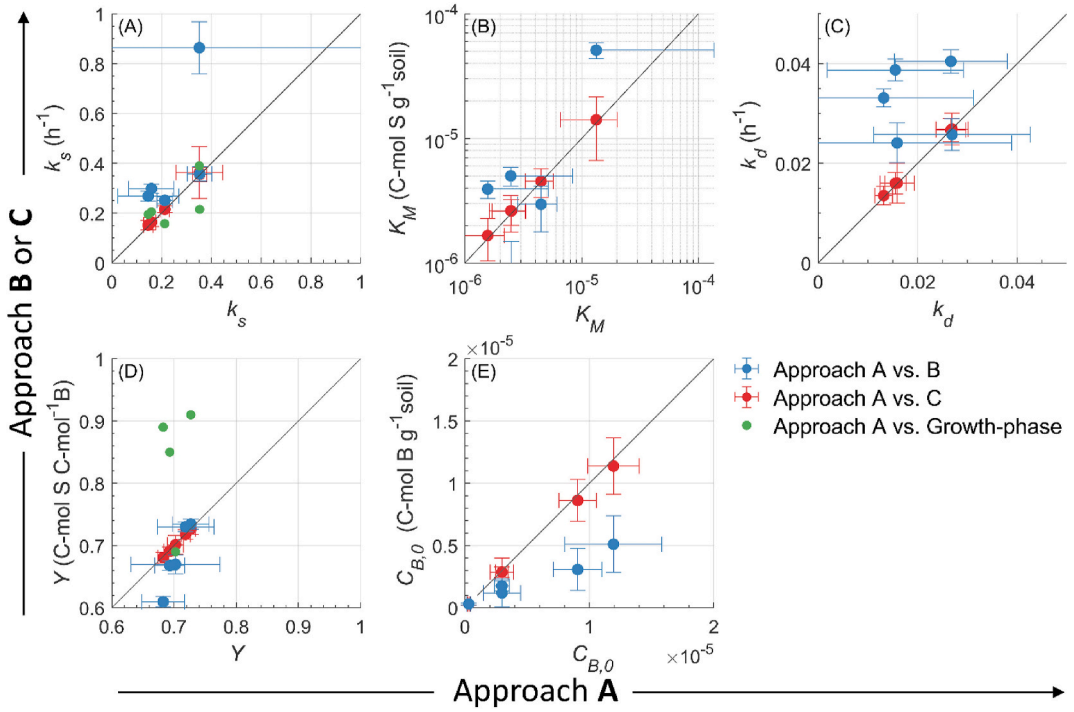

Fig. 4. Comparison of parameters estimated from the simplified physiological model using three estimation approaches (Table 1). (A) Maximum uptake rate k_s, (B) half-saturation constant K_M, (C) microbial mortality constant k_d, (D) growth efficiency Y, and (E) initial microbial biomass $C_{B,0}$. Parameter values from approach A are plotted on the y-axes, whereas values from approaches B and C are plotted on the x-axes. The horizontal and vertical bars are the 95% confidence intervals for the estimated parameters; 1:1 lines are indicated by black solid lines.

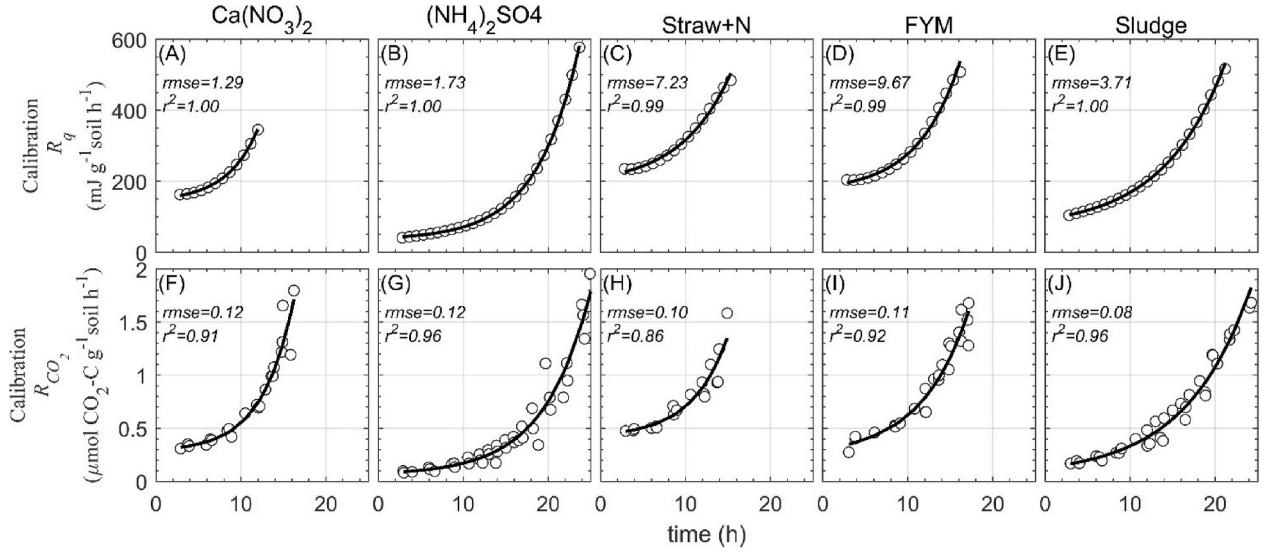

Fig. 5. Calibration of the growth-phase model using heat dissipation rates (R_q) (top panels A–E) and respiration rates (R_{CO_2}) (bottom panels F–J) from the GPI dataset. Modeled and observed heat (and respiration) rates are shown as a solid line and open circles, respectively. The root mean square errors (RMSE) and coefficients of determination (r^2) are shown for each model fit.

growth-phase of the GPI experiment are presented in Fig. 5 and the estimated parameters are summarized in Table S6. As it is expected from Eqs. (28) and (31), the estimated values of the maximum specific growth rates (β_{H2} and β_{C2}) were similar, indicating that microbial growth rates were comparable when estimated from an energetic or C flow standpoints, even though in this model the two types of data were used independently. The maximum uptake rate k_s (given by β_{H2} or β_{C2} divided by the growth efficiency Y, where Y is obtained from Eq. (35)) was close to the estimated values from the calibration done using the simplified physiological model (green points in Fig. 4A). This lends further support to our parameter estimation approach. Moreover, the growth efficiency values estimated from the growth-phase model were similar to those estimated from the simplified physiological model for the soil treated with Ca(NO$_3$)$_2$, but overestimated for other treatments (green points in Fig. 4D). Therefore, short-term measurements during the growth-phase can be used to estimate growth rates, but not always growth yields.

The observed and simulated CR for the LPI experiments are shown in Fig. 6. The CR for the five soil treatments from the GPI experiment are

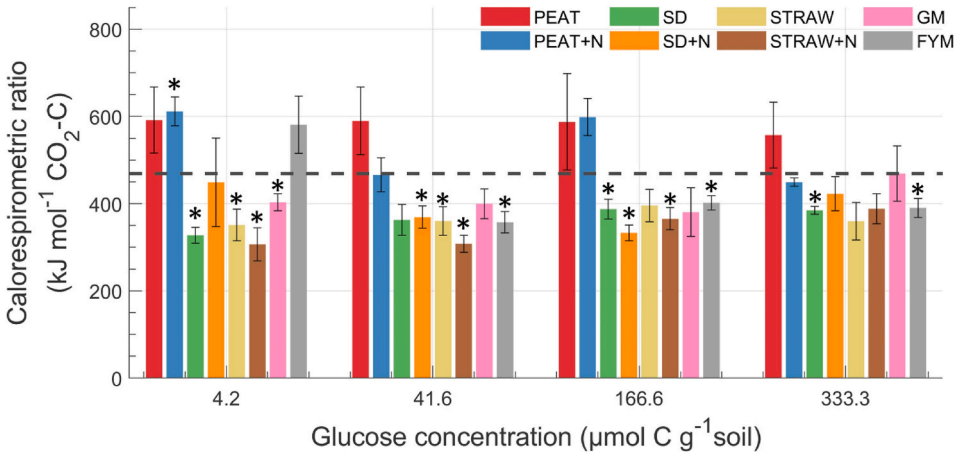

Fig. 6. Calorespirometric ratios (CR) from the lag-phase incubation experiment: different color bars represent soil treatments, and the whiskers are the standard errors (four replicates). The horizontal dashed line represents the constant value of CR equivalent to the enthalpy change in the complete combustion of glucose (CR_L = 469 kJ mol^{-1} CO_2–C). Asterisks denote significant differences between the experimental and model simulated CR values ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

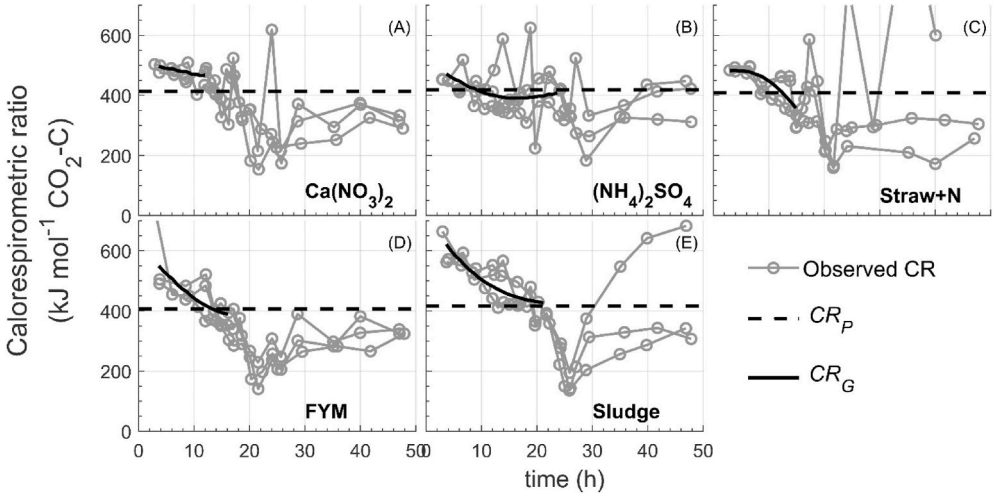

Fig. 7. Calorespirometric ratios (CR) from the growth-phase incubation experiments: different gray lines represent the observed CR (three replicates), whereas the black dashed and solid lines represent the model simulated CR values using the simplified physiological model (CR_P, Eq. (32)) and the growth-phase model (CR_G, Eq. (34)), respectively.

shown in different panels (Fig. 7). We note that the observed CR in the GPI experiment are time varying quantities because they were calculated from the ratio of heat dissipation and respiration rates, which were measured at the same time, different from the LPI experiment. The simulated CR from the simplified physiological model (dashed lines, Fig. 7) are time-invariant values that depend only on the microbial growth yield, whereas the simulated CR using the growth-phase model (solid black curves) varied through time and better captured the observed CR, but only during the exponential growth-phase (i.e., the solid black curves stop at the end of this phase).

4. Discussion

In this study, we presented a coupled mass and energy balance model to interpret the observed heat dissipation and respiration rates from substrate-induced responses from soils. Different from earlier energetics-based models (Braissant et al., 2013; Hansen et al., 2004; Matheson et al., 2004; von Stockar and Birou, 1989), here we proposed a dynamic model and a systematic parameter estimation approach that is relevant to the quantification of functional traits of microbial communities in soils. Using this model, we estimated the maximum rate of substrate uptake (k_s or k_s'), the half-saturation constant for Monod kinetics (K_{M1} or K_{M2}), growth efficiency (Y), microbial mortality constant (k_d), and the initial (active) microbial C (C_{B0}) from two different datasets (Table S2–S6). Additionally, we used our model to interpret the observed CR in both datasets. Model parameters were estimated using different approaches (Table 1) to answer the three questions posed in the introduction.

4.1. Can heat dissipation rate alone constrain soil C cycling models?

Our comparison of parameters estimated from different approaches shows that: i) approach A provides accurate estimates despite being based only on heat exchange data, and ii) approach C leads to more uncertain estimates compared to A and B (Fig. 4). This means that the heat dissipation rates alone can be used to characterize microbial properties, and information regarding C fluxes can be inferred from the energy fluxes, but not vice versa. The main advantage of this approach is that it allows leveraging the high-temporal resolution data from calorimeters that are becoming more available.

To place these results in a broader context, our estimated values of microbial traits were compared to those reported in other studies (Table S7). The compiled studies focused on microbial communities from the top organic soil horizon amended with a single or a range of glucose concentrations, using respiration or heat dissipation rate measurements to estimate microbial traits. The range of glucose concentrations in the literature compilation is similar to that in both LPI and GPI datasets, thereby allowing direct comparisons with our results. In

only two of these studies, heat dissipation data were used to estimate the parameters of Monod kinetics for glucose mineralization (Nuñez et al., 1994; Barja and Núñez, 1999). The other studies were based on respiration rate measurements from either a single pulse addition or a range of concentrations of glucose. The parameters K_{M1} and k_s', estimated using the lag-phase model are of a similar order of magnitude ($\sim 10^{-6}$ and $\sim 10^{-7}$ C-mol S g^{-1} soil, respectively) as in the previous studies. The parameter μ_{max} is equivalent to $Y \times k_s$ in the GPI dataset, and the estimated values are similar ($\sim$0.3 h^{-1}) to previous studies except one (Barja and Núñez, 1999). The growth efficiency values estimated using the simplified physiological model from the GPI dataset are towards the high range of literature values. The microbial mortality rate ($k_d \sim 0.02 - 0.04\ h^{-1}$) and half-saturation constant ($K_{M2} \sim 10^{-6}$ C-mol S g^{-1} soil) estimated using the simplified physiological model are also of a similar order of magnitude as values reported in previous studies. Therefore, based on this comparison with independent studies, we can conclude that our estimation approach provides reasonable parameter values.

We also attempted to parameterize the complex physiological model (Eqs. (1)–(7)) for the entire duration of GPI experiments (similar to Blagodatsky et al., 1998; Blagodatsky and Richter, 1998); however, reliable estimation was not possible due to the larger number of parameters. This suggests that simplified models using only heat dissipation data can achieve good performance and provide robust estimates of microbial traits comparable across studies.

4.2. Can we estimate selected microbial traits from the growth-phase data?

Our analysis suggests that the fitting of the growth-phase model is more accurate than that of the simplified physiological model during the first part of the incubations (cf. Figs. 3 and 5). In fact, despite the overall good performance, in most soil treatments in the growth-phase incubation dataset, the simplified physiological model failed to capture the initial heat dissipation rate for both calibration scenarios. In these incubations, substrates were provided at saturating concentrations, as shown by the estimated half-saturation constant (K_{M1}) being almost one order of magnitude lower than the amount of glucose added. Under these conditions, the kinetics of microbial growth represented by Eq. (11) reduces to exponential kinetics, which is mathematically similar to the form obtained with the growth-phase model (Eq. (22)). However, the parameters in Eq. (11) are estimating using more data points than when fitting Eq. (22). The additional data points around the peak and along the declining branch of the substrate-induced response constrain the parameter values in such a way that the simplified physiological model is not capable of capturing both the initial lag-phase and the final steep decline. In contrast, the growth-phase model is calibrated using data points essentially aligned along the exponential growth curve, which allows a more accurate fit at the cost of neglecting information from data around the peak and the following decline.

In samples amended with labile substrates, the substrate-induced response has been described in previous studies as the sum of lag- and growth-phase contributions (Panikov, 1996; Blagodatsky et al., 2000; Wutzler et al., 2012). Here, the growth-phase model accounted for both these terms and was able to capture the heat dissipation and respiration rates in both lag-phase and growth conditions (Fig. 5). As a result, the growth-phase model is more flexible and performs better than the simplified physiological model when focusing on growth-phase data.

In the exponential growth-phase, the estimated apparent efficiency Y is a good approximation of the actual growth efficiency because of the minimal maintenance load on microorganisms under these conditions (Bradley et al., 2018). Therefore, it is not surprising that Y values obtained from the growth-phase model are larger than those obtained using the other models. In the case of soil treatments with straw, Y is erroneously estimated to be higher than 1 (Table S6). This could be caused by the relationship between Y and CR (i.e., Eq. (35)), which only accounts for aerobic conditions, and thus, is not valid if other metabolic pathways and/or priming of SOM are also active. The effect of other metabolic pathways or priming on CR is further discussed in the next section.

4.3. Can a simplified model help to interpret the observed variability in CR?

In the LPI experiment, the added glucose is used mainly for maintenance respiration, so that glucose is only catabolized without resulting in growth. Thus, during maintenance, complete oxidation of 1-C mol of glucose to 1-C mol of CO_2 takes place, and 469 kJ C-mol^{-1} glucose of heat is produced. Therefore, we would expect CR = CR_L = 469 kJ C-mol^{-1} CO_2, which is also the CR value implicitly assumed by the lag-phase model. However, most soil treatments show significant differences between observed CR and CR_L (Fig. 6, dashed line), and a two-way ANOVA indicates that observed CR differ significantly across soil treatments but not across glucose levels (Fig. 6). Without microbial growth, these deviations can be explained either by the presence of other metabolic pathways or priming of soil organic matter (Hansen et al., 2004; Boye et al., 2018; Chakrawal et al., 2020).

In our experiments, CR values are above 200 kJ C-mol^{-1} CO_2, indicating mostly aerobic conditions (Chakrawal et al., 2020). Because CR is the ratio of heat to CO_2 production rates, values higher than 469 kJ C-mol^{-1} CO_2 indicate either additional heat production or lower CO_2 production than complete oxidation of glucose. Additional heat could be released as a result of priming because the metabolism on soil organic matter may produce large amounts of heat, but a smaller amount of CO_2, if highly reduced organic matter is undergoing decomposition (Chakrawal et al., 2020). Evidence from Arcand et al. (2017) further suggests that the long-term amendment of soils with organic materials (e.g., straw) can reduce nutrient availability for the microbial community compared to inorganic amendments (e.g., $Ca(NO_3)_2$). As a result, N mining via priming of soil organic matter would be induced along with glucose mineralization (Arcand et al., 2017). This could be the case in the Peat and Peat + N treatments because peat has high lignin content with a higher degree of reduction compared to carbohydrates (Turetsky et al., 2000; Worrall et al., 2016). Moreover, peat samples have large organic matter content, so that some priming is likely (Fig. 6).

In contrast, CR values lower than 469 kJ C-mol^{-1} CO_2 indicate either lower heat production or higher CO_2 production compared to complete glucose oxidation. For example, when glucose is not completely oxidized to CO_2, both the amount of heat dissipated and CO_2 produced would be reduced. This would be the case during fermentation or combined fermentation and aerobic decomposition. However, their ratio (CR) could still decrease (see Chakrawal et al. (2020) for details). This could explain the CR values lower than 469 kJ C-mol^{-1} CO_2 for treatments other than Peat and Peat + N (Fig. 6).

In the GPI experiment, CR values vary in the range of 150–700 kJ C-mol^{-1} CO_2 during the incubation, generally decreasing during the growth-phase and recovering after the peak of heat dissipation rate (Fig. 3). CR values are higher than 469 kJ C-mol^{-1} CO_2 during the early phase of the incubation but becoming lower than this threshold during the growth-phase. This indicates a transition from the early lag-phase, when microbial metabolism is uncoupled from growth and substrate is catabolized only for maintenance, to the growth-phase, when metabolism is coupled with growth and substrate is catabolized in association with anabolism. Since the coupling of catabolism and anabolism is different at different stages of microbial metabolism, the rates of CO_2 and heat production are not always proportional; thus, their ratio, CR, also varies through time. Under different assumptions on the type of microbial metabolism, the time variation of CR can be explained using the growth-phase model, but not the simplified physiological model.

In fact, in the simplified physiological model, a time-invariant value of CR is assumed, which misses the observed variability (dashed line

Fig. 7). This is because the CR from the simplified physiological model depends only on the microbial growth efficiency (Eq. (32)), which is comparable across soil treatments (Table S3). For growth efficiency in the range of 0.4–0.8, Eq. (32) would predict CR ranging from 364 to 440 kJ C-mol^{-1} CO_2; more importantly, CR would always be less than 469 kJ C-mol^{-1} CO_2 for glucose. Similar to systems with negligible microbial growth, the deviation of CR from the 364–440 kJ C-mol^{-1} CO_2 range in systems under aerobic growth conditions is indicative of the presence of other pathways of glucose metabolism or of priming of soil organic matter, which we have not included in our model. Interestingly, without incorporating the details of other glucose metabolic pathways and the priming of soil organic matter, the relatively simple growth-phase model explained the observed CR in the exponential growth-phase better than the physiological model (Fig. 7). The improved performance was the result of independently fitting heat dissipation and respiration rate data, which provided the needed flexibility to capture time-varying CR.

4.4. Ecological implications

In addition to the findings presented above, the microbial traits estimated using energy fluxes can be used to study the ecological implications of different management of soils, such as the consequences of N addition, and of organic *vs.* inorganic amendments to soils. Moreover, the estimated parameters can also be used to study the trade-offs (fast *vs.* efficient growth; Lipson, 2015) in the microbial responses to glucose additions among different soil treatments.

In the LPI experiment, the addition of N generally led to increased mineralization of glucose as reflected in the higher amount of heat and CO_2 released; e.g., in the case of Peat (red line) vs. Peat + N (blue line) in Fig. 2B. The estimated microbial traits using the lag-phase model were able to capture this difference as shown by higher values of the maximum rate of substrate utilization in soils receiving additional N (k'_s in Table S2). This suggests either higher microbial biomass or higher maintenance respiration in soils with more organic N.

Previously, data from the sludge and $(NH_4)_2SO_4$ treatments in the GPI experiment were used to study the effects of long-term stress by heavy metal toxicity and low pH on soils (Harris et al., 2012). The authors concluded that microbial communities under stress perform at lower thermodynamic efficiency and thermal yield (analogous to Y; Soares, 2019). In contrast, the growth efficiency estimated in our study does not show any strong differences among soil treatments indicating that microbial responses were dominated by glucose metabolism rather than soil treatments. Further, Arcand et al. (2017) also reported similar values of thermodynamic efficiency among organic and inorganic treatments based on glucose additions.

Lastly, we did not find any significant trade-offs between the maximum growth rate and growth efficiency among different soil treatments (Figure S1), possibly because of the relatively small range in these traits resulting from the glucose dominated microbial metabolism. In fact, it is possible that growth-efficiency trade-offs emerge only across very different microbial communities as a consequence of contrasting resource availabilities (Lipson et al., 2009; Lipson, 2015), while in our experiments, conditions were comparable and determined by the amount of added glucose.

5. Conclusions

We proposed a hierarchy of coupled C mass-energy balance models to estimate microbial traits from calorespirometric experiments. While our models are based on glucose metabolism, they can also be used to study functional traits of microbial communities utilizing multiple substrates or decomposing SOM, as long as the typical microbial response is observed; i.e., the lag-phase followed by exponential growth and then decay. In the cases of multiple peaks of microbial activity, as often observed during diauxic or drying-rewetting experiments, the data could be separated into different time periods, and then our models could be used to estimate microbial traits in each of them. Thus, our approach can be generalized depending on the experimental setup.

Based on our findings, we advocate using energy fluxes as reliable complements or alternatives to mass fluxes such as respiration rates for estimating microbial traits and constraining model parameters. Moreover, combining energy to mass fluxes in the calorespirometric ratios provides a metric to characterize microbial metabolism that could be used to test microbial-explicit models of soil C dynamics.

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Declaration of competing interest

The authors 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.2021.108169.

Author Contributions

Arjun Chakrawal: Formal analysis, Data curation, Writing – original draft, Writing – review & editing, conceived the study and designed the modeling framework, analyzed the data and took the lead in writing the manuscript with feedback from SM, Anke M. Herrmann: Data curation, Writing – review & editing, designed both the LPI and GPI incubation experiments, Stefano Manzoni: Formal analysis, Writing – review & editing, conceived the study and designed the modeling framework.

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