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Abstract

Microbial carbon use efficiency (CUE) affects the fate and storage of carbon in terrestrial ecosystems, but its global importance remains uncertain. Accurately modeling and predicting CUE on a global scale is challenging due to inconsistencies in measurement techniques and the complex interactions of climatic, edaphic, and biological factors across scales. The link between microbial CUE and soil organic carbon relies on the stabilization of microbial necromass within soil aggregates or its association with minerals, necessitating an integration of microbial and stabilization processes in modeling approaches. In this perspective, we propose a comprehensive framework that integrates diverse data sources, ranging from genomic information to traditional soil carbon assessments, to refine carbon cycle models by incorporating variations in CUE, thereby enhancing our understanding of the microbial contribution to carbon cycling.

Introduction

Earth System Models (ESMs) are indispensable tools for predicting the planetary response to climate change [1]. The accuracy and reliability of ESMs are crucial for informing climate projections that guide policy decisions. Soils store more carbon (C) than plants, the surface ocean or the atmosphere, and thus are critical for the functioning of the Earth system [2]. While ESMs are becoming increasingly complex, their predictions of soil organic C (SOC) stocks have improved only marginally in recent decades [3,4].

Microbial communities process most of the C entering the soil, thereby shaping its fate [5,6]. Microbes metabolize multiple C sources, including detritus, root exudates, and microbial metabolites [7]. The energy needed to acquire C depends on whether the compounds can be taken up directly or require prior enzymatic degradation [8]. Additionally, microbial community composition and functioning are influenced by prevailing climatic conditions [9–11]. The general omission of microbial community structure and related processes in C cycle models has been suggested as one of the causes for their poor performance in predicting SOC stocks and their responses to climate change [12,13].

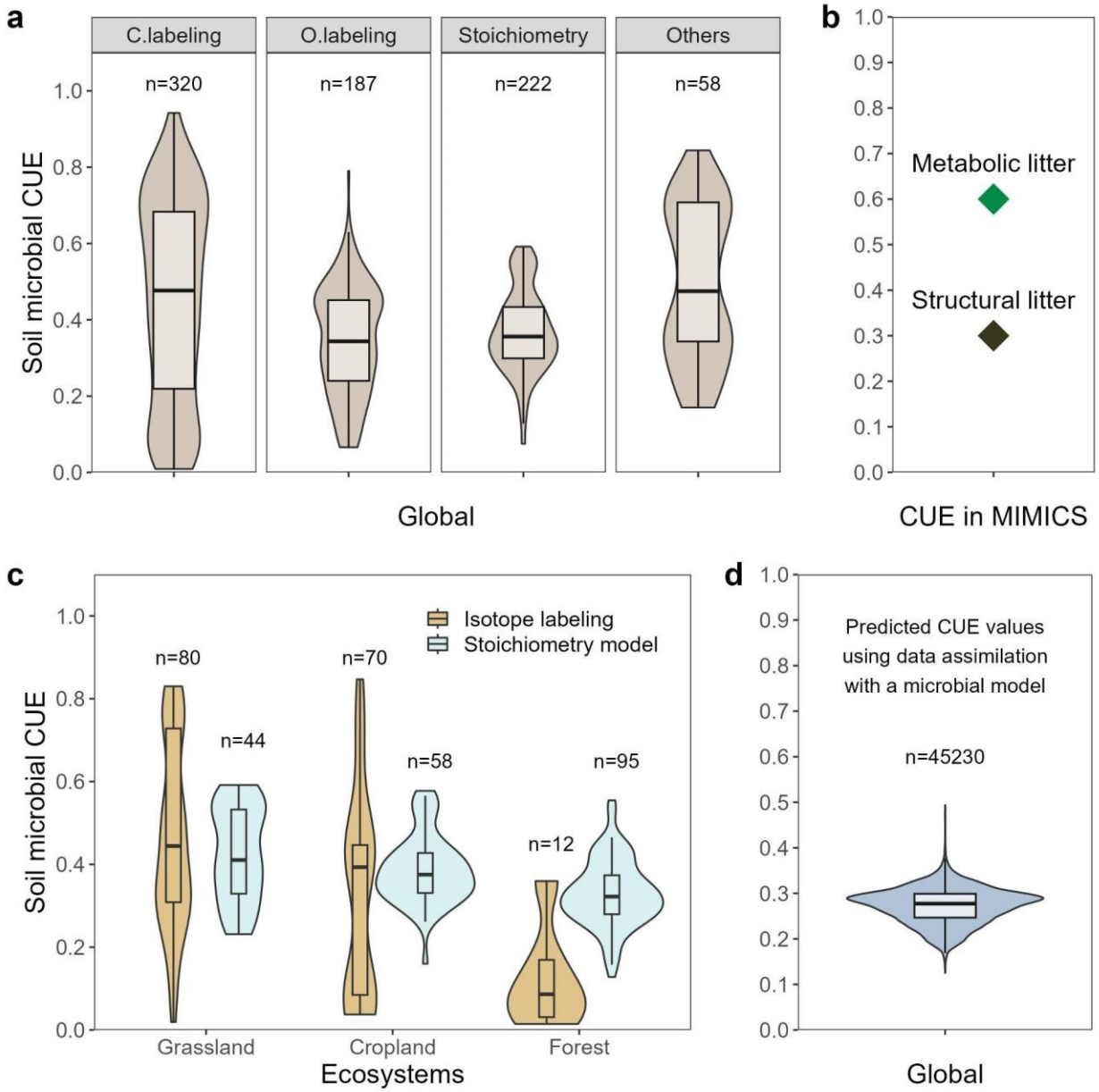
Recognizing the impracticality of representing every conceivable microbial metabolic pathway, many models combine a spectrum of microbial processes into a single metric referred to as microbial C use efficiency (CUE) [14,15]. CUE, as a model parameter or as a system property emerging from multiple co-occurring processes, represents the fraction of C uptake allocated to the production of new microbial biomass [16]. Using this definition, CUE declines as more C is used for respiration to generate energy (for substrate uptake, cellular maintenance, enzyme production) or for exudation (extracellular enzymes, polysaccharides) [17,18]. This pragmatic approach streamlines the modeling of soil C cycling by incorporating the diverse fates of microbial C, including biomass production, respiration, and exudation, thereby providing a more comprehensive understanding of microbially-mediated C-pathways.

However, accurately integrating the spatial or temporal dynamics of microbial CUE into soil C models remains a significant challenge. Most of the current C cycle models either lack explicit representation of CUE or treat it as a constant value [4], despite our understanding that CUE varies under different environmental conditions. For example, observations indicate significant variability in CUE at the global scale [8], which may be partially attributed to inconsistencies among measurement techniques (Figure 1a). Moreover, comparisons across

ecosystems reveal that CUE is generally higher in grasslands than in croplands, with forests consistently showing the lowest CUE values, regardless of the measurement approaches used [19,20] (Figure 1c). CUEs derived from data assimilation [21] are also lower than those from more direct measurement approaches (Figure 1d).

Several attempts have been made to reflect or incorporate CUE variations into models of litter [22] or soil organic matter [9,13] decomposition with the aim of assessing the implications for soil C cycling. For example, incorporating an empirically-derived negative relationship between microbial CUE and temperature into a microbial-explicit SOC model improved the simulation of contemporary soil C stocks [23]. Zhang et al. [24] introduced the effects of substrate quality and soil fertility on microbial respiration, highlighting the joint control of litter quality and quantity on the steady-state SOC stocks. Wieder et al. [25] enhanced the understanding of CUE variation by including two types of decomposers with differing substrate preferences and CUE (Figure 1b). These examples suggest that more realistic representations of microbial C transformations have the scope for improving model predictions of soil C [23,26]. However, these predictions were poorly constrained by observational data, calling their reliability into question [21,27,28].

In this Perspective, we synthesize our understanding of CUE regulatory factors and databases for constraining numerical models, with the aim of clarifying complexities, addressing controversies, and providing a holistic perspective on pathways to adequately reflect CUE variations in C cycle models and their consequences for simulated soil C stocks.



111

112 **Figure 1: Variability of carbon use efficiency (CUE) at a global scale**. a): Observation-based
113 CUE estimates at the global scale from C (^{13}C and ^{14}C) and ^{18}O isotopic labeling, stoichiometric
114 modeling and other methods. Data were collected from [19,21,29–31]. b): CUE constants used in the
115 MIcrobial-MIneral Carbon Stabilization model (MIMICS) for two litter types (diamonds).
116 Metabolic litter comprises plant litter that decomposes easily, whereas structural litter is more
117 resistant to decomposition [32]. c): Observation-based estimates for different ecosystems using
118 isotopic labeling [29] or stoichiometric modeling [19]. d): CUE values predicted using a microbial

model assimilating information on SOC profiles [21]. Data assimilation integrates observed data into predictive models to refine model parameters and improve estimation accuracy.

Data availability and challenges

Terminology and definitions of microbial CUE

The concept of microbial CUE, the fraction of C uptake that is used to produce microbial biomass [16–18], is intuitively straightforward, but CUE definitions vary depending on the ecological processes involved, measurement methods, and scales of biological organization (e.g., population, community and ecosystem) [14,17]. Therefore, CUE can be regarded as an emergent parameter, encapsulating multiple processes within a single metric. It is useful in modeling as the number of processes that can be modeled is constrained by practical limitations (e.g. availability of data for calibration). Consequently, ecosystem models often simplify microbial process complexity, which in reality, escalates from the genomic to the ecosystem level (Figure 2).

CUE is quantitatively expressed as the ratio of microbial growth (μ) to C uptake (U) [16,33], that is, CUE = μ/U. This ratio encapsulates the efficiency with which microorganisms convert assimilated C into biomass. Microbial uptake involves C assimilation for growth (μ), respiration (R), and the secretion of extracellular enzymes and metabolites (EX). Geyer et al. (2016) introduced a nested conceptual framework for understanding CUE across different biological organization levels: population (CUE_P), community (CUE_C), and ecosystem (CUE_E). This framework is useful for integrating C fluxes mediated by soil microbes into models at various ecological scales (Figure 2).

CUE_P reflects the species-specific functioning of microbial taxa (e.g., biosynthesis rate, exudate production) and thermodynamics of C substrate metabolism that limits the proportion of C uptake used for biosynthesis versus C lost from the cell (e.g., mineralized or exuded as metabolites). Typically measured in cultured populations, the CUE_P formula adjusts for respiration (R) and exudation (EX) losses from the uptake, expressed as $CUE_P = \frac{U-R-EX}{U}$. CUE_C incorporates additional environmental and community factors influencing microbial metabolism in natural communities consisting of multiple populations. It focuses on gross microbial

production prior to the recursive substrate recycling of necromass and exudates, capturing the metabolic response of microbial communities to substrates over short durations (hours), and is similarly expressed as $CUE_C = \frac{U-R-EX}{U}$.

CUE_E considers C retention as net microbial growth over longer time scales (days to months), taking into account the drivers of CUE_P and CUE_C as well as microbial biomass turnover. On these time scales, a significant proportion of microbial biomass is converted to necromass following microbial death (MD) [32] such that $CUE_E = \frac{U-R-EX-MD}{U}$, encompassing all aspects of microbial C processing, including death and recycling processes.

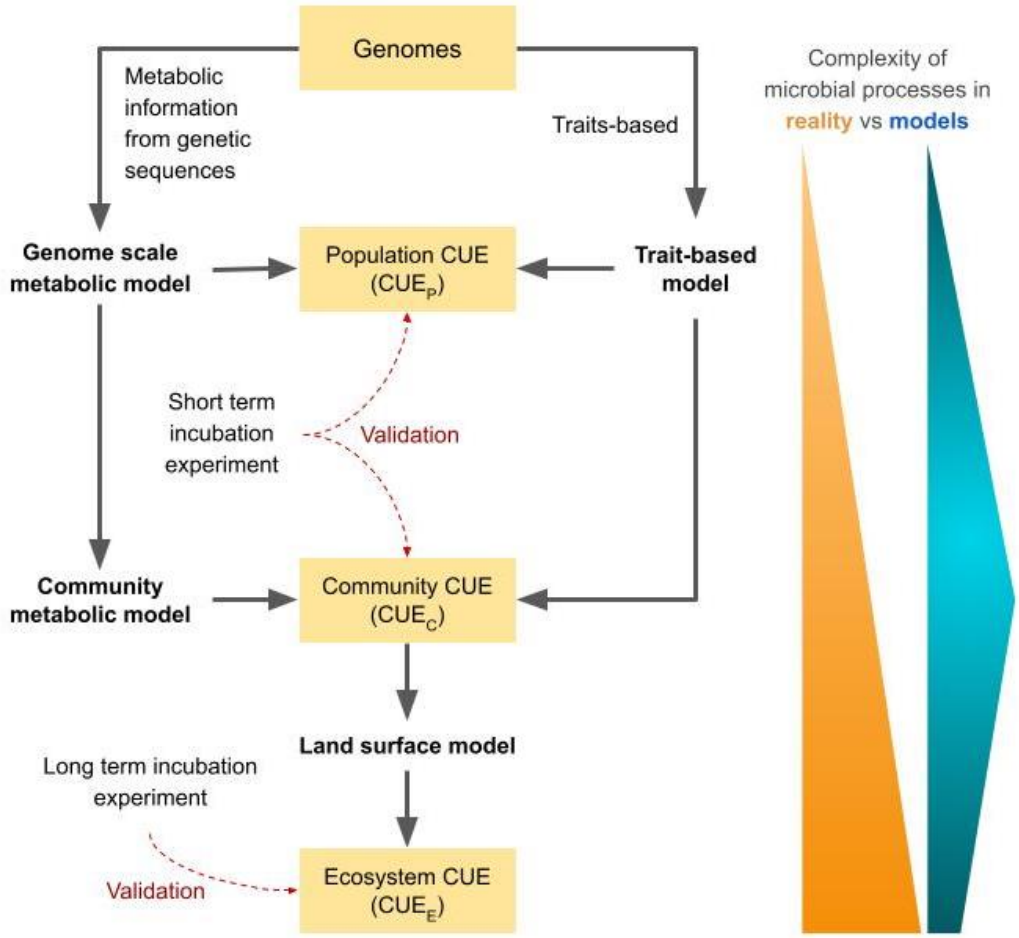


Figure 2. Schematic representation of a cluster of models integrating observational constraints on CUE at population (CUE_P), community (CUE_C) and ecosystem (CUE_E) scales. The genome-scale metabolic model predicts the movement of metabolites within a cell based on its genomic information. CUE_P and CUE_C can be validated by short-term incubation measurements, while CUE_E requires long-term incubation measurements. Although the scales

and processes governing CUE expand from individual cells to entire ecosystems, there is a practical limit to the extent they can be resolved in C cycle models.

Methods for measuring microbial CUE

Multiple approaches can be used to quantify CUE, such as isotopically labeling substrates [35,36], stoichiometric modeling [22,37] and others [38]. These methods rely on different assumptions and capture distinct microbial processes, which can explain the variability in CUE estimates across methods [8,39,40] (Figure 1a), including differences in the response of CUE to environmental changes [41], and the relationship between CUE and SOC (Figure 3a and b).

The most common approach for measuring CUE is the tracking of isotopically labeled compounds (^{14}C, ^{13}C labeled substrate, or ^{18}O water) introduced to the system. Carbon isotopes in microbial substrates enable the differentiation between C allocated to microbial biomass and that released through respiration. Although this labeling technique is widely used, its results can be influenced by the choice and combination of substrates [35], as well as the incubation period [14,42]. A significant limitation of this approach is that measured CUE reflects only the efficiency of those microbes that use the introduced substrates, not the entire microbial community. Furthermore, the variation in incubation times and temperatures across different studies (Figure 3c and d) presents a substantial obstacle to standardizing CUE measurements.

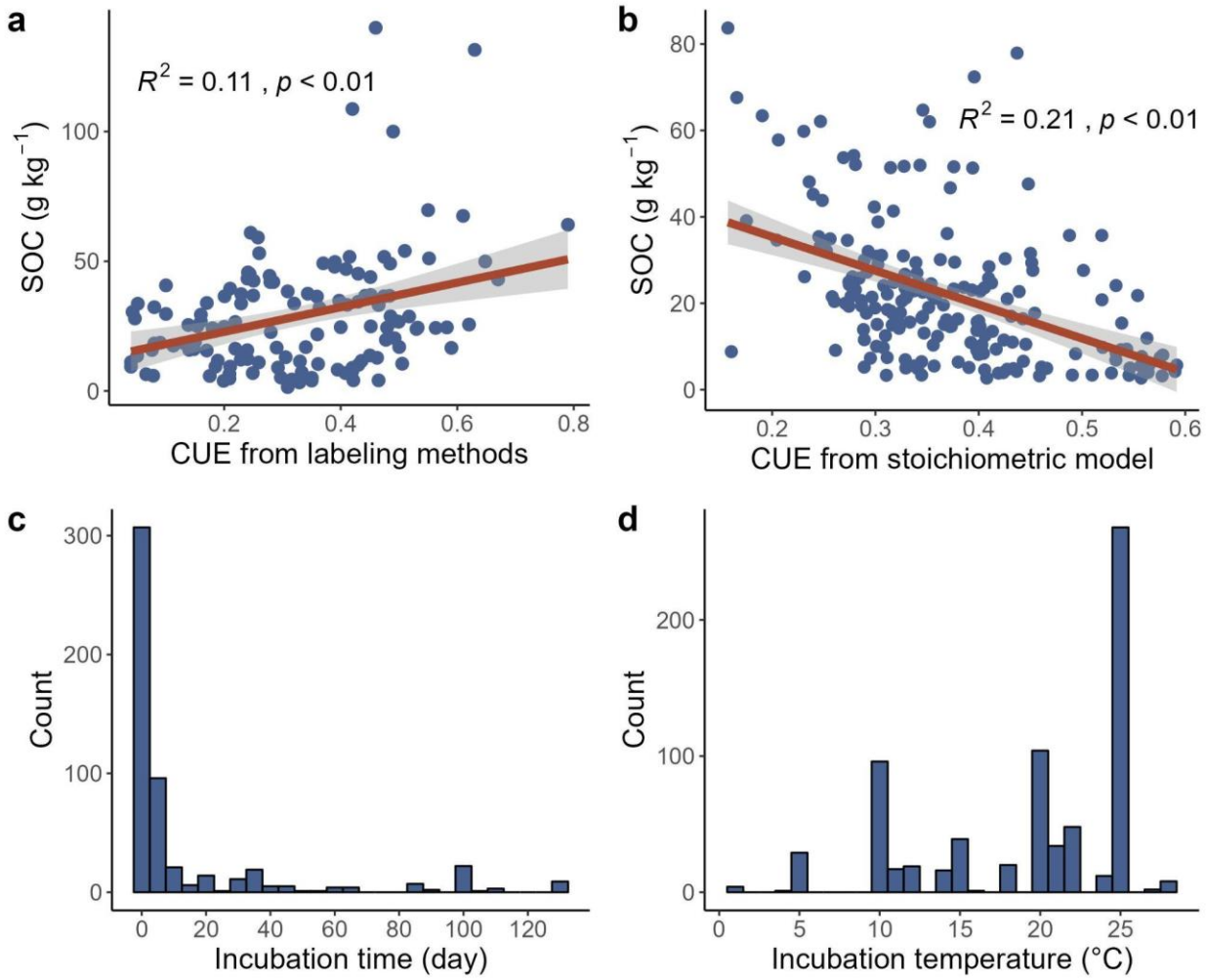


Figure 3. The relationships between soil organic carbon (SOC) concentration and CUE from (a) isotopic labeling methods (^{14}C, ^{13}C labeled substrate, and ^{18}O water) and (b) stoichiometric modeling. The figure also shows (c) the incubation duration and (d) temperature employed in studies using labeling and incubation methods. Data in the panels are from (a) [21], (b) [19], and (c and d) [20].

The method using ^{18}O-labeled water is based on the incorporation of the ^{18}O-atom into microbial DNA as a measure of growth as compared to catabolic C losses as CO_2 [36,43]. This method has higher accuracy than the C labeling method as it is not substrate specific, does not perturb microbial metabolism like methods involving substrate addition, and exhibits comparatively less variability over time [39]. Nonetheless, this method faces limitations such as higher cost and demanding technical procedures. Concerns also arise regarding the method's

foundational assumptions, e.g., the presumption that water is the sole oxygen source for microbial DNA synthesis and the hypothesis that all microbial cells maintain a consistent DNA to biomass C ratio [44]. Furthermore, its applicability in dry soils is challenging [45].

Stoichiometric modeling is a common method for indirectly estimating CUE, which is based on the assumption that microbes growing on plant detritus allocate C to produce enzymes and other necessary components to acquire nutrients in the appropriate elemental ratios at the whole-community scale [33,37]. This approach offers the advantage of requiring only a limited number of parameters, such as the activities of enzymes targeting C versus nitrogen (N) or phosphorus (P) acquisition and the C:N:P composition of the substrate and microbial biomass, which can be constrained by existing observations. However, it relies on highly simplified assumptions regarding elemental ratios and C allocation [40]. This approach inherently suggests lower CUE in soils with high SOC due to its focus on the metabolic costs of nutrient acquisition under conditions where nutrients are scarce relative to C. This outcome (Figure 3b) starkly contrasts with the positive correlation between CUE and SOC observed using isotopic labeling techniques (Figure 3a), which are commonly considered to provide a more realistic insight into the relationship between CUE and SOC. The isotope labeling method estimates microbial growth and CUE by tracking the incorporation of labeled atoms into biomass or DNA, reflecting intracellular biochemical transformations. In contrast, the stoichiometry model method estimates CUE by analyzing the activities of extracellular enzymes and the stoichiometric balance between organic matter and microbial biomass, focusing on extracellular metabolic processes [46]. Therefore, caution is advised when comparing results obtained from these two methods, even though they use the same term (CUE). We do not yet know the extent to which the stoichiometric and isotope methods are comparable. Until we understand which patterns can be accurately captured by the simpler stoichiometric method, we should rely on the more robust ^{18}O method for measuring actual CUE and the ^{13}C method for CUE associated with specific substrates.

In addition to the methods mentioned above, there are other less commonly used approaches, including the use of ^{18}O in water vapor to minimize impact on soil moisture [45], metabolic flux analysis [17], and calorespirometry [47]. Each method offers unique advantages and faces specific limitations, grounded in their underlying assumptions and theoretical bases [39–41]. These limitations not only affect the accuracy of these methods but also introduce significant comparability issues. Consequently, there is an urgent need to improve current methodologies and integrate innovative techniques to more accurately assess soil microbial CUE.

Data gap

Given the methodological challenges in measuring CUE in situ, field assessments of microbial CUE are rare. The vast majority of existing CUE observations have been obtained from lab incubations. Yet, these CUE observations remain scarce at the global scale, a situation which is exacerbated by the lack of harmonization of observations from different measurement approaches. For some ecosystems, observations are few or even nonexistent, including ecosystems that play a critical role in the global C cycle, such as tropical rainforests, wetlands, and peatlands [48,49].

Existing CUE measurements mostly come from studies of the litter and surface mineral soil [16]. Thus, our understanding of microbial CUE in subsurface soil remains limited, which is problematic as large amounts of C are stored in subsoils globally, and especially those of wetlands and peatlands. The few existing studies indicate that microbial CUE decreases with soil depth [50,51] and that subsurface CUE may be less sensitive to warming [35] but more sensitive to nutrient variations [52].

Moreover, data on temporal variations in CUE are lacking. A commonly overlooked factor that may contribute significantly to CUE variability in soil ecosystems, regardless of methodology, is seasonality in CUE. Seasonal changes are associated with significant variations in substrate availability, temperature and moisture, all of which may have a substantial impact on the growth and respiration of soil microorganisms, thereby altering microbial CUE [43]. For example, CUE estimated using the ^{18}O incorporation method ranged from 0.1 to 0.7 in soils from an agricultural field site and from 0.1 to 0.6 at a forest site within one year [31]. It has also been reported that soil microbial CUE exhibits significant fluctuations within a short period (daily) after rewetting [53,54]. This temporal dynamic in CUE values could contribute to the significant variability observed in CUE measurements.

Regulatory factors governing microbial CUE

The incorporation of soil microbial CUE dynamics into process-based models necessitates a comprehensive understanding of a range of regulatory factors influencing CUE

(Figure 4). CUE at a specific biological level is influenced by features of both the microbial community itself (biological controls) and its external environment (abiotic controls). These factors frequently interact, particularly at the community and ecosystem levels: abiotic controls can modify CUE_C or CUE_E by regulating biological controls, while biological controls may induce adaptation to abiotic factors, thereby influencing the impact of abiotic controls.

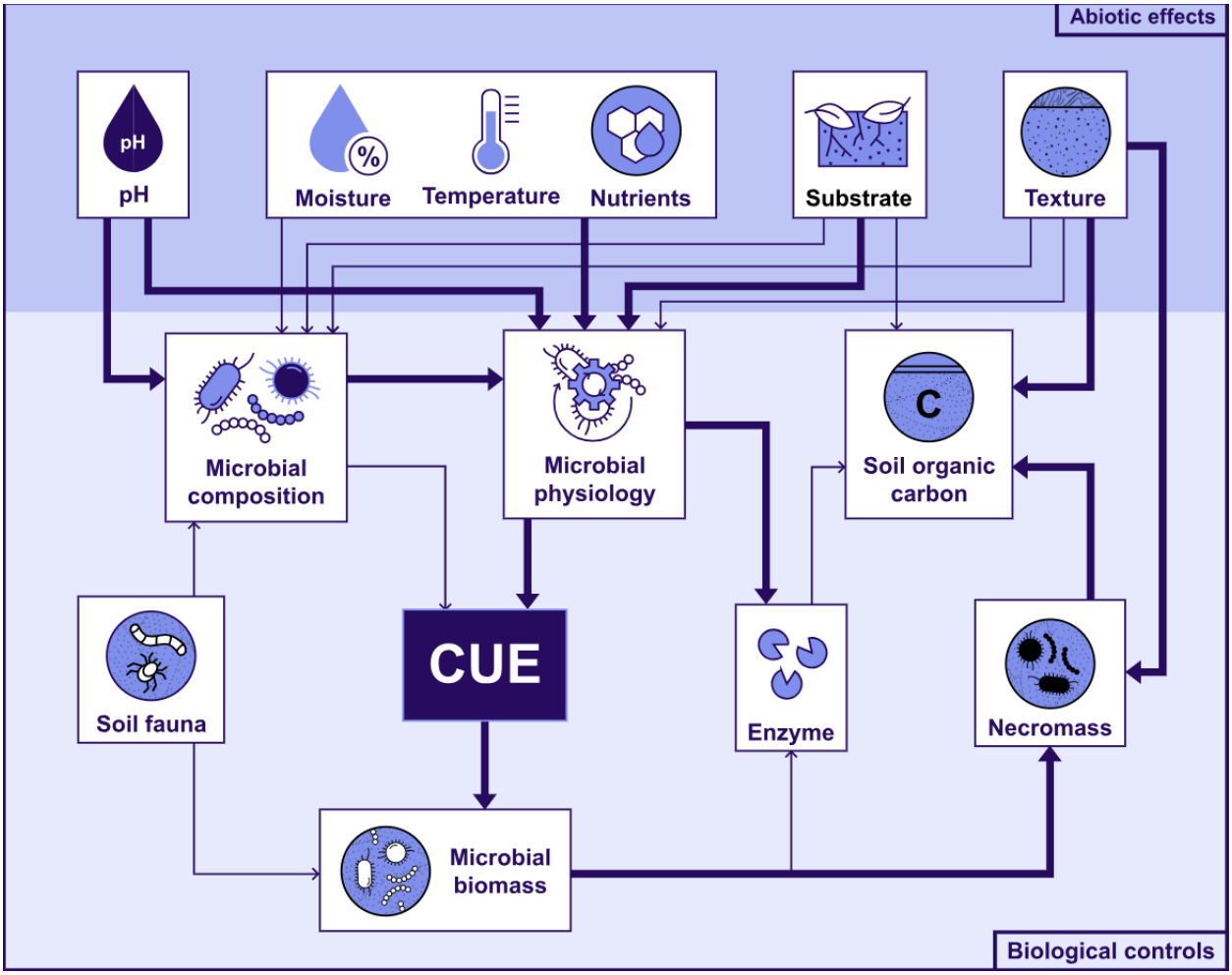


Figure 4. Framework of biological and abiotic determinants of CUE in a carbon cycle context. The darker-colored area in the figure indicates biological controls; the lighter-colored area indicates abiotic effects. The arrows depict implicit relationships and the width of the arrows corresponds to the levels of scientific certainty: confident assertions are represented by thick lines, while less confident assertions are indicated by thinner lines. These confidence levels are based on the expertise of the authors.

Biological controls:

Microbial physiological state

Microbial CUE reflects the physiological state of microorganisms. Under natural conditions, only a small proportion (values vary from 1% to >20% in different studies [55,56]) of soil microbial cells are metabolically active, and soil respiration primarily originates from these metabolically active cells [56]. Nonetheless, a high fraction of microbial cells in the soil are in a potentially active state (10 to 60% of the total microbial biomass), meaning that they are ready to start using available substrates within a few hours after easily available substrate is added. The shifts in physiological states of these microbial cells, resulting from changes in temperature, moisture, or substrate availability, significantly impact CUE [57]. Consequently, CUE_P or CUE_C measurement methods relying on substrate addition may overestimate CUE [14], and shifts in physiological state can lead to seasonal variations in CUE [31].

Microbial community diversity and composition

Increased microbial diversity enriches the spectrum of metabolic functions within a community, potentially leading to greater microbial growth [58] and CUE_C by facilitating more efficient use of varied C sources [10,59]. The composition of microbial communities, notably the ratio of fungal to bacterial biomass (F:B), plays a critical role in determining CUE_C [60]. Communities dominated by fungi can show higher CUE_C, attributed to their higher biomass C to N) ratios (C:N) and their proficiency in decomposing complex organic materials [61], or lower CUE due to the high costs associated with resource acquisition by decomposer fungi [60]. Therefore, this contrasting evidence from plant litter studies indicates that the relationship between F:B ratio and CUE is context-dependent [60,62]. Alternatively, an approach categorizing microorganisms into copiotrophs (*r*-strategists with low CUE) versus oligotrophs (*K*-strategists with high CUE) has been promising for estimating CUE [63]. For example, shifts from *r*-strategists to *K*-strategists explain increased CUE_C along a successional gradient in the southeastern Tibetan Plateau [64].

Changes in community composition may also enable microbial communities to alter their CUE in response to environmental changes or fluctuations [65,66]. For instance, long-term warming experiments indicate a decline in the temperature sensitivity of CUE_C, suggesting that shifts in microbial composition can maintain CUE_C despite changes in temperature and

substrate quality [35]. Similarly, modeling studies suggest that changing microbial community composition can reduce the sensitivity of CUE_C to substrate quality [67] and soil moisture fluctuations [68].

Biotic interactions

In the soil food web, biotic interactions such as mutualism, facilitation, competition, and predation can shape CUE_C [59]. Interspecific microbial competition drives accelerated growth rates, accompanied by the release of secondary metabolites that can negatively affect CUE_C [69]. Antagonistic interactions may trigger stress responses, further diminishing CUE_C [70]. Conversely, facilitation enhances CUE_C by broadening species-realized niches, alleviating environmental stress, and reducing extracellular enzyme production costs [67]. Biotic interactions at higher trophic levels, such as predation, can variably affect CUE_C by altering microbial density and influencing the outcomes of interspecific competition [71,72].

Abiotic controls:

Temperature

Temperature significantly affects soil microbial CUE, with respiration often increasing more than growth in short-term incubations, resulting in a decrease in CUE_P [9,38,73]. The impact on CUE_C and CUE_E is less clear [66], likely due to varied responses among microbial taxa [74,75] and interactive effects with other environmental factors [42,43,50,76]. Temperature shifts can lead to changes in community traits or select for taxa with distinct life strategies, known as trait modification and trait filtering, respectively [77,78]. However, limited research on how CUE_P varies among different taxa in response to temperature impairs our ability to accurately predict changes in CUE_C [79–81].

The interplay between direct and indirect temperature effects on soil microbial CUE_C and CUE_E complicates our understanding of the impact of warming on CUE. Warming can intensify C-nutrient imbalances, potentially diminishing microbial CUE [82], but it can also improve the efficiency of substrate utilization, thereby enhancing CUE [36,75]. Expected reductions in soil moisture due to increased evapotranspiration under warming conditions [83] add another layer of complexity, with the combined impacts of temperature and moisture on microbial CUE

328 remaining inadequately explored [10,84]. Some soil C models, including Millennial [85] and MIMICS [25]
329 have begun to account for the temperature dependency of CUE_C, indicating a growing
330 recognition of the importance of including the dynamic response of microbial CUE to fluctuations
331 in temperature.

332 Soil water availability

333 Increased soil moisture promotes microbial growth and CUE by improving substrate
334 diffusivity and accessibility, and lowering investment in osmolyte synthesis, as long as
335 conditions remain oxic [8,10,86]. Prolonged water stress reduces soil substrate accessibility and
336 increases the need to synthesize osmolytes to survive during dry periods, leading to lower
337 CUE_C [86], even though the taxa that remain active in dry conditions can maintain relatively high
338 growth rates [87]. Furthermore, drought reduces plant C inputs to the soil [86], thus potentially
339 leaving microbes with fewer lower resources, resulting in lower CUE. The intricate interplay of
340 drought-induced changes in microbial respiration and growth may leave CUE unchanged if the
341 affected processes balance each other [81]. High levels of soil moisture may also reduce microbial
342 CUE. As soil pores fill with water, air spaces and oxygen diffusivity decline, potentially leading to
343 anaerobic conditions if saturation occurs. Under O_2 limitation, soil microbes shift from aerobic to
344 anaerobic respiration or fermentation, significantly reducing energy yield and leading to
345 decreased microbial growth and CUE while having little impact on CO_2 production rate due to
346 upregulated biochemical rates [86].

347 Microbial responses to rewetting of a dry soil also cause rapid changes in CUE, as
348 shown in modeling studies [53] and confirmed by empirical evidence [54]. Upon rewetting,
349 respiration increases while growth lags behind, especially when the soil has been dry for a long
350 period [54]. As a result, just after rewetting, CUE is low and then increases as growth recovers
351 during the first days after rewetting. However, after this initial pulse of microbial activity, CUE
352 peaks and decreases again as substrates released during rewetting are consumed [54].

353 Nutrient availability

354 The availability of nutrients such as N and P significantly affects microbial growth and
355 respiration according to the concept of stoichiometric homeostasis which assumes constrained
356 biomass C:N:P ratios of microbial cells [33,67]. Consequently, CUE decreases with increasing
357 substrate C-to-nutrient ratios and increases with nutrient amendment when organic substrates

are nutrient-poor [22,33]. Several C cycle models, such as the one proposed by Manzoni et al. [88] and its later implementation [24], have integrated CUE dynamics as a function of stoichiometry. In contrast to the homeostasis concept, recent findings highlight the capability of microbes to store and use nutrients dynamically, contributing to a stable CUE across different environments by separating growth and respiration processes from immediate nutrient availability [89]. This resilience to nutrient stress suggests that future C modeling should incorporate microbial nutrient storage dynamics for enhanced predictive accuracy.

Soil pH

Soil pH influences microbial CUE_C and CUE_E by affecting the bacterial community composition and acting as a potential stressor [90]. It also impacts CUE by altering microbial community composition [91], nutrient solubility [86], and metal toxicity (e.g., aluminum [90]). Habitats with neutral pH generally have higher bacterial diversity and biomass compared to acidic or alkaline soils [7]. The response of community composition to a shift in soil pH from acidic to neutral corresponded with a significant increase in CUE_C [90,92]. However, recent research indicates a complex interplay between soil pH, microbial community composition, and CUE dynamics, evidenced by both negative correlations [93] and a U-shaped response curve, pinpointing a critical threshold at pH 6.4 [93], although the calculations to document this are complex and may necessitate refinement.

Soil texture and structure

Microbial growth is intricately linked to substrate accessibility, which is influenced by soil environmental conditions like texture and soil structure. Approximately 40–70% of soil bacteria are associated with microaggregates and clay particles [95]. The structural complexity of the soil environment also plays a crucial role in shaping the community structure and function of soil microorganisms at the ecosystem level [96]. Heterogeneity of soil structure and composition creates diverse microhabitats that influence microbial interactions, diversity, distributions, and activity, as well as ecosystem processes like nutrient cycling and organic matter decomposition [97]. Still, limited information exists on the relationship between soil texture or structure and microbial CUE. A recent meta-analysis found a significant positive link between microbial CUE_C or CUE_E for glucose and soil clay content [30], which was attributed to increased clay content enhancing substrate adsorption [98], thereby limiting substrate availability to microbes [99], and resulting in higher microbial CUE_C or CUE_E.

Substrate quality

Substrate quality, defined by the chemical characteristics of organic matter that influence its decomposability, such as the C:N ratio and molecular composition, significantly impacts soil microbial CUE [100]. A "high-quality" substrate typically has a lower C:N ratio, indicating a balanced N content relative to C, and a lower content of recalcitrant compounds, which generally leads to faster decomposition and higher CUE by providing C and nutrients that microbes require for growth and metabolism [8]. Compounds requiring multiple enzymatic steps for degradation can lead to reduced efficiency in building biomass. Polymeric substrates like lignin and cellulose need depolymerization before cellular uptake, whereas smaller substrates readily diffuse across membranes [65]. Takriti et al. (2018) found a positive association between soil CUE_C and ratios of cellulase to phenol oxidase enzyme activity potential, which was considered to be indicative of soil organic matter (SOM) substrate quality [50]. Different substrates necessitate distinct metabolic pathways, resulting in different respiration rates per unit C assimilated [8,101]. Frey et al. (2013) observed lower microbial CUE_C when soils were amended with oxalic acid or phenolic compounds compared to glucose, despite similar molecular sizes [35].

Microbial CUE increases with the chemical energy per mole of C in the substrate, highlighting the importance of substrate chemistry for microbial CUE variability in soil [8]. This relationship is akin to the concept of energetic imbalance [102], which parallels the idea of stoichiometric imbalance. The energy content of soil microbial biomass and substrate can be quantified by the degree of reduction (γ), which refers to the average number of electrons available per C atom for biochemical reactions, indicating the energy density of the substrate or biomass [8]. The degree of reduction of soil microbial biomass (γ_B) is typically around 4.2, while that of substrate (γ_S) usually varies between 1 (e.g., for oxalate) and 8 (methane) [8]. Most of the substrates used by soil microorganisms have a γ_S of 3 (e.g., various organic acids), 4 (e.g., glucose and other carbohydrates), and rarely 5 or higher (e.g., leucine, polyhydroxyalkanoates or lipids) [8]. When γ_S is lower than γ_B, the substrate's energy content is insufficient to meet microbial demand, necessitating the oxidation of more substrate per unit of C assimilated, thereby reducing CUE [103]. These insights form the basis of the stoichiometric modeling for indirect CUE estimates.

SOC-CUE relationship

The relationship between CUE and SOC concentration at the ecosystem level can be positive, negative, or non-existent, depending on the interactions among multiple processes [21,95,98,104–106]. Higher CUE can lead to increased SOC through biosynthesis and accumulation of microbial by-products — facilitating SOC formation via the entombing effect [16,104,107] — or conversely, trigger SOC decline through the priming effect by ramping up microbial biomass and enzyme activity [9]. While some studies suggest a negative correlation between CUE and SOC [105,106,108], the majority of research supports a positive relationship [21,77,109,110], indicating that higher CUE is often linked to increased SOC levels. In a recent study, Tao et al. [21] employed observational data and data assimilation algorithms and found that, on a global scale, CUE is positively correlated with SOC concentration, arguing for CUE as the major determinant for SOC formation. However, subsequent arguments have raised methodological concerns which might have obscured the importance of microbial community dynamics [27] and SOC stabilization processes [111].

Indeed, the link between microbial CUE and SOC is contingent upon the stabilization of microbial necromass within soil aggregates or its association with minerals [98,104,107]. This stabilization process, pivotal for enhancing SOC, is significantly influenced by physico-chemical soil properties, which vary greatly and determine the potential for necromass protection [112,113]. Positive SOC-CUE relationships could be anticipated in soils with high physicochemical C stabilization potential and microbial communities that convert simple chemical substrates into necromass [113]. Conversely, when soil microbes face environmental stress, the relationship between CUE and SOC becomes less predictable. Particularly under conditions where nutrients are limited relative to carbon, the increased microbial respiration required to maintain stoichiometric balance leads to a decreased CUE [33,37]. Further reductions in CUE may be driven by environmental challenges such as low oxygen or pH [91,108], as well as the physiological costs of microbial competition [69]. However, these stressors on microbial activity may differently affect SOC, potentially leading to either a negative or negligible correlation between CUE and SOC [108]. It's worth noting that in organic-rich soils, such as peat, C stabilization relies more on the accumulation of undecomposed plant material than on necromass formation [114], making the link between CUE and SOC less direct. Therefore, the CUE-SOC relationship in organic soils is expected to differ from mineral soils where C is mainly stabilized by mineral associations.

Additionally, it is important to recognize the distinct sensitivities of microbial CUE and SOC to environmental changes, as their responses are not synchronized. Microbial CUE can adjust rapidly, from days to months, in contrast to SOC, which may take years or even decades to respond to a measurable extent [31,115]. Data from two meta-analyses highlight this disparity, showing that although fertilization positively affects both CUE_C and SOC [29,41], the response ratios of CUE_C were not significantly correlated with the response ratios of SOC, or even microbial biomass C content (Figure 5a and c). Here, the "response ratio" is calculated as the ratio of the measured value in the treatment to the value in the control. Furthermore, the response ratios of microbial CUE_C were not significantly related to treatment duration (within ten years of treatment) (Figure 5b), whereas the response ratios of SOC increased significantly with experiment duration (Figure 5d). Therefore, SOC gradually approaches a new equilibrium over several decades, whereas CUE achieves equilibrium almost immediately. This discrepancy underscores the importance of considering the state (SOC and microbial biomass) dynamics of an ecosystem when evaluating the interplay between microbial CUE and SOC dynamics.

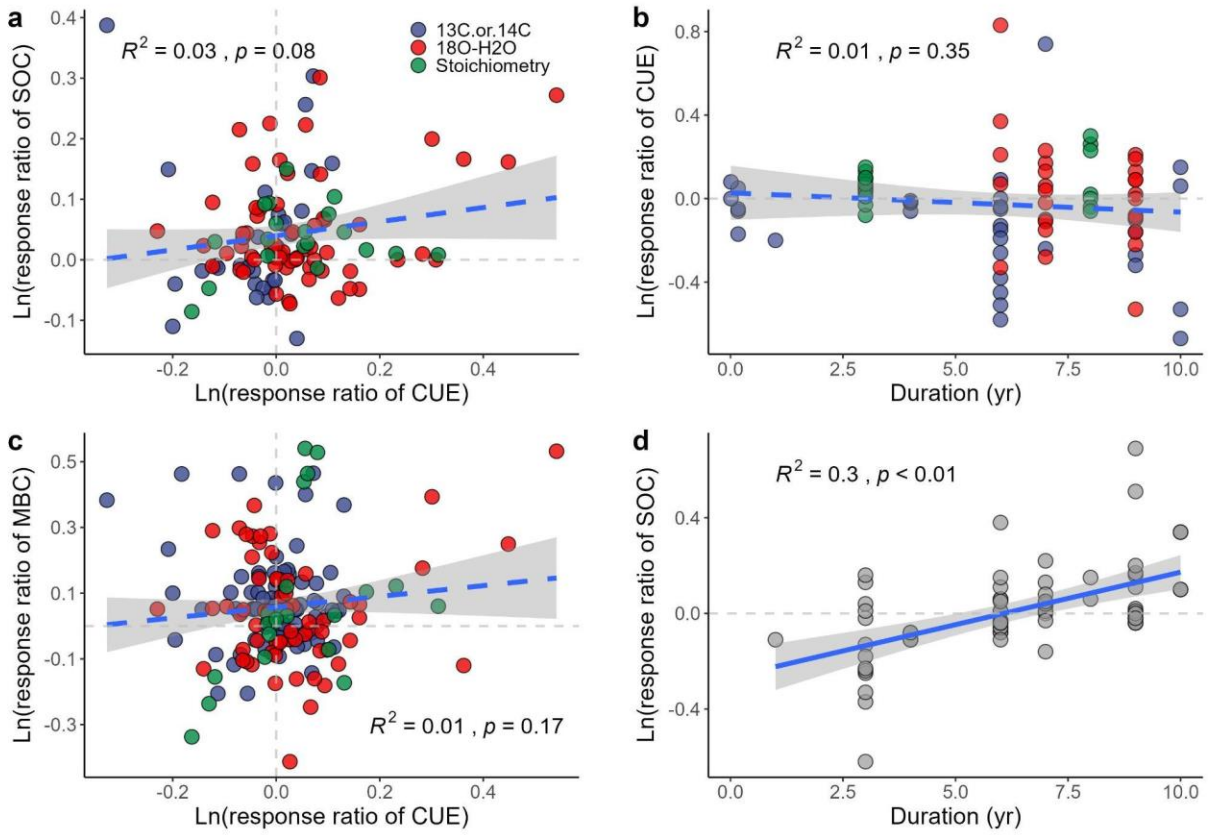


Figure 5. Contrasting responses of SOC and CUE to fertilization. Correlations between ln-transformed response ratios of microbial CUE and ln-transformed response ratios of (a) SOC and (c) microbial biomass C (MBC); and the correlation between experiment duration and ln-transformed response ratios of (b) CUE and (d) SOC. The response ratio is calculated as the ratio of the measured value in treatment to the value in the control. Data are from meta-analyses [27, 41 29]. Both datasets include observations from all three methods of CUE measurement, i.e., C labeling, O labeling, and stoichiometry modeling as indicated by symbol colors in panels a, b and c.

Using models and data across scales to clarify the microbial role in C cycling

Integrating genomic data with CUE and C models

With the rise of high throughput sequencing technology, the use of genomic datasets to help calibrate or validate C models has become both feasible and affordable. This capacity is especially valuable when predicting CUE [116]. As genomic data related to microbial traits becomes more readily available at both the population [117] and community levels through metagenomics [118], there is a growing need to effectively integrate this data into C cycle models. This integration requires models that can handle complex microbial interactions, from individual populations to entire communities (Figure 2).

One way to integrate genomic data is by converting the genetic sequences of microbes into information on metabolic pathways (e.g. cellulose degradation, lignin degradation, nitrogen reduction, and fermentation) using genome-scale metabolic models (GEMs) [119]. GEMs take into account the microbe's environment, such as substrate availability, and predict the transformation of metabolites within a cell based on its genomic information. This process allows for the calculation of CUE at the population level by analyzing substrate use and CO_2 production [119]. For community-level CUE, GEMs can be combined into microbial community models that simulate interactions between different microbial taxa: The ‘computation of microbial ecosystems in time and space metabolic modeling platform’ (COMETS) extends GEMs to include dynamics of microbial growth and interactions, providing a tool for predicting CUE_C under various environmental conditions [116].

An alternative modeling approach at the community level is based on traits (e.g., quantity of cellulase produced, maximum rate of reaction (V_{max}) of cellulose decay by cellulase, V_{max} of cellulose-monomer uptake, and turnover rate), such as the DEMENT model, which uses data on microbial traits to simulate substrate use and CO_2 production [120]. This model can predict both CUE_P and CUE_C under different environmental conditions and over time. However, translating genomic data into traits remains challenging [121]. Genomic datasets typically indicate the presence or absence of certain genes or pathways, but additional information, such as that from GEMs or experimental data, is necessary to accurately map these genes to functional traits in the models.

Validating genomic and trait-based models is crucial and can be achieved using community-level genomic datasets, which offer insights into microbial strategies that affect CUE, such as nutrient recycling and stress tolerance [118,122]. Combining these models with traditional CUE measurements and omics data allows for the creation of detailed maps of community-level CUE, offering new insights into C cycling dynamics and providing input information for C cycle models.

A major challenge in this field is the high computational demand of integrating omic data into complex models. One solution is the development of computational emulators that can simulate the dynamics of microbial models more efficiently, bridging the gap between detailed, small-scale models and broader applications in C cycle studies [123]. This approach promises to improve our understanding of microbial contributions to C cycling, leveraging the power of genomic data to inform and validate complex ESMs.

Harmonization of CUE measurements and aligning measured and modeled CUE

Harmonizing soil microbial CUE measurements across different methods, i.e., aligning results from different methodologies, poses a challenge due to the differences across measurement techniques. While adopting a universal protocol for CUE measurement—a single, standardized measurement method— would be ideal, it may not be feasible given the complexities of CUE. Therefore, a more practical approach involves providing a clear and comprehensive description of the methodologies used in different studies. This detailed reporting should include information on the physiological processes considered, such as maintenance, enzyme production, biomass generation, and mortality rates. This level of detail

helps in understanding and comparing results across studies, as well as in selecting appropriate data for model calibration [17].

In contemporary soil C models that explicitly incorporate microbial processes [25,85], the CUE is close to empirically measured CUE_C. To achieve a uniform approach to CUE measurement, microbial models that resolve key processes influencing CUE, such as uptake, respiration, exudation, and microbial death could be used [17]. Such models can generate CUE metrics that align with different measurement methodologies by incorporating a complete or partial set of these processes into their calculations. Furthermore, these models can be adapted to conduct numerical experiments with specific substrates or to incorporate isotopic tracers (e.g., ^{13}C, ^{14}C, ^{18}O) to simulate outcomes from labeling experiments. This adaptability allows for the exploration of hypotheses regarding discrepancies in measurements under diverse conditions by modifying model boundary conditions. Additionally, microbial models serve as foundational tools for integrating microbial metabolism into broader global C models, potentially enhanced by machine learning emulators for improved scalability and applicability.

Constraining CUE using model-data fusion

Data assimilation encompasses a collection of techniques, including Bayesian inference, that refine biogeochemical models by integrating observational data. This process not only updates model parameters to reflect the most likely values based on available data but also quantifies their uncertainties, thus bridging the gap between empirical observations and theoretical models [109]. This approach is particularly valuable for parameters like microbial CUE, which are challenging to measure directly in the field due to technical limitations. An innovative application of data assimilation is demonstrated by Tao et al. [21], who developed the PROcess-guided deep learning and DAta-driven (PRODA) approach [124]. This method integrates global-scale SOC data with a microbially explicit model to produce a global map of microbial CUE. PRODA employs traditional Bayesian data assimilation to estimate parameters at specific sites and then uses deep learning to extrapolate these site-specific parameter estimates to a global scale. The result is a set of parameters that optimally align with observed data, offering a detailed view of microbial CUE and SOC storage patterns worldwide, along with other soil C cycle dynamics such as decomposition rates, environmental impacts on soil respiration, and vertical C transport [21].

Despite the potential of approaches like PRODA to harness large datasets for enhancing our understanding of the soil C cycle, their computational intensity—stemming from the extensive data sampling required by Bayesian inference—may limit their application in models with complex structures. The next wave of data assimilation techniques will likely integrate process-based models with deep learning algorithms more seamlessly [122]. Such advancements could offer quicker parameter optimization and facilitate comparisons across different models, paving the way for more accurate and comprehensive assessments of microbial CUE and C cycle dynamics on a global scale.

Long-term SOC records and ecosystem manipulation experiments

Ecosystem manipulation experiments and observations of natural gradients offer invaluable insights into how microbial communities and CUE adapt to global change factors. Especially insightful are field experiments (or studies leveraging natural gradients) that alter environmental factors such as soil temperature, precipitation patterns, or nutrient levels [79,126] over long durations. These experiments provide critical data on the enduring effects of global change drivers on CUE, while simultaneously highlighting the limitations of current models and enhancing our comprehension of ecological processes. Integrating the results from these experiments with model simulations, supported by proven site modeling protocols and extra observational data, is crucial for steadily enhancing the accuracy and complexity of models [127].

Incorporating radiocarbon (^{14}C) data and long-term SOC records into models is also vital for refining CUE forecasts across longer (decadal to centennial) time scales. This temporal information is essential for capturing the dynamics of CUE over time, thereby improving the precision of models in depicting spatial and temporal fluctuations [128].

Diagnosing CUE from existing models or simulation archives

In global C modeling, approaches to quantify the environmental impact on organic matter decomposition and stabilization differ significantly. An effective method for estimating microbial CUE at the ecosystem level as emerging from model simulations involves the calculation of the ratio between soil heterotrophic respiration (R) and gross decomposition (D) within these models. Gross decomposition refers to the sum of all C fluxes transferred between the modeled soil C pools that are mediated by microbial processes, excluding physically mediated transfers (e.g., sorption, aggregation, or leaching). This includes all C removed from

587 organic matter pools, whether it is lost as CO_2 or transferred to another pool (SI-Text 1). This
588 ratio effectively quantifies microbial-mediated C losses from SOC pools, integrating both growth
589 (anabolic processes) and respiration (catabolic processes). Under steady-state conditions, it is
590 assumed that heterotrophic respiration aligns with microbial C uptake, resulting in the formula:
591 CUE = 1 - R/D. The steady-state assumption implies that microbial communities and SOC stock
592 are stable in time (i.e., in equilibrium with boundary conditions). This is an approximation of real
593 systems where SOC varies due to anthropogenic and natural changes (e.g., Holocene climatic
594 variations). This diagnosed CUE, emerging as a property inherent to the model, is not
595 susceptible to the equifinality issues that can affect the underlying intrinsic model parameters
596 (like CUE_C), and it does not necessitate the incorporation of explicitly microbial models, offering
597 a simplified yet insightful metric. These model-based CUE estimates, derived from long-term
598 flux averages (e.g., 20 years), represent stable C stocks. In contrast, measurement-based
599 estimates, taken over shorter periods, are more susceptible to significant CUE variations due to
600 asynchronous fluctuations in components such as respiration and degradation, potentially
601 introducing estimation inaccuracies. This timescale discrepancy likely accounts for the greater
602 variability observed in measurement-based CUE compared to model-based CUE. We propose
603 this "model-diagnosed CUE" as a novel metric, designed to estimate microbial CUE from model
604 outputs without direct measurements of microbial uptake.

605 Analyzing diagnosed CUE and its relationship with SOC across various models, such as
606 those evaluated in the Trends in the land carbon cycle (TRENDY) model intercomparison
607 project [2], facilitates the identification of differences attributable to unique model structures and
608 assumptions. For example, warming-induced CO_2 emissions should be higher in models with
609 low diagnosed CUE compared to high CUE as the warming-induced stimulation of microbial
610 activity will result in relatively more C being respired than cycled within the soil systems. This
611 approach further allows the benchmarking and subsequent refinement of diagnosed CUE
612 estimates using observed CUE_E data.

613 For instance, we derived CUE estimates from simulations conducted with two different
614 versions of the Organising Carbon and Hydrology In Dynamic Ecosystems (ORCHIDEE) land
615 surface model [129], which differ in the SOC model deployed. The CENTURY SOC model (Fig.
616 S1a), which is widely used but does not resolve microbial processes, uses first-order decay,
617 while the MIMICS model (Fig. S1b) resolves microbial physiology, providing a more mechanistic
618 understanding of microbial processes. The resulting global CUE maps (the average of
619 simulation results over 20 consecutive years) revealed significant spatial variability (Fig. 6a & b).

While the two maps showed a good correlation (Fig. 6c), the CUE values diagnosed from the MIMICS model were higher than those from the CENTURY model (Fig. 6d). These findings underscore the importance of incorporating observational data into model calibration efforts to enhance the accuracy and reliability of SOC predictions by realistically resolving CUE.

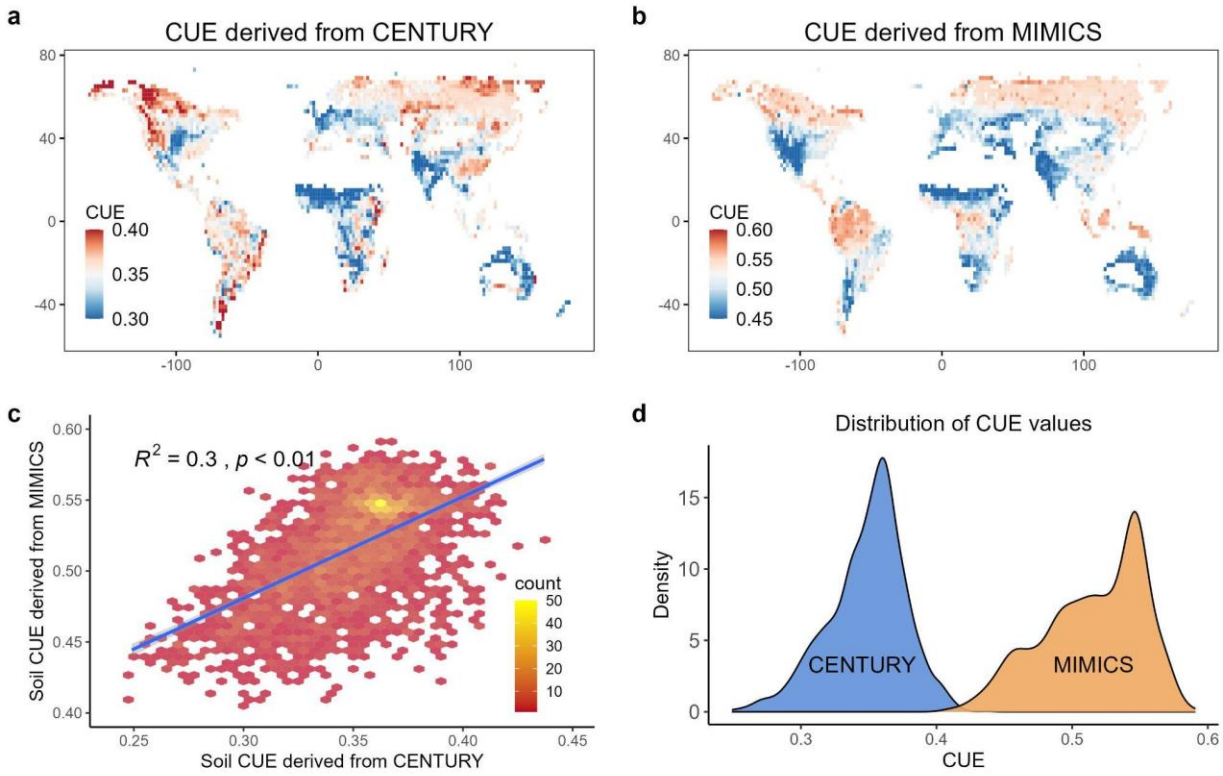


Figure 6. Diagnosed CUE from two existing soil C models. CUE diagnosed from a nutrient-enabled version of the the Organising Carbon and Hydrology In Dynamic Ecosystems land surface model (ORCHIDEE-CNP) deploying a soil module based on (a) the CENTURY model [129], or (b) the MIMICS model with constant intrinsic CUE_C [130]. (c) Correlation between diagnosed CUE values from the CENTURY-based model and the MIMICS-based model. (d) Distribution frequency of CUE for the two scenarios.

In conclusion, the inherent structure of a model significantly shapes its outcomes, making the integration of empirical data with data-constrained models a fundamental step toward realistic predictions [131,132]. Precisely delineating the spatial and temporal dynamics of CUE in models that specifically address microbial activities is crucial for the reliability of their predictions of SOC status and dynamics. Moreover, future soil C models must navigate the

intricate balance between the complex regulatory mechanisms of CUE, other processes governing SOC formation and stabilization, and the practicality of model use to promote more precise projections of CUE responses under diverse environmental scenarios. This Perspective underscores the importance of combining different data sources with sophisticated modeling techniques to refine global CUE predictions. By incorporating genomic data, standardizing measurement protocols, applying data assimilation practices and critically evaluating CUE within existing frameworks, our comprehension of the global dynamics of microbial CUE can be markedly improved. This Perspective provides a roadmap for establishing an effective modeling approach to accurately represent global soil microbial CUE and its interactions with other biological and abiotic processes that regulate SOC dynamics.

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975 The authors declare no competing interests.

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977 **Author contributions**

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