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Challenges in Integrating DOM Chemodiversity into Kinetic Models of Soil Respiration for Improved Carbon Cycling Predictions

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1. Abstract:

Chemodiversity of dissolved organic matter (DOM) has been proposed as an ecosystem property controlling the microbial metabolism; thus, the fate of carbon (C) in soils. Recent research suggests that accounting for DOM chemodiversity can improve the accuracy of process-based C cycling models; however, this approach has never been validated at continental U.S. scale. In this study, we used statistical and kinetic modeling approaches to evaluate how DOM chemodiversity affects soil respiration and whether incorporating it in kinetic models improves respiration prediction. We utilized paired high resolution FTICR-MS descriptions of DOM chemistry and soil respiration rate measurements from 63 topsoils across the USA, provided by the Molecular Observation Network. Regression analysis revealed that DOM alpha diversity (defined as the number of detected organic compounds) interacted nonlinearly with dissolved organic C (DOC) and water-extractable total nitrogen (WETN) concentrations. Soils with high DOC and WETN concentrations, showed decreased soil respiration with increasing alpha diversity, while soils with low DOC and WETN concentrations showed increased respiration. Therefore, DOM chemodiversity controlled the plausible tradeoff in microbial metabolism leading to either loss of C through respiration or SOM buildup through increased microbial growth. This finding implies that chemodiversity, as a parameter, has the potential to increase the accuracy of soil C cycling models. To evaluate respiration rate dependence on chemodiversity as a parameter, we tested three kinetic models: (1) as a function of DOC concentration only, (2) with model parameters informed by average DOM chemodiversity and (3) by chemodiversity calculated within chemical classes of DOM. All three models predicted respiration with similar accuracy. This inability suggests that current kinetic formulations do not adequately represent chemodiversity–microbial metabolism interactions. Therefore, we advise future studies to explore the effects of DOM chemodiversity, with consideration of its interactions with tradeoffs in microbial traits and environmental conditions.

Keywords: FTICR-MS, bioenergetic, soil organic matter, soil respiration, chemodiversity, carbon cycle modeling

2. Introduction

Dissolved organic matter (DOM) plays a significant role in terrestrial systems, fueling microbial metabolism, has been a key source of uncertainty in Earth Systems Models. Accurate characterization and representation of DOM in Earth System Models is essential for quantifying carbon fluxes and assessing the impacts of climate and environmental disturbances on soils (Tanentzap and Fonvielle, 2024). Most soil C cycling models aiming to predict greenhouse gas fluxes simplify SOM pools into a few chemically and/or physically distinct pools, and then may further constrain DOM dynamics using steady-

state assumption due to its putative high bioavailability. Indeed, the DOM pool has faster turnover time compared to more refractory C fractions in soil (Wutzler et al., 2017), and its chemodiversity may directly relate to the balance of soil organic matter decomposition vs. persistence (Ding et al., 2020; Kothawala et al., 2021). Yet, effectively integrating DOM chemistry in soil C cycle models to improve predictions of C stocks and fluxes beyond DOM pool size remains unresolved (Graham and Hofmockel, 2022).

Traditional soil carbon cycle models incorporate the chemical complexity of soil organic matter through discrete pools representing varying degrees of SOM physicochemical recalcitrance, characterized using linear kinetic parameters (Parton et al., 1994). More recent models have improved upon this abstraction by conceptually dividing SOM into plant-derived, mineral-associated, dissolved, and microbial carbon pools (Abramoff et al., 2018; Robertson et al., 2019; Wang et al., 2013; Wieder et al., 2014). Despite numerous studies highlighting the chemodiversity of organic compounds found in particulate organic matter (Witzgall et al., 2021), mineral-associated organic matter (Anderson et al., 2023; Lv et al., 2020) and DOM (Ayala-Ortiz et al., 2023; Bahureksa et al., 2021), most models still consider these pools to be chemically homogeneous and define their decomposition rates using fixed kinetic parameters. The fixed parameters approach may be problematic for DOM pool metabolism because the choice of a parameter may not represent the chemodiversity found in DOM. Furthermore, models based on Monod kinetics typically use bulk chemistry (i.e., DOC concentration) to define the rate of microbial utilization (Camino-Serrano et al., 2018; Yu et al., 2020). Such simplification overlooks the microbially induced transformations of organic matter, and the intricate interactions between microbial uptake, release (Amenabar et al., 2017; Marschmann et al., 2024), and sorption of DOM on mineral reactive surfaces (Keiluweit et al., 2015; Sokol et al., 2019), all of which ultimately dictate chemical diversity and microbial metabolism of DOM.

New measurement capabilities have also advanced our empirical understanding of DOM–high resolution mass spectrometry methods can now detect tens of thousands of organic compounds–coincident with emerging modeling approaches to account for this extraordinary chemical and thermodynamic diversity (Ayala-Ortiz et al., 2023; Bahureksa et al., 2021). Recent advancements have shown the potential of integrating the chemodiversity of DOM into C cycling models using bioenergetic to predict its uptake and microbial growth rates (Chakrawal et al., 2022; Desmond-Le Quéméner and Bouchez, 2014; LaRowe and Van Cappellen, 2011; Song et al., 2020). These approaches only account for the average thermodynamic properties of DOM despite the fact that different chemical classes of DOM have varying bioavailability and thermodynamic properties (Ahamed et al., 2023; Song et al., 2020). Despite a strong theoretical basis, these model formulations have not been widely tested with empirical datasets. Overall, the integration of high resolution FTICR-MS data into models is still new in the field of modeling soil biogeochemical processes, with considerable uncertainty regarding how effectively these new data-model integration approaches can capture the complexity of DOM chemodiversity and its impacts on ecosystem processes.

Here, we adopt a two-pronged approach to (1) assess the nature of relationships between the chemodiversity of DOM and soil respiration across the continental United States and (2) determine if recent advances in modeling DOM chemodiversity improve continental-scale predictions of soil

respiration. Our overarching goal is to assess the ability of current-generation, DOM-chemistry-explicit soil C models to improve predictions of soil respiration, and to identify potential interactions between DOM chemodiversity and edaphic factors that may benefit from explicit representation. First, we use regression analysis to evaluate various metrics of DOM chemodiversity (e.g., abundances and functional diversity) to predict soil respiration in the context of common soil biogeochemical variables. We then evaluate the predictive ability of three types of kinetics-based model simulations: Monod kinetics, a single homogeneous DOM pool model, and a multi pool model. Monod kinetics formulates respiration as a function of DOC concentration only and remains the most common method in soil carbon models. The single homogeneous DOM pool model and the multi pool model are based on metabolic transition state theory, which uses parameters that link soil respiration rates directly to the thermodynamic properties of DOM (Desmond-Le Quéméner and Bouchez, 2014). In the homogeneous DOM pool model, we define a single DOM pool by the average thermodynamic properties of the DOM. The multi pool model incorporates a more detailed representation, with thermodynamic properties defined for individual chemical classes of DOM. Our main hypothesis is that DOM chemodiversity is a crucial driver of soil respiration; therefore, incorporating chemical diversity into kinetic parameters will improve model predictions of soil respiration relative to models based on soil C concentration alone.

3. Methods and Materials

3.1. Data

We used standardized data collected from topsoil (0-10 cm) in 63 cores across the continental U.S. (CONUS) (Figure 1A). A comprehensive set of data on soil respiration rates, water-extractable OM concentration and chemistry, and over twenty additional biogeochemical parameters were collected by the 1000 Soil Pilot program of the Molecular Observation Network (MONet), described by Bowman et al. (2023) and Shi et al. (2024). Soil respiration rate was measured using the CO_2 burst method, and dissolved SOM chemistry was assayed with Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS). Please refer to Bowman et al. (2023) for detailed methodologies regarding data collection.

As previously described by Shi et al. (2024), FTICR-MS detected over 7,000 unique dissolved SOM molecules, which were then assigned chemical formulas using CoreMS. We then used Van Krevelen analysis to group molecules into nine broad molecular classes: Amino Sugar-like, Carbohydrate-like, Condensed Hydrocarbon-like, Lignin-like, Lipid-like, Protein-like, Tannin-like, Unsaturated Hydrocarbon-like, and Other. Van Krevelen analysis assigns an organic compound (OC) to a class if its H/C and O/C ratios fall within the specified upper and lower limits for that class (Ayala-Ortiz et al., 2023; Bahureksa et al., 2021; Bailey et al., 2017). Chemical classes defined only represent a class-like category because they rely on elemental composition and do not capture structural complexity (e.g., lignin-like or carbohydrate-like). Still, FTICR-MS provides unmatched resolution in DOM composition, enabling the detection of the full range of DOM compounds using any available analytical techniques.

3.2. Statistical analysis of soil respiration

We used linear regression models to predict soil respiration as a function of biogeochemical variables and DOM chemodiversity related variables calculated from FTICR-MS data (Table A1). To represent the

chemical nature of DOM, we used the mean nominal oxidation state of C, mean double bond equivalent, and mean molecular weight of DOM (see supplementary information for more details). Further, to quantify the chemodiversity of DOM, we used alpha diversity, indicating the number of detected organic compounds and the Shannon diversity index. In the initial model, we included soil moisture content, clay content (%), DOC and water-extractable total N concentrations, total C (%), pH, thermodynamic favorability factor (λ see eq A2), alpha diversity, and Shannon diversity index as predictors of soil respiration rates. Potential respiration was log-transformed, and all predictors were centered and scaled. We selected the best-fit linear regression following a sequential model selection approach, beginning with the most complex model (including all interaction terms) and simplifying the model based on the Akaike information criterion and log-likelihood test. We note that removing insignificant predictors can increase the Akaike information criterion; however, if the resulting model had a marginal reduction in the coefficient of determination, then we selected the simpler model regardless of the increase in the Akaike information criterion. More details on model construction are provided in the supplementary information.

3.3. Microbial growth reaction and growth kinetics

To account for the chemical diversity of DOM and its concentration while estimating soil respiration rate, we used the bioenergetic approach that allows integrating thermodynamic properties of different organic compounds as parameters in kinetic models. We used the bioenergetic framework as described in Chakrawal et al. (2022) and Song et al. (2020) to estimate the stoichiometric coefficients of the metabolic growth reaction of microorganisms under aerobic conditions and predicted microbial respiration rates from DOM chemistry (Graham et al., 2023; Zheng et al., 2024). Under aerobic conditions, the carbon balance in the metabolic growth reaction for building 1 C mole of microbial biomass ($\mathrm{CH_{1.8}N_{0.2}O_{0.5}}$) from a general organic compound ($\mathrm{C_aH_bN_cO_dP_eS_f^z}$) can be written as follows,

$$y_{OC}\ \mathrm{C_aH_bN_cO_dP_eS_f^z} \rightarrow \mathrm{CH_{1.8}N_{0.2}O_{0.5}} + (ay_{OC} - 1)\mathrm{CO_2}, \quad (1)$$

where y_{OC} and $y_{CO_2} = (ay_{OC} - 1)$ are the stoichiometric coefficients of organic compound and CO_2, respectively, calculated using the Gibbs energy balance of catabolic, anabolic, and metabolic reactions. The stoichiometric coefficient of the metabolic reaction is estimated by doing a Gibbs energy balance of catabolic (ΔG_{cat}) and anabolic parts (ΔG_{an}) of metabolic reactions (see Song et al. (2020) for details). A higher value of y_{OC} indicates a thermodynamically less favorable substrate because more substrate is utilized to produce 1C mol of biomass requiring more energy to be generated from catabolism. The carbon use efficiency of microbial growth (CUE) can be calculated as $\mathrm{CUE} = \frac{1}{a \times y_{OC}}$.

We used the metabolic transition state theory (MTS) (Desmond-Le Quéméner and Bouchez, 2014) to calculate the respiration rate as a function of stochiometric coefficients (y_{OC} and y_{CO_2}) and DOC concentration (S_{DOC}) (Table 1). For a single pool of DOM, we used average values of y_{OC} and y_{CO_2}, and the total concentration of DOC from each soil sample for estimating soil respiration rate, R_{MTS}^{mean}. In the case of multi pool model, we calculated soil respiration (R_{MTS}^{multi}) as the sum of respiration from all chemical classes of DOM considering the variation in their relative abundance and variations in model parameters y_{OC}, y_{CO_2}, and μ_{max} values for each chemical class (Table 1). Relative abundance was

calculated as the number of peaks within each chemical class as a percentage of total peaks. We also considered Monod kinetics with a single pool DOM as a reference model where model parameters were directly fitted using observed respiration (Table 1). Additional details on bioenergetic framework and kinetics are provided in the supplementary information.

In the two models based on MTS kinetics (single and multi pool), there are four model parameters: the stoichiometric coefficients y_{CO_2} and y_{OC} maximum growth rate μ_{max}, and a volume harvest parameter V_h representing accessible volume by microorganisms to acquire chemical energy from the surroundings. For each chemical class, we calculated stoichiometric coefficients directly from the growth reaction described above (eq. 1). For μ_{max}, first, we calculated the maximum substrate uptake rate (q_{max}) as a function of number of electrons (N_e) transferred from organic C to electron acceptor during catabolism following González-Cabaleiro et al. (2015). Equations for q_{max} and μ_{max} are as follows,

$$q_{max} = \frac{3}{N_e} \tag{2}$$

$$\mu_{max} = CUE\ q_{max} \tag{3}$$

Using eqs 2 and 3, in the single pool, the average μ_{max} is calculated for each soil sample, while in the multi pool model μ_{max} varies among chemical classes as well as for soil samples. We used the same approach for stoichiometric coefficients y_{CO_2} and y_{OC} in the single and multi pool models. Parameters V_h and normalization factor *N* in MTS, and the maximum respiration rate (V_{max}) and half saturation (K_m) in Monod kinetics were estimated as best fitted parameters by fitting the model to observed rates of soil respiration. Parameter values, model performance indices, the coefficient of determination (R^2), and root mean squared error (rmse) are provided in Table 1.

Table 1 Kinetic rate expression for soil respiration as a function of stochiometric coefficients of DOM (y_{OC}) and CO_2 (y_{CO_2}), and DOC concentration (S_{DOC}) for three kinetic formulations. Estimated model parameters and model performance indices (coefficient of determination (R^2) and root mean squared error (rmse) is also included.

Model	Rate expression	Maximum rate constant (V_{max} in day^{-1}) or Normalization constant (N, in mg C g^{-1} soil)	Half saturation constant (K_M in mg C g^{-1} soil) or volume harvest parameter (V_h in (mg C g^{-1} soil)$^{-1}$)	R^2 [-]	rmse [mg C-CO_2 g^{-1} soil day^{-1}]
Monod	$R_{Monod} = \frac{V_{max} S_{DOC}}{S_{DOC} + K_m}$	$V_{max} = 1.58$	$K_M = 0.935$	0.55	0.123
MTS* (Single pool)	$R_{MTS}^{mean} = \mathrm{N}\,\bar{\mu}_{max} \overline{y_{CO_2}} \exp\left(-\frac{\overline{y_{OC}}}{V_h S_{DOC}}\right)$	$N = 1.8$	$V_h = 0.57$	0.57	0.120

MTS** (Multi pool)	$R_{MTS}^{multi} = \mathrm{N}\sum_i y_{CO_2,i}\,\mu_{max,i}\exp\left(-\frac{y_{OC_i}}{V_h S_i}\right)$	$N = 0.84$	$V_h = 1.42$	0.56	0.121

* Overline on symbol denotes average quantities.

** S_i is the concentration of a i^{th} chemical class of DOM, and N is the normalization constant used in the data-model fitting to convert simulated respiration units to observed respiration, i.e., mg C-CO_2 g^{-1} soil day^{-1}.

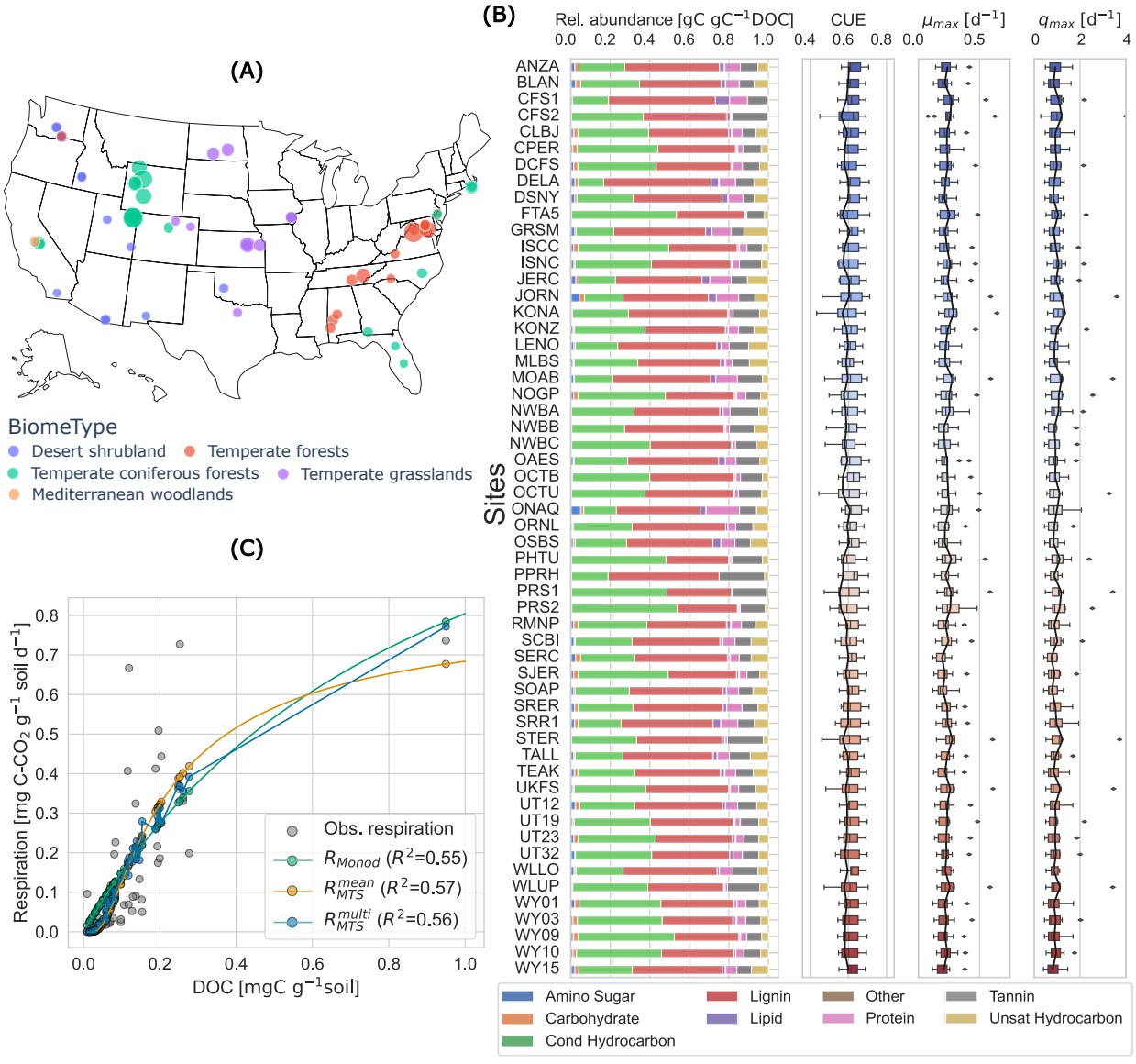

Figure 1 (A) Location of collected soil cores with colors and size of circles illustrating biome type and soil respiration rate, respectively, (B) Bar chart with relative abundance of each class of dissolved organic C

(in gC g^{-1}C DOC) and boxplots of carbon use efficiency (CUE), maximum substrate uptake rate (q_{max}) and maximum growth rate (μ_{max}) across chemical classes. The solid black line denotes the mean value. (C) Soil respiration as a function of DOC concentration. Observed (grey) and modeled soil respiration rates using Monod kinetic R_{Monod} (green) and metabolic transition state kinetics, R_{MTS}^{mean} (single pool, yellow) and R_{MTS}^{multi} (multi pool, blue).

4. Results and Discussion

We start by describing the observed chemodiversity of DOM in soil samples across the continental U.S. (section 4.1). Next, we discuss the results from regression analysis relationships between the chemodiversity of DOM and soil respiration rates (section 4.2) and compare the predicted soil respiration using three kinetics models (section 4.3). We end with a broad discussion on the implications and challenges of incorporating chemodiversity of DOM in biogeochemical models (section 4.4).

4.1. Chemodiversity of DOM across CONUS

Soil respiration rates varied spatially, with temperate forests having relatively higher rates compared to other biomes (Figure 1A). Although soils displayed a high relative abundance of lignin- and condensed hydrocarbon-like SOM compared to other chemical classes, there was substantial variation in the proportion of these chemical classes in various soils (Figure 1). In turn, the stoichiometric coefficients y_{OC} and y_{CO_2} in metabolic reaction (Figure A1) and maximum carbon use efficiency (CUE), substrate uptake rate (q_{max}) and growth rate (μ_{max}) (Figure 1B) also varied across compound classes and across different soils. Further, the alpha diversity of DOM varied by more than one order magnitude (range of 914-26933), reflecting significant chemodiversity differences in soils across CONUS (Figure A1).

4.2. DOM chemodiversity interacts with biogeochemistry to explain rates of soil respiration

Our goal in using the regression analysis is to build a baseline model for predicting respiration rate as a function of DOM, its chemodiversity, and other biogeochemical variables. We use the regression analysis to evaluate the relationship, especially, between respiration rates and DOM chemodiversity, and test whether DOM chemodiversity significantly contributed to improving the prediction of respiration rates. Our regression model included DOC and water-extractable total N concentrations, soil moisture, DOM alpha diversity, and interactions of DOM alpha diversity with these three predictors (R^2=0.73, fivefold cross-validation R^2=0.62$\pm$0.09). Of these predictors, soil moisture had the highest contribution in predicting soil respiration, followed by DOC concentration, DOM alpha diversity, and interaction between DOC concentration and DOM alpha diversity (Table A2). Thus, supporting our hypothesis that the chemical diversity of DOM can improve predictions of soil respiration, DOM and its chemical traits were some of the most important statistical predictors of soil respiration rates across the continental U.S. While soil moisture and DOC concentration are known drivers of soil respiration , chemodiversity is not widely recognized to be an important control over organic matter decomposition rates.

In the following text, we discuss soil respiration as a function of DOC and water-extractable total N concentrations and DOM alpha diversity. Overall, the relationship between respiration and DOM alpha diversity varied with DOC and water-extractable total N concentrations (Figure 2). To explain these patterns, first, we defined low resource conditions as soils with low water-extractable C concentrations

(DOC< avg. DOC) vs. with low water-extractable N concentrations (WETN< avg. WETN). High resource conditions are assigned to soils corresponding to opposite values of C (DOC> avg. DOC) and C:N (WETN>avg. WETN).

Under high resource conditions, DOM alpha diversity was negatively correlated with soil respiration (Figure 2A for DOC>0, and Figure 2B for WETN >0). Higher alpha diversity also corresponded to a greater proportion of putative labile compounds and high carbon use efficiency, thus favoring anabolic pathways and low CO_2 production (Figure A3A-B). We can use the Yield-Acquisition-Stress (YAS) trait-based paradigm of microbial metabolic strategies to explain some of these results. This paradigm defines microbial communities based on their functional traits—communities adapted to maximize resources towards biosynthesis with high CUE referred to as Y-strategists, communities adapted to maximize resources acquisition with uptake rate referred to as A-strategists, and communities adapted to divert resources into acquisition with uptake rate referred to as A-strategists (Malik et al., 2020). Here, the decrease in soil respiration with increasing DOM alpha diversity under high resource conditions may indicate the dominance of a Y-strategist microbial community that metabolizes bioavailable C and N for growth purposes. Increased growth of microbial biomass via increased CUE has been associated with the buildup of SOM through increased necromass production pathway (Tao et al., 2023); thus, chemodiversity of DOM can be a critical driver of soil C persistence.

In contrast, under low resource conditions, DOM alpha diversity was positively correlated to soil respiration (Figure 2A for DOC<0, and Figure 2B for WETN <0). The YAS trait-based paradigm offers a plausible explanation for this relationship. Under resource limitation, microbial communities are likely to invest more in the production of extracellular depolymerization enzymes that increase the bioavailability of C and N (i.e., the A strategy) (Malik et al., 2020). Higher DOM alpha diversity under low resource conditions may therefore trigger expression of a diverse set of enzymes, enabling the microbial community to access a broader array of substrates. Consequently, under low concentrations of DOC conditions with high alpha diversity of DOM, a microbial community adapted for resource acquisition may dominate, and the increase in enzymatic activity in such communities can lead to elevated respiration through catabolic processes that are uncoupled from biosynthetic pathways. Alternatively, high DOM alpha diversity may arise from the depolymerization processes, in which chemically complex DOM is decomposed into different monomers of DOM. This results in a high diversity of molecules as a product of decomposition. In either case, increasing DOM alpha diversity at low DOC concentrations may open new niche spaces for complementary organisms to bolster rates of metabolism (D'Andrilli et al., 2019; Logue et al., 2016; Wang et al., 2022). High enzymatic activities under low resource conditions indicate an increased loss of C from soils, thus suggesting that the chemodiversity of DOM may indirectly control soil C loss via increased respiration.

The regression analysis reveals a significant nonlinear relationship between chemodiversity and soil respiration, even though it does not provide mechanistic insights into how chemodiversity affects soil respiration. This finding suggests that kinetic models predicting respiration rates need to account for the dependency of these rates on chemodiversity. The results further indicate that DOM chemodiversity could be a key factor influencing shifts in microbial metabolism and, consequently, in determining overall

soil respiration rates. These findings provide a basis for generating hypotheses in future studies about how DOM chemodiversity may affect microbial community responses, such as increased investment in enzymes, biosynthesis, or respiration. For instance, testing how varying degrees of DOM chemodiversity by introducing different types of labile organic compounds, while maintaining a constant DOC concentration, influences microbial metabolic tradeoffs. We anticipate that such empirical investigations will be crucial for establishing a mechanistic link between DOM chemodiversity to functional traits in microbial explicit models.

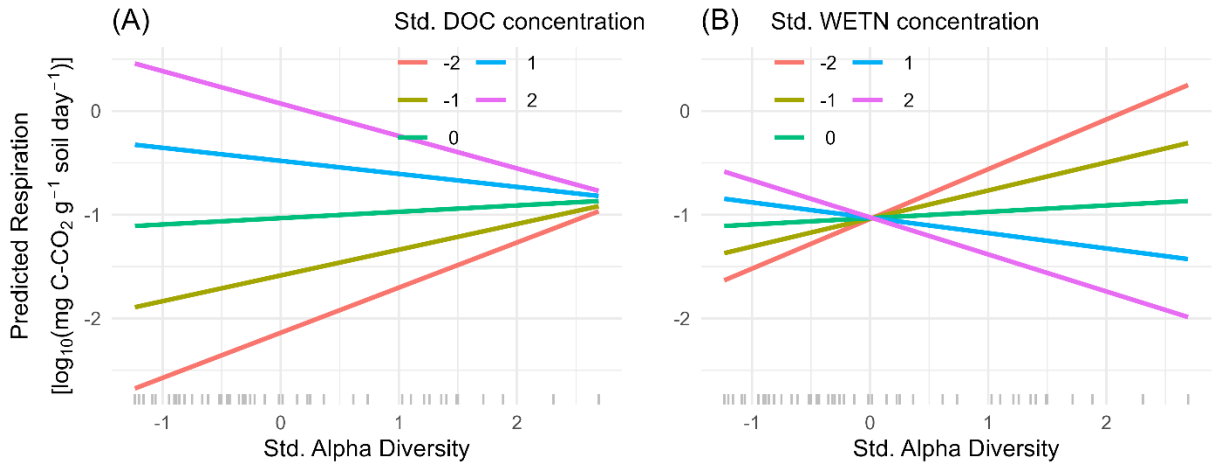

Figure 2 Interaction plot showing the variation of soil respiration with alpha diversity for varying degrees of dissolved organic C, DOC (A), and water-extractable total N (WETN) concentrations (B) from the linear regression model. The respiration rate is in the log10 transformed scale, and DOC concentration, CN ratio, and alpha diversity are on a standardized scale with a mean of zero and a standard deviation of one.

4.3. Comparison of kinetic models

In the previous section, we used empirical evidence to conclude that chemodiversity of DOM exhibits contrasting relationships to soil respiration under resource excess versus depleted conditions. This finding implies that kinetic models informed by chemodiversity should be capable of capturing this rate dependence on chemodiversity. In this section, we address this question using three kinetic models that are representative of current state-of-science modeling approaches to test whether model predictions of respiration rates improve the ability of model parameters to account for the chemodiversity of DOM. Our analysis is the first study to evaluate the calibration of kinetic models using observed soil respiration rates while also estimating model parameters using high resolution DOM chemistry at the CONUS scale. Moreover, the use of FTICR-MS data to estimate kinetics parameters can be used to reduce parameter uncertainty related to microbial processes in Earth System scale models.

The Monod kinetics model, which simulates respiration only as a function of DOC concentration, predicted soil respiration rates with an R^2 of approximately 0.55 (Figure 1C). When thermodynamic properties of DOM were incorporated with single pool (average μ_{max} and stoichiometric coefficients y_{OC}

and y_{CO_2}) or with multi pool (varying μ_{max}, $y_{OC,}$ and y_{CO_2} for discrete chemical classes), MTS kinetics model predictions were similar to the Monod model (R^2 = 0.57 and 0.56, respectively, Figure 1C and Table 1). Overall, all kinetic models predicted rates of soil respiration less accurately than regression analysis, suggesting that emerging kinetic models may still lack key processes such as tradeoffs in microbial metabolic strategies (i.e., YAS traits) that may result in the observed nonlinearity in the relationship between DOM alpha diversity and soil respiration.

Compared to other recent studies, our approach of using FTICR-MS data to predict soil respiration had higher predictive power. Previous studies by Song et al. (2020) and Ahamed et al. (2023) employed a similar bioenergetic approach to leverage DOM chemodiversity in predicting microbial respiration rate from river systems, utilizing a fixed value of volume harvest parameter times substrate concentration, i.e., $V_h S_{DOC}$ (denominator in the exponent of MTS kinetics, see Table 1). This approach does not account for the variability in DOC concentration across soil samples and has resulted in a poor correlation between observed and simulated respiration rates. Building on Song et al. and Ahamed et al., Zheng et al. (2024), incorporated initial DOC concentration into $V_h S_{DOC}$ an MTS kinetic model using observed water extracted organic C concentration while tested different values of V_h to find best correlation between simulated maximum specific growth rate as a proxy for respiration with observed respiration rates. Since the maximum growth rate (unit d^{-1}) and respiration rate (unit μmol CO_2 g^{-1} soil d^{-1}) are not directly comparable with each other, a best fitted parameter estimation of V_h was not performed in their stud, which may be the reason for low correlation and R^2 (Pearson r = 0.56, R^2 = 0.19) between modeled maximum microbial growth rates and measured soil respiration rates.

Conversely, in our analysis, we used directly modeled CO_2 production, not maximum microbial growth rate, and compared modeled predictions to measured CO_2 production. The conservation of units between predicted and observed respiration allows for a more accurate comparison of model performance. Furthermore, in our study, y_{OC} and y_{CO_2} in metabolic reaction (eq 1, and Figure A1), and maximum carbon use efficiency (CUE), substrate uptake rate (q_{max}, eq 2 and Figure 1B) and growth rate (μ_{max}, eq 3 and Figure 1B) varied across compound classes and different soils; thus, improved refinement of DOM pool representation should lead to enhanced model predictions. However, we did not find significant improvement across models.

Results from all three kinetic modeling approaches notably contrast with statistical regressions that suggest that the chemodiversity of DOM is a controlling factor for shifts in microbial metabolism and, thus, overall soil respiration rates. The lack of improvement in model prediction from MTS parameters might be influenced by the domination of lignin- and condensed hydrocarbon-like DOM across all soil samples. Consequently, MTS-predicted respiration rates are based mainly on parameters derived from the same two chemical classes of DOM regardless of soil sample. Furthermore, all three kinetic models deviated significantly from the 1:1 line (Figure A2), likely due to variability in soil properties, climate, microbial communities, and other factors that were not included in the model.

Overall, regression analysis supported our hypothesis that incorporating the chemical diversity of DOM improved predictions of soil respiration; however, results from kinetic models did not. This disparity

indicates that kinetic models for incorporating advanced DOM analytics still fail to capture process-based relationships between DOM chemodiversity and soil respiration. In the next section, we explore the implications of these results on the development and improvement of biogeochemical models.

4.4. Implication and challenges for incorporating chemodiversity of DOM in biogeochemical models

Factors driving the chemodiversity of DOM and its effects on microbial decomposition processes are closely intertwined. Microbial activities contribute to the chemodiversity of DOM, while, in turn, the chemodiversity of DOM influences microbial metabolism (Davenport et al., 2023; Lehmann and Kleber, 2015). The niche separation of microorganisms by variation in substrates, mineralogy, moisture, and other environmental factors, along with the DOM chemodiversity resulting from microbial decomposition (D'Andrilli et al., 2019; Logue et al., 2016; Wang et al., 2022), plays a crucial role in soil respiration (Kothawala et al., 2021). This constitutes a major challenge for soil C models, specifically in identifying the level of detail required to represent DOM chemodiversity while maintaining model simplicity.

The representation of DOM chemodiversity in process-based models is usually based on a spectrum of kinetic parameters used to represent slow or fast decomposition of organic matter or slow or fast growing microbes (Camino-Serrano et al., 2018; Khurana et al., 2023; Wieder et al., 2015; Yu et al., 2020). These approaches use kinetics parameters as a proxy for either chemical recalcitrance or shift in microbial community and do not estimate model parameters as a function of the chemical properties of organic matter. Newer studies are increasingly using the nominal oxidation state of organic matter to characterize the decomposition and persistence of organic matter (Boye et al., 2017; Garayburu-Caruso et al., 2020; Lin et al., 2021; Naughton et al., 2021). Recent efforts to include chemodiversity in soil C cycle models have diverging conclusions on its control of the decomposition of DOM. For example, in their theoretical model, Weverka (2023) found that microbes may invest in a diverse set of enzymes to assimilate heterogenous DOM pools, or ignore some substrates in favor of others that are more favorable. Both strategies result in reduced overall C assimilation rates, leading to lower DOM decomposition and microbial respiration. While Khurana et al. (2023) found a positive correlation between DOM chemodiversity and its decomposition rate when the chemodiversity was represented using the number of organic compounds, but not when the chemodiversity was represented using variation in the nominal oxidation state of C in organic compounds. These two examples highlight the need for improved representation of chemodiversity of DOM in models to capture the nonlinear effects of chemodiversity with soil respiration.

Another challenge in incorporating the effect of chemodiversity of organic matter in models is the tradeoff on microbial traits with varying DOM chemodiversity. For instance, our results hint towards potential tradeoffs in microbial metabolism at the community level from maximizing yield under resource excess conditions vs. maximizing enzyme production under resource depleted conditions. A possible solution for incorporating tradeoffs in models might be using dynamic optimization that estimates optimal changes in functional traits based on the chemical composition of organic matter (Chakrawal et al., 2024) or using the dynamic energy budget model that is able to resolve the tradeoff between microbial growth rate and carbon use efficiency. These challenges highlight the need for further

investigation of novel approaches for including DOM chemodiversity in soil C cycling decomposition models and developing models that are compatible with integrating the chemical diversity of soil organic matter.

New studies have suggested the use of a more refined representation of DOM pools in mechanistic models (Muller et al., 2024). However, incorporating detailed information on DOM chemistry into soil C cycling models also prompts the question of whether integrating more complex representations enhances model performance, especially when considering multifaceted interactions of DOM with factors like soil mineralogy and microbiology (Graham and Hofmockel, 2022). In a recent work by Muller et al. (2024), authors implemented multi pool representation of DOM chemodiversity in a reactive transport model (Lambda-PFLOTRAN) that can be coupled with other biological and hydrological processes at the watershed scale. Such coupling between C cycling and watershed scale models that are able to integrate interactions with other biogeochemical processes is a promising avenue for improving the next generation of soil C cycling models, particularly if they are also extended to represent variation in microbial functional traits explicitly. We note that while explicit DOM chemistry in reactive transport or other ecosystem-scale models is appealing, these larger-scale frameworks often introduce additional parameters, which can lead to issues like equifinality and parameter unidentifiability (Marschmann et al., 2019). To address this challenge, we need widespread datasets with high molecular resolution measurements. Initiatives such as the Molecular Observation Network (MONet) are facilitating high-throughput molecular-scale data collection, which could mitigate this challenge. Future model development efforts capable of effectively integrating these comprehensive datasets hold promise for advancing our understanding of molecular scale processes and determining the requisite level of detail needed in large-scale Earth System Models.

5. Conclusions:

In this study, we examined whether incorporating dissolved organic matter (DOM) chemodiversity into predictive models of soil respiration enhances model performance. Our findings indicate a statistically significant relationship between DOM alpha diversity as a measure of chemodiversity, and measured soil respiration rates. However, when chemodiversity informed models were parameterized to reflect DOM chemodiversity, their performance was comparable to the reference model (Monod kinetics). These contrasting results suggest that the impact of DOM chemistry on soil respiration is not adequately captured by emerging kinetic modeling approaches. Accurately capturing the nonlinear effect of chemical diversity under varying resource conditions is essential for predicting soil C persistence, as our results showed that chemodiversity controlled the plausible tradeoffs in microbial metabolism leading to either loss of C through respiration or SOC buildup through increased growth.

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7. Data availability

The data used in this analysis is available from the 1000 Soils Pilot Dataset repository https://zenodo.org/records/7706774 with additional information on https://www.emsl.pnnl.gov/news/1000-soils-digs-data-belowground-ecosystems.

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Supplementary information

Challenges in Integrating DOM Chemodiversity into Kinetic Models of Soil Respiration for Improved Carbon Cycling Predictions

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1. Microbial growth reaction and growth kinetics

Under aerobic conditions, the carbon balance of a metabolic growth reaction for building 1 C mole of microbial biomass from a general bioavailable dissolved organic compound (DOC, $\mathrm{C_aH_bN_cO_dP_eS_f^z}$) can be written as follows,

$$y_{OC}\ \mathrm{C_aH_bN_cO_dP_eS_f^z} \rightarrow \mathrm{CH_{1.8}N_{0.2}O_{0.5}} + y_{CO_2}\mathrm{CO_2} + \Delta G_{met} \quad \text{(A1)}$$

where $\mathrm{C_aH_bN_cO_dP_eS_f^z}$ represents a generic OC, $\mathrm{CH_{1.8}N_{0.2}O_{0.5}}$ is a chemical representation of microbial biomass, and y_{OC} and y_{CO_2} are the stoichiometric coefficients of DOC and CO_2. The stoichiometric coefficient of the metabolic reaction is estimated by doing a change in Gibbs energy balance of catabolic (ΔG_{cat}) and anabolic parts (ΔG_{an}) of metabolic reactions. The ratio of the sum of Gibbs energy dissipated and conserved in biomass to Gibbs generated from catabolism is defined as the thermodynamic favorability factor λ as follows,

$$\lambda_i = \frac{\Delta G_{diss\,i} + \Delta G_{an_i}}{-\Delta G_{cat\,i}}. \quad \text{(A2)}$$

The subscript i represent the class of organic carbon. For details in calculating the change in Gibbs energy values for catabolic and anabolic reaction reader are referred to (Kleerebezem and Van Loosdrecht, 2010; LaRowe and Van Cappellen, 2011; Song et al., 2020; Chakrawal et al., 2022). The change in Gibbs energy was also corrected for observed pH in soil pore water (Amend and LaRowe, 2019; Song et al., 2020).

A higher value of λ_i denotes less favorable substrate because catabolism needs to run a greater number of times to produce 1C mol of biomass, requiring more energy to be generated from catabolism. The stoichiometric coefficients of DOC and CO_2 can be calculated as a function of λ as follows,

$$y_{OC_i} = \lambda_i + y_{OC_i}^{an}, \quad \text{(A3)}$$

$$y_{\mathrm{CO_2}\,i} = y_{OC_i}a\ - 1, \quad \text{(A4)}$$

where $y_{OC_i}^{an}$ is the stoichiometric coefficient of DOC in the anabolic reaction, and a is the number of C atoms in the chemical formula of DOC. Note that the change in Gibbs energy values was corrected for measured soil pH (Kleerebezem and Van Loosdrecht, 2010).

Next, we used the metabolic transition state theory (Desmond-Le Quéméner and Bouchez, 2014) to calculate the respiration rate ($R_{CO_{2_i}}$),

$$R_{CO_{2}i} = \mathrm{N}\, y_{CO_{2_i}} \mu_i^{max} \exp\left(-\frac{y_{OC_i}}{V_h S_{DOC,i}}\right), \quad \text{(A5)}$$

where μ_i^{max} is the maximum growth rate (d^{-1}) and $S_{DOC,i}$ is the concentration of DOC (mg C g^{-1} soil) of each class of organic compound, and V_h is a kinetic parameter representing accessible volume by microorganisms to acquire chemical energy from the surroundings ((mg C g^{-1} soil)$^{-1}$) and N is a normalization factor (mg C g^{-1} soil). The concentration of each class of compound was calculated as their relative abundance multiplied by the total concentration of DOC for the given soil sample.

For a single pool DOM kinetic model, DOM is considered to be chemically homogeneous, and kinetic parameters are estimated using the average thermodynamic properties of the DOM. The respiration for a single pool model can be written as follows,

$$R_{MTS}^{mean} = \mathrm{N}\, \bar{\mu}_{max} \overline{y_{CO_2}} \exp\left(-\frac{\overline{y_{OC}}}{V_h S_{DOC}}\right) \quad \text{(A6)}$$

The overline symbols represent average statistics across nine classes of OCs. The respiration for a multi-pool model is calculated as the sum of respiration from all classes considering the variation in their relative abundance and thermodynamic favorability, and can be written as follows,

$$R_{MTS}^{multi} = \mathrm{N} \sum_i y_{CO_2,i} \mu_{max,i} \exp\left(-\frac{y_{OC_i}}{V_h S_i}\right) \quad \text{(A7)}$$

Modelled respiration in both MTS based models have the units of mg C g^{-1} soil day^{-1}. As a reference model that does not include aspects of the chemodiversity of DOM, we used Monod kinetics to predict respiration as a function of DOC concentration. The respiration rate using Monod kinetics can be written as,

$$R_{Monod} = \frac{V_{max} S_{DOC}}{S_{DOC} + K_m} \quad \text{(A8)}$$

where V_{max} and K_M are maximum respiration rate (mg C g^{-1} soil day^{-1}) and half saturation constant (mg C g^{-1} soil), respectively.

2. Statistical analyses

We used linear mixed effect models for predicting soil respiration as a function of other biogeochemical variables, and DOM chemodiversity related variables were calculated from FTICR-MS data. To represent the chemical nature of DOM, we used the mean thermodynamic favorability factor, lambda (eq A2, (Song et al., 2020)), mean double bond equivalent (DBE), and mean molecular weight of DOC (calculation provided in following section). Further, to represent chemodiversity of DOM, we used alpha

diversity indicating the number of detected organic compounds, coefficient of variation in lambda, and Shannon diversity index. For robust model selection, first, we removed predictors with insignificant correlation with respiration using p-value>0.05 threshold in Pearson correlation coefficient, and then, we removed multicollinear predictors with correlation coefficients of more than 0.7.

For a preliminary model, we included soil moisture content, clay content (%), DOC and water-extractable total N (WETN) concentrations, total C (%), pH, thermodynamic favorability factor (λ see eq A2), alpha diversity and Shannon diversity index as predictors. To enhance interpretability, respiration was log10-transformed, and all predictors were centered and scaled. In the linear mixed effect model, biome type was used as a random effect on intercept; however, later, it was dropped due to the high Akaike information criterion compared to the linear model as a base model. We selected the best-fit linear regression following a sequential model selection approach, beginning with the most complex model (including all interaction terms) and simplifying the model based on the Akaike information criterion and log-likelihood test. We note that removing insignificant predictors can increase AIC; however, if the resulting model had a marginal reduction in coefficient of determination, then we selected the simpler model regardless of the increase in AIC.

Regression analysis was performed using R statistical software (R Core Team, 2023), version 4.3.2. The analysis utilized the nlme package (Pinheiro et al., 2023) for mixed-effects models. For estimating individual contribution of predictors in predicting respiration rate, we used lmg function from relaimpo package (Groemping, 2007) that decompose overall R-squared for fit to R-squared explained by individual predictors while accounting for the correlation structure among predictors and predictors ordering in the regression formula. Lastly, for summarizing regression results in tabular format, the modelsummary package (Arel-Bundock, 2022) was employed.

2.1. Nominal oxidation state of carbon and double bond equivalent

The nominal oxidation state of carbon (NOSC) in an organic compound ($\mathrm{C_aH_bN_cO_dP_eS_f^z}$) is defined as following,

$$NOSC = 4 - \frac{4a + b - 3c - 2d + 5e - 2f - n_z}{a} \quad \text{(A9)}$$

The NOSC ranges from -4 for most reduced state of C in CH_4 and +4 for most oxidized state of C in CO_2 (LaRowe and Van Cappellen, 2011).

The double bond equivalent (*DBE*) is used to estimate the degree of unsaturation (i.e., the presence of double, triple bond or rings in chemical structure) in an organic compound (Koch and Dittmar, 2006) and calculated as follows,

$$DBE = 1 + 0.5\,(2a - b + c) \quad \text{(A10)}$$

2.2. Shannon diversity index

Shannon diversity index (Sh) provides a measure of entropy of the population, here, the population is chemically diverse DOM, and calculated as,

$$Sh = \sum_{i=1}^{n} p_i \ln p_i \quad \text{(A11)}$$

where *n=9* is the total number of chemical classes, and p_i is the relative abundance of each chemical class.

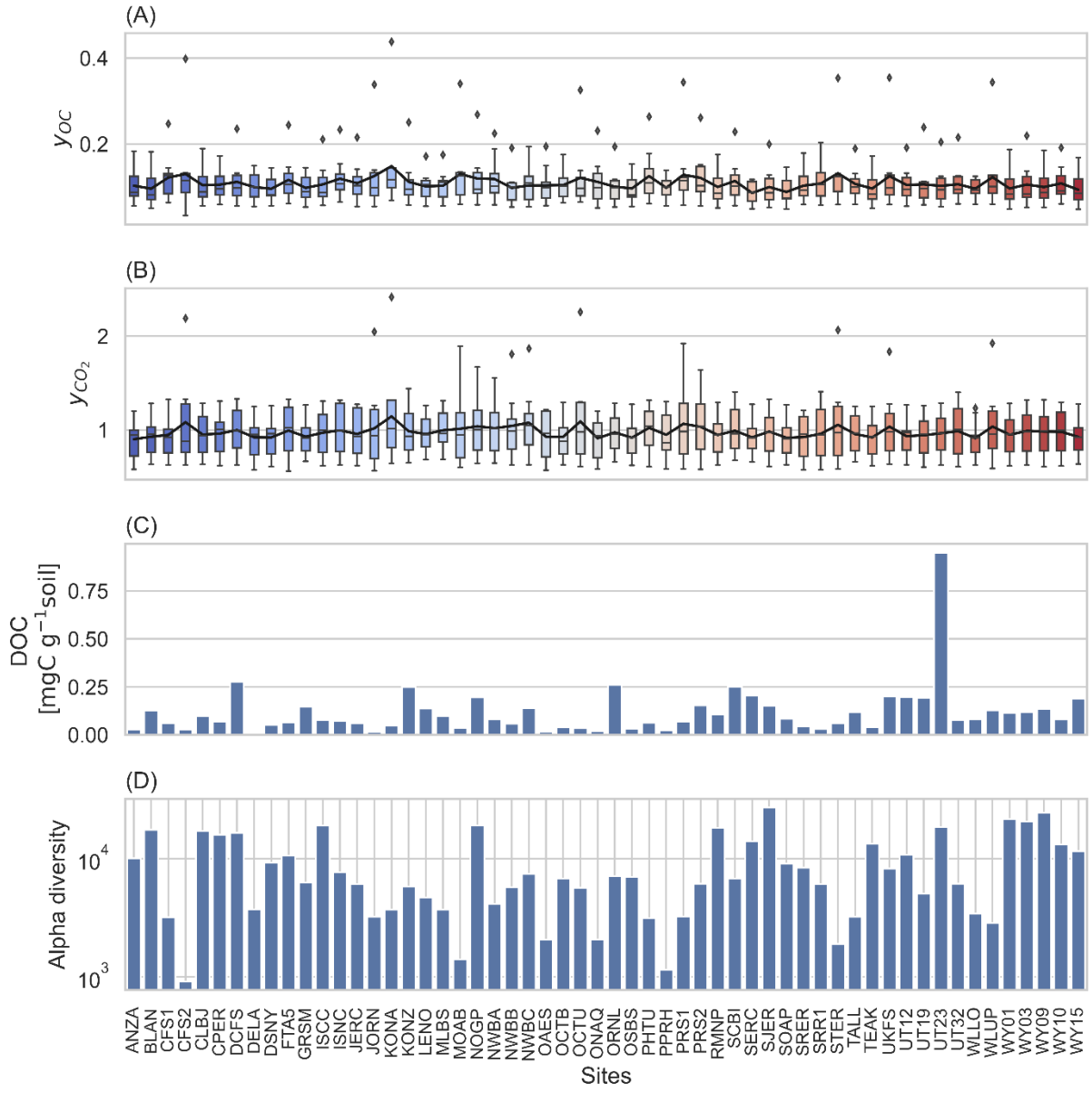
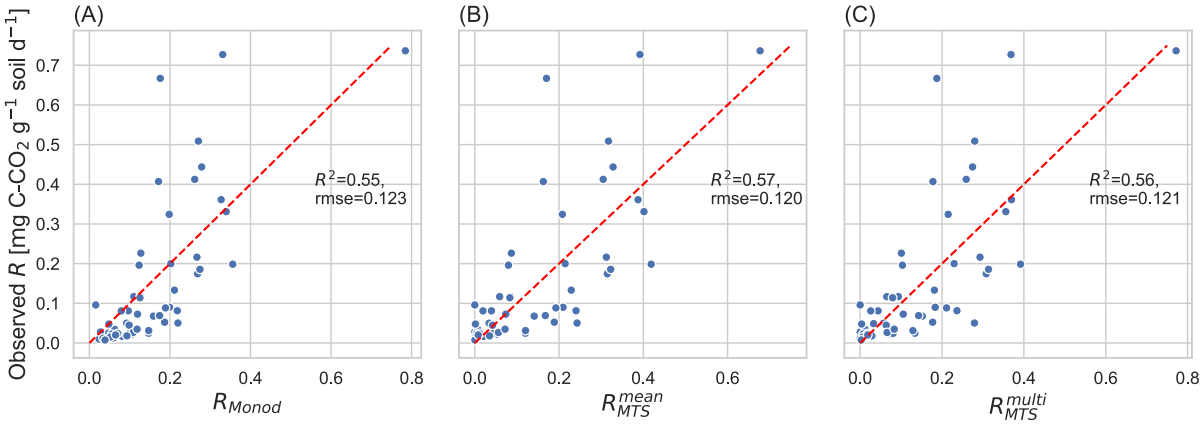


Figure A1 Stoichiometry of organic compound y_{OC} (A) and CO_2 y_{CO_2} (B) in the metabolic growth reaction, DOC concentration (C), and alpha diversity, i.e., species richness of DOM for each soil sample (D). The box plots of y_{OC} and y_{CO_2} show the variation across different chemical classes, and the solid black line is the mean value.



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668 Figure A2 Scatterplot of observed and simulated respiration rates using Monod kinetics (A), using
669 metabolic transition state kinetics with single pool DOM (B), and multiple pool DOM (C). The annotation
670 text, R^2, and rmse are the coefficient of determination and root mean square error, respectively.

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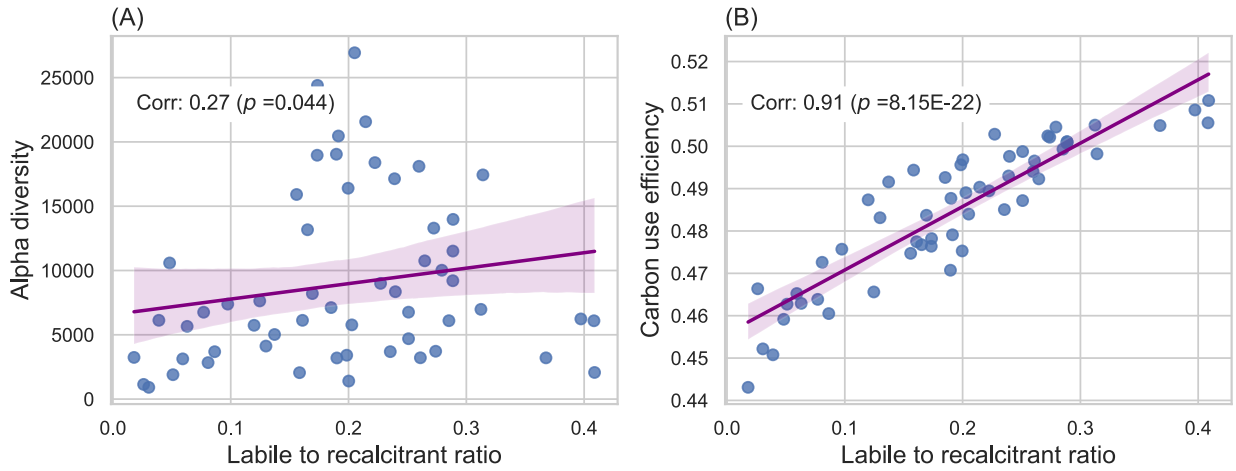

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674 Figure A3 Alpha diversity of DOM (A) and carbon use efficiency (B) as a function of the ratio of labile to
675 recalcitrant organic compounds. Carbohydrates, proteins, amino sugars, lipids, unsaturated
676 hydrocarbons, and other compounds were taken as labile pools, whereas lignins, condensed
677 hydrocarbons, and tannins were considered as recalcitrant compounds. The annotated text, *Corr* and *p,*
678 denote the Spearman correlation and corresponding p-value, respectively.

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Table A1 Coefficient estimates and model fit statistics from linear regression for predicting respiration across different models during model selection process

	Alternate Model	*Final model*
(Intercept)	−0.98 (0.05)***	−1.03 (0.05)***
DOC	**0.39 (0.08)*** **	**0.55 (0.10)*** **
Total C (%)	0.24 (0.07)***	
WETN		0.00 (0.07)
alpha diversity	0.04 (0.05)	0.06 (0.05)
DOC × Total C (%)		
DOC × alpha diversity	**−0.20 (0.06)****	**−0.19 (0.09)***
WETN × pH		
WETN × alpha diversity		**−0.21 (0.10)***
soil moisture	0.07 (0.06)	**0.13 (0.05)***
Total C (%) × soil moisture	−0.11 (0.05)*	
soil moisture × alpha diversity		**0.13 (0.05)***
Num.Obs.	52	*52*
R2	0.750	0.733
AIC	26.7	32.3
DOC: dissolved organic C concentration, **SD**: standard deviation, **Num.Obs**.: number of observations, R^2 coefficient of determination in linear regression. Values within parentheses represent the standard error of the estimate. Significance levels: *** p < 0.001, ** p < 0.01, * p < 0.05, . p < 0.1, + p > 0.1		

Table A2 R-square decomposition among predictors of final model in Table A1

Predictor	R^2
soil moisture	0.20
DOC	0.18
alpha diversity	0.11
DOC × alpha diversity	0.09
WETN × alpha diversity	0.07
WETN	0.06

soil moisture × alpha diversity	0.02
sum of R^2	0.73

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