**Fail safe:** 1) Maybe we can use some aspect of (Wutzler et al., 2017)or (Sistla et al., 2014), (Weverka et al., 2023) optimal resource allocation to enzyme model with variable CNB to have some sort of optimization then simply sue molecular mixing approach to identify litter pools and do dynamic simulation. Use the output to look at dynamic DR.

2) kind of a cohort one pool simple CN dynamic model for whole litter with chemical diversity based quality index, and some way of doing dynamic DR.

Paragraph 1: Why study litter decomposition, Controls of litter decomposition, especially how litter chemical traits (diversity) affect its decomposition and microbial activity

* Improved understanding of role of N management in terrestrial ecosystems. In many ecosystems, above and below ground species compete for nutrients which is linked to carbon storage capacity of such systems. For example, the C sequestration in forests and grasslands under eCO2 conditions are related to their belowground nutrient conditions. Often N in OM is explicitly modeled as a separate pool but N does not exist in elemental form but as organic compound containing N. We represent N directly in the protein pool without needing to track N separately.
* Most studies on litter decomposition are not comprehensive that they do investigate the decay rate of litter component other than cellulose, lignin, proteins. For instance, lipids are often neglected. Our study gives a broad range of lipid decay rates. (Warren and Butler, 2023)
* Maybe link to biofuels?

Paragraph 2: Bring the energetic constraints: From Gunina and Kuzyakov 2021, that as microbes decompose litter, nosc of litter decreases. Here we show how!! With MMM model pool description of litter, we can vary microbial growth efficiency under C limited condition based on its degree of reduction. This is feedback from bioenergetics. Otherwise, CUE under C limited condition is always considered constant.  
more recent models addressing chemodiversity issue (examples from Waverka, Khurana), FTICR data example from soil (energetic return on investment decreases with depth) lipids also protect? (Spaccini et al., 2002)

**Paragraph 3:** Models and data used to parametrize of litter decomposition

Talk about how litter decomposition models have evolved to use microbial metabolism; microbial succession models (Moorhead), optimal enzyme allocation model (Wutzler, Chakrawal), cohort models (C-stability, Manzoni 2012, Göran Ågren 2021)

* Traditional models: Old Century, Yasso, Moorhead models, recent MIMICS, SEAM, MEMS, Dement, more recent models addressing chemodiversity issue (examples from Waverka, Khurana), FTICR data example from soil (energetic return on investment decreases with depth)
* OMDY with NMR data
* CUE decreases with lignin content because of increased oxidative enzyme demand, but what if we can predict decomposition patterns in sugars, proteins, lignin without this feedback?
* Add more on C, N mass loss from litter bag incubation (examples from Sierra, Moorehead, Manzoni), proximate analysis (Moorehead, MEMS)?

## Model limitations and Sources of Uncertainty

Despite strong model-data agreement, estimated parameters varied across several orders of magnitude, reflecting underlying equifinality and uncertainty in observational constraints. A major source of this uncertainty lies in the limited availability and inconsistency of input data. Because ¹³C NMR measurements are resource-intensive, datasets often lacked synchronized reporting of total C, total N, and molecular composition, necessitating selective inclusion of available variables during model calibration. Additionally, chemical shift assignments used to estimate the five compound classes were not standardized across studies, requiring harmonization of spectral boundaries that likely introduced additional uncertainty into the derived chemical fractions. These data limitations reduce the ability to uniquely constrain kinetic parameters and may lead to compensatory effects among them, even in chemically explicit models. Although the incorporation of a lignin-based rate modifier improved mechanistic representation of decay inhibition, the model structure does not account for dynamic environmental drivers such as temperature and moisture, and estimated rate constants reflect average rather than time-varying conditions.

These findings underscore the need for harmonized, high-resolution litter chemistry datasets with consistent temporal coverage, alongside structural model improvements, to better constrain decomposition dynamics and reduce uncertainty in model predictions.

4o

* Data limitation and uncertainty in assigning spectra to OC
  + We are modeling the state of art accuracy for chemical composition of litter, but it comes with its own limitations, bring the point about uncertainty in assigning spectra to OC-> probably in limitations
* Not accounting for temperature and soil moisture variability in estimated rate constants
* Include limitation from lignin modifier story

## Paradigm shift in modeling litter decomposition, where to go from here?

What can you do with this model?

* Plant chemical diversity is linked with soil C stabilization.
* Application of the new model to understand the energetic status of C entering soil?
  + Model can simulate dynamic changes in DR of overall litter which can be used to study the
  + Microbial necroass as precursor to SOM. Use your model output to estimate the **relative contribution of each chemical class (e.g., protein, lignin, lipid)** to microbial necromass or dissolved organic matter (DOM), which are precursors to SOM formation. This helps demonstrate how chemical resolution links to **long-term C persistence pathways**. How would different litter type under different N adaptation condition change necromass chemistry?

High resolution mass spectroscopy methods such as the 13C NMR allow us to accurately describe the molecular scale chemical diversity of plant litter and thus develop process-based models capable of incorporating more detailed process descriptors of the role of litter chemical composition on its turnover rates.

novel modeling framework that incorporates and takes advantage of these data to understand the mechanisms of inhibition of carbohydrate and protein decay due to lignin protection in litter decomposition by incorporating a lignin rate modifier. We further analyzed how such a rate modifier would affect simulations of microbial-driven processes related to litter turnover and compared model performance with and without using lignin modifiers.

Such models lack the resolution necessary to capture intra-molecular interactions (5) like lignin protection, which can create a bottleneck during litter decomposition (3,4). Our model framework bridges this gap as it can readily include NMR spectral data converted into five traditional litter pools using molecular mixing models (5). Furthermore, it allows us to explore the effects of chemodiversity by more accurately describing the litter fractions in each type of litter material. The use of highly resolved litter descriptions has not only been suggested from modeling but also empirical studies looking at the effects of distinct lignin chemistries on litter turnover rates (9).

However, high resolution in litter data does not fully resolve the persistent issue of equifinality in biogeochemical models (6). This becomes evident by the remaining uncertainty in kinetic parameter fits, which vary across many orders of magnitude and are even comparable to parameter fits using more traditional models. The many reasons why different models can fit empirical observations with comparable performance metrics can be summarized as either the data not providing sufficient information to constrain model parameters or because of compensation among parameter combinations (6). By adding high-resolution litter data, it is possible that equifinality issues in our results are mainly driven by compensatory effects of the kinetic parameters.

There four nobs in the model.

1. Protection on and off: Protection of Ch and P decreases their uptake.
2. Oxidative enzyme on and off: Investment in oxidative enzymes decreases CUE, thus G, thus T-> less microbial turnover in substrate pool.
3. Oxidative enzyme time invariant vs variant. If variant (vO) then having more lignin increases its uptake
4. N limitation strategy N retention vs flexible CUE: N retention tends to keep more N in the by recycling it, flexible CUE increases waster of C through overflow respiration when there N limitation

Figure S2:

* useful figure to show how range of variations in NMR fractions across compounds. Gives a perspective there is mostly carbs, lipids and lignins in litter. (it does not add too much to story so I am open to push it to SI, if needed)

Figure 2: I would like this figure to be presented as model exploration figure to describe model capabilities. See figure S3 as well.

* We focus mainly on N Retention strategy and CUE regulation is used for model performance comparison
* model exploration figure. How does C [gC] in different pool changes with different scenarios under N retention strat.
* In NPNE, no protection and no incurred cost to oxidative enzyme, allowed microbes to uptake C containing compound at a maximum rate leading to prolonged period of N limitation (>0 for a year). This also implied longer period of N retention in necromass increasing protein pool size.
* In NPWE, no protection, but incurring cost to oxidative enzyme, reduced CUE to more than half of max CUE, which lead to low growth and necromass recycling, thus overall loss of protein pool. Interestingly, lower growth rate reflected lower N demand as the N limitation period was smallest in this case.
* In PWOE, protection of Ch and P relatively reduced their availability so there is lower decay of Ch.
* IN PWOV, time variant vO is lower than its max value in PWOE, which reduced lignin decay.

Figure 3:

* We obtained comparable model predictions of the five litter fractions, despite the high variability in the litter pools estimated using the molecular mixing model and NMR data.
* However, model fits are heavily influenced by data availability or by the uncertainty in localizing certain NMR spectra to the litter fractions. Thus, fractions like protein and carbonyl do perform the worst regardless of the model scenario used.

Figure 4:

* Model performance figure: comparing NSE/RMSE . NSE = 1: Perfect match between model and observations. 0 < NSE < 1: Model is better than the mean but not perfect. NSE = 0: Model predictions are as accurate as using the mean of the observed values. NSE < 0: Model is worse than simply using the mean of the observations.
* Model does a poor job in predicting protein dynamics. Figure had large negative values of NSE so I had to cut the y axis to -2 to make it visually appealing. That’s why I included % NSE<0 to be transparent.
* Large number of litter datasets shows NSE >0, RMSE close to zero-> robust model. But then there are datasets where model is performing very poorly- > might be associated with data issue rather than model robustness.
* Model performed similarly under both N limitation strategies -> even this detailed chemistry is not sufficient to understand which N strat is active under which condition -> link to Manzoni 2021 frontier.