



1 **The Implications of Microbial Functional Diversity on the**
2 **Response of Soil Organic Matter Decomposition to Nitrogen**
3 **Fertilization: A Theoretical Model**

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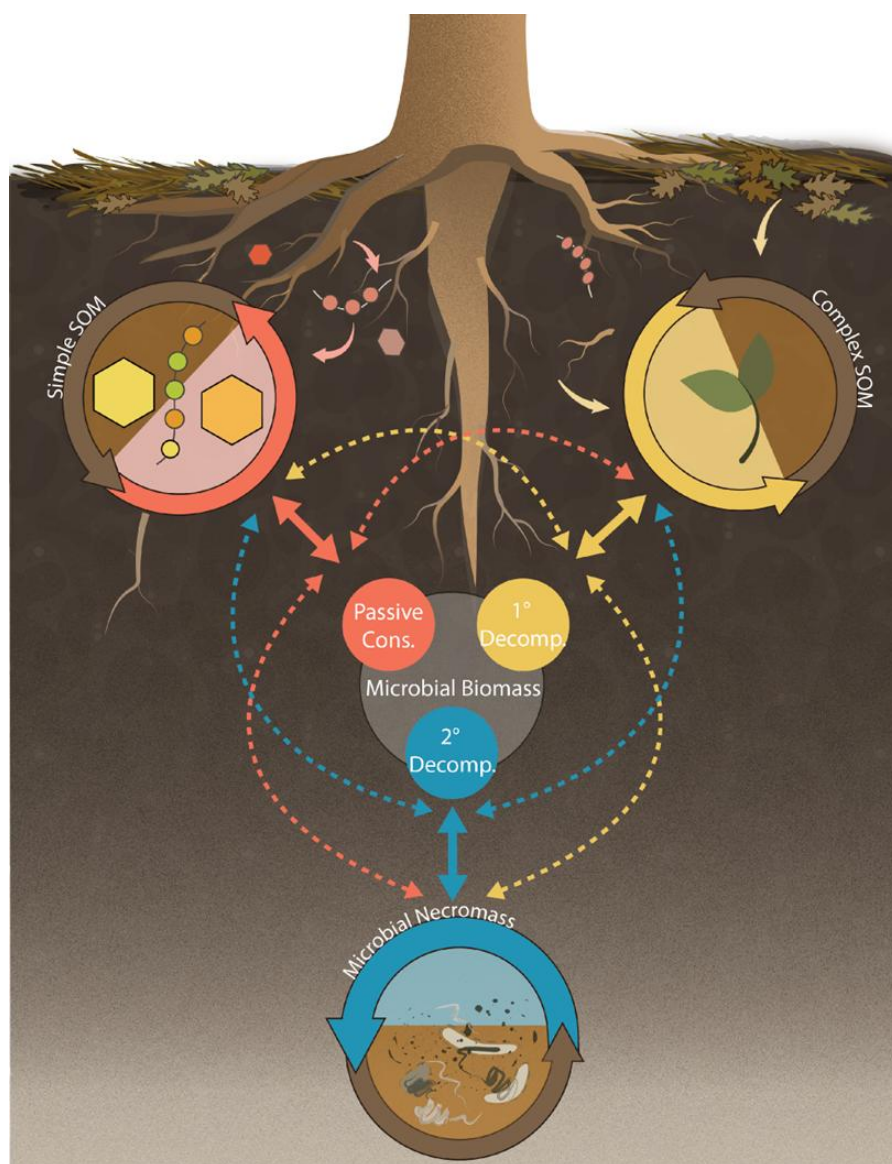
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32 **ABSTRACT.** Nitrogen (N) fertilization decreases carbon (C) decomposition, but there remain competing
33 hypotheses underlying this response including reduced belowground C allocation, shifts in microbial diversity, and
34 altered microbial function. Further, current soil organic matter (SOM) decomposition models lack the ability to test
35 these hypothesized mechanisms. To theoretically test these mechanisms, we integrated the Carbon Acquisition
36 Ecological Strategies (CAES) framework into the Carbon, Organisms, Rhizosphere, and Protection in the Soil
37 Environment (CORPSE) model. CAES represents a pathway for integrating functional diversity into microbial
38 explicit soil decomposition models, with three microbial functional groups that specialize in decomposing and
39 utilizing a different SOM pool: primary decomposers (1° decomposers) target complex SOM, secondary
40 decomposers (2° decomposers) target microbial necromass, and passive consumers target simple SOM. We then
41 used the refined model (Functional Group-CORPSE) to theoretically test hypotheses by leveraging long term data
42 from a 30 year, watershed scale N fertilization experiment at the Fernow Experimental Forest in Parsons, WV. We
43 performed modeling experiments to evaluate the microbial community response to varying C and N inputs, and to
44 simulate hypothesized mechanisms of microbial response to N deposition. Varying substrate composition led to
45 distinct microbial communities, reflecting substrate preferences between functional groups. However, only when
46 both the function and abundance of complex C decomposers was reduced could FG-CORPSE capture the SOM
47 dynamics observed at the Fernow. Collectively, our results show that FG-CORPSE enables the simulation of both
48 functional and compositional shifts in microbial communities to facilitate the testing of microbe centric hypotheses
49 for decomposition and C storage under N enrichment.



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52 *Figure 1: Conceptual diagram of the C pools (circles) and fluxes (arrows) represented in the functional group*
 53 *CORPSE (FG-CORPSE) model. Soil C pools include the living microbial biomass pool and three soil organic*
 54 *matter (SOM) pools classified as Complex (complex plant detritus), Simple (dissolved organic matter), and*
 55 *Necromass (microbial necromass) that are either protected (brown) or unprotected (yellow, red, or blue). Microbial*
 56 *biomass is comprised of three functional groups based on carbon acquisition ecological strategies (CAES). Each*
 57 *microbial functional group specializes in degrading and utilizing C from one of the three SOM pools; primary*
 58 *decomposers (1° decomposers) target complex SOM, secondary decomposers (2° decomposers) target necromass,*
 59 *and passive consumers target simple SOM. The size and activity of the microbial biomass controls decomposition of*
 60 *available SOM, with protection of SOM determining availability.*



61 1 Introduction

62 Nitrogen (N) fertilization reduces soil organic matter decomposition (Liu et al., 2017), yet a cohesive mechanistic
 63 understanding linking reduced microbial-mediated decomposition to increased soil carbon (C) storage remains
 64 lacking. Empirical studies suggest multiple, potentially independent mechanisms underlying this response, including
 65 direct inhibition of specific microbial groups that decompose complex plant detritus (Fog, 1988 and DeForest et al.,
 66 2004), declines in root-driven priming (Carrara et al., 2017), and reductions in microbial biomass (Treseder, 2008).
 67 This mechanistic uncertainty is reflected in soil C models, particularly first-order decomposition models that fail to
 68 capture observed declines in decomposition under N enrichment. Instead, these models often simulate increases in
 69 soil C primarily through enhanced plant inputs, rather than reduced microbial decomposition (Eastman et al., 2024).
 70 As a result, they do not adequately represent the microbial processes that are hypothesized to drive soil C
 71 accumulation under N fertilization. Microbial-explicit soil C models offer a potential path forward but remain
 72 limited by simplified representations of microbial taxonomic and functional diversity. In particular, current
 73 frameworks often lack sufficient resolution to capture shifts in microbial community composition and function in
 74 response to N enrichment. Addressing this limitation will require the development of a theoretical microbial
 75 framework that can better represent the complexity of soil microbial communities within process-based soil C
 76 models. Given that theoretical modeling frameworks have advanced our conceptual understanding of soil
 77 decomposition responses to C vs. N limitation (Schimel and Weintraub 2003) and warming (Allison et al. 2010
 78 Nature Geoscience), there is an opportunity to use a similar approach to examine how microbial functional diversity
 79 controls N fertilization responses.

80 Current microbially explicit soil C models have moved the field forward by incorporating microbial physiology
 81 functional diversity into their model structure (e.g., Weider et al., 2014). However, many of these models still lack
 82 the capabilities to mechanistically test the leading hypotheses thought to govern decomposition declines with N
 83 fertilization. For example, MIMICS (Microbial-Mineral Carbon Stabilization) represents two microbial functional
 84 groups, r and K strategists (McArthur and Wilson 2001), that have unique physiologies: R strategists that have high
 85 growth and turnover rates that allow them to exploit pulses in resource availability vs. k strategists that have slow
 86 growth and turnover that invest heavily in enzymes to drive decomposition. As a result, MIMICS can capture shifts
 87 in microbial physiology but is unable to link physiological changes to the decomposition of different soil organic
 88 matter fractions (i.e., particulate organic matter vs. mineral associated organic matter, (Eastman et al., 2022) or shifts
 89 in root C investment belowground (Carrara et al., 2017) that are key to resolving N fertilization responses. In
 90 addition, the CORPSE model (Carbon Organisms Rhizosphere Protection in the Soil Environment) has moved the
 91 field forward by representing SOM protection process, rhizosphere priming, and empirically measurable C pools in
 92 its model structure. Similar to MIMICS, CORPSE also cannot capture N fertilization responses because it only has
 93 one microbial functional group which precludes its ability to capture shifts in microbial function and traits. While
 94 these models have advanced our predictive understanding of how microbial physiology impacts soil decomposition,
 95 there remains a clear need for a new theoretical model structure where substrate specific microbial functional groups
 96 are linked to measurable soil C pools in order to test the competing hypotheses controlling soil decomposition
 97 responses to N fertilization.

98 Integrating substrate specific microbial functional groups into SOM decomposition models would enable the
 99 theoretical testing of hypothesized mechanisms of microbial community response to N deposition that drive SOM
 100 accumulation. The Carbon Acquisition Ecological Strategies (CAES) framework, recently proposed by Morrissey et
 101 al. (2023), suggests four ecological strategies for microorganisms based on their preferential use of chemically
 102 distinct SOM substrates; primary decomposers (1° decomposers) primarily utilize C from complex plant derived
 103 SOM, secondary decomposers (2° decomposers) utilize microbial necromass, passive consumers assimilate
 104 dissolved organic C (DOC), and microbial predators assimilate live microbial biomass. Microbial C acquisition
 105 strategies are influenced by many covarying traits, but substrate assimilation can represent the aggregate product of
 106 these traits. By linking microbial functional groups directly to distinct SOM pools, the CAES framework provides a
 107 tractable means of incorporating microbial functional diversity into process-based models to test hypotheses on the
 108 response of soil C cycling to N fertilization. N enrichment has been shown to reduce decomposition of complex
 109 plant-derived C, a process associated with primary decomposers. Primary decomposers depend on plant litter inputs
 110 and are sensitive to changes in root allocation and turnover, both of which are altered under N deposition. Nitrogen
 111 fertilization may also reduce root-driven priming of SOM decomposition, which can be represented by passive
 112 consumers that rely on labile C inputs. Declines in simple C additions from root exudates may reduce the energetic
 113 capacity of microbial communities to decompose more chemically complex C substrates (Bengston et al., 2012).
 114 Additionally, reductions in microbial biomass under N fertilization influence the production and turnover of



115 microbial necromass, a process captured by secondary decomposers. Because microbial necromass is a major
116 contributor to stable SOM formation, alterations in necromass dynamics may mechanistically explain persistent soil
117 C accumulation following N deposition. By explicitly representing these functionally distinct microbial groups, the
118 CAES framework enables mechanistic testing of how shifts in microbial community composition and function
119 contribute to soil C accumulation under N deposition.

120 Data from the long-term N fertilization experiment in the Fernow Experimental Forest in WV provides a theoretical
121 testbed for evaluating hypotheses of microbial community response to N deposition. The Fernow N fertilization
122 experiment comprises two whole watersheds and ran from 1989-2019 (Adams et al., 2012; Eastman et al., 2021),
123 providing an opportunity to validate ecosystem level model predictions over decadal scales. Increases in SOM,
124 especially POM, and reductions in belowground C allocation, soil respiration, and microbial biomass were observed
125 at the Fernow (Eastman et al., 2021; 2022; Carrera et al., 2018). These results led to the development of several
126 hypotheses for microbial response to N deposition. Under N fertilization, reduced belowground C allocation could
127 decrease SOM decomposition by negatively impacting 1° decomposer abundance, efficiency, extracellular enzyme
128 production, or a combination thereof (Fig. 2). Perhaps the simplest hypothesis is that 1° decomposer abundance
129 declines as belowground inputs of complex plant derived C decrease (Freedman et al., 2016; Morrison et al., 2016;
130 Carrara et al., 2018; Zak et al., 2019) (H1). Alternatively, if labile C inputs (root exudates) decrease the energy
131 decomposers expend on enzyme production and substrate acquisition (Manzoni et al., 2012; Frey et al., 2014; Gill &
132 Finzi 2016), reduced root exudation could decrease the efficiency of complex SOM decomposition by 1°
133 decomposers (H2). Lastly, given the often-positive relationship between substrate availability and extracellular
134 enzyme production (Freedman et al., 2016; Carrara et al., 2018), 1° decomposers could respond to reduced
135 belowground C allocation by decreasing extracellular enzyme production (H3). While these hypotheses are difficult
136 to test in the field, integrating the CAES framework into a process-based decomposition model enables the
137 evaluation of these hypotheses *in silico*.

138 Our overarching objective was to use a theoretical modeling approach to determine if integrating the CAES
139 framework into an existing SOM decomposition model would enable it to capture the response of soil C cycling to
140 N fertilization observed at the Fernow. We developed FG-CORPSE, a version of the CORPSE model that was
141 modified by integrating microbial functional groups derived from the CAES framework. We then performed a series
142 of simulation experiments to evaluate the potential for FG-CORPSE to depict microbial diversity and substrate
143 specialization. First, we modeled decomposition of SOM inputs with varying composition to demonstrate the
144 substrate specific degradation and assimilation traits that distinguish the functional groups. Next, we used the model
145 to test the hypothesized mechanisms of SOM accumulation under N fertilization, using empirical measurements of
146 SOM in soils from a long-term, whole-watershed N fertilization experiment to validate soil C predictions. Finally,
147 we used the model to project soil C storage during 30 years of recovery after enhanced N fertilization ceased to
148 determine if excess soil C accumulated under enhanced N deposition is vulnerable to rapid decomposition.

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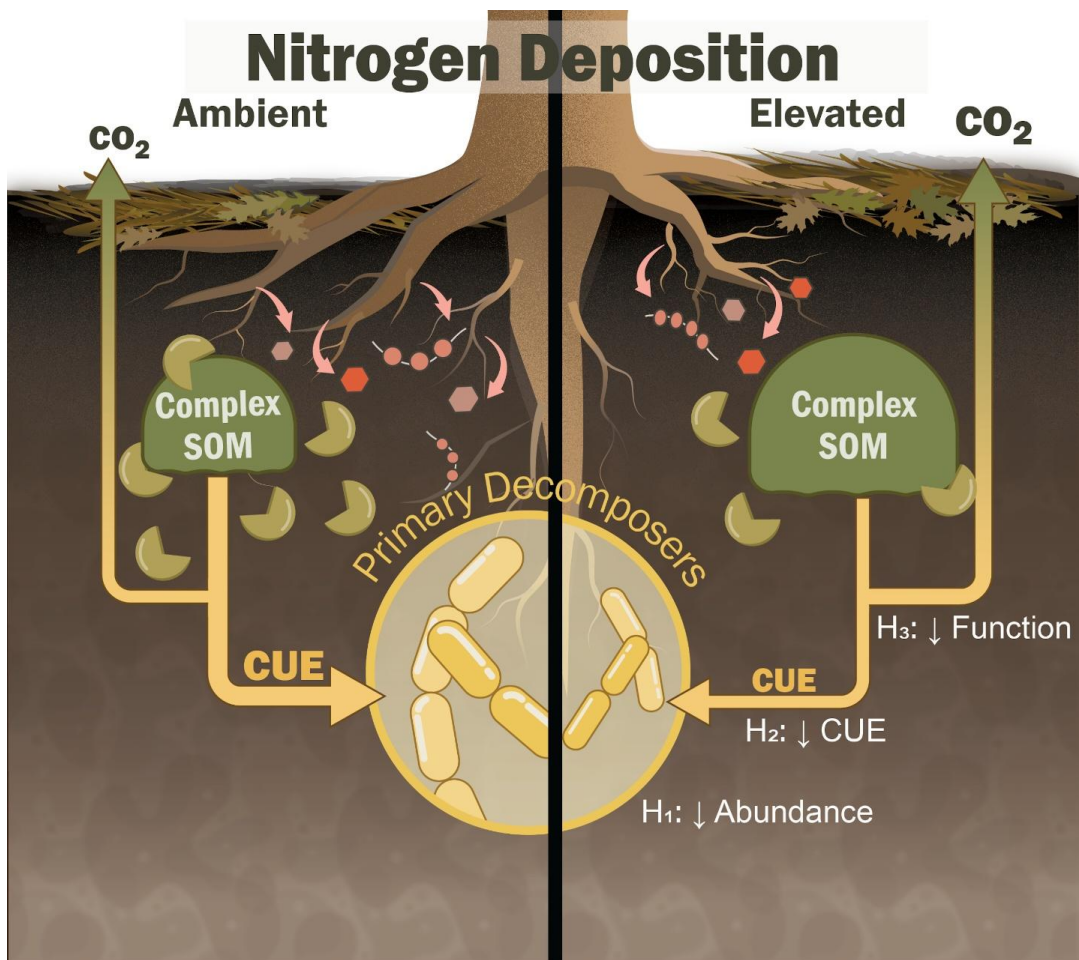
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162 *Figure 2: Hypothesized microbial response to belowground carbon (C) allocation under ambient and enhanced*
163 *nitrogen (N) deposition. Increased SOM resulting from N fertilization may result from reduced primary (1°)*
164 *decomposer abundance (H1), reduced 1° decomposer complex SOM C use efficiency (CUE; H2), decreased 1°*
165 *decomposer complex SOM degrading functional ability (H3), or a combination of H1 and H3.*

166 **2 Methods**

167 **2.1 CORPSE model description**

168 The CORPSE model is a microbial explicit SOM decomposition model that includes representations of
169 microbial physiology, the chemical and physical protection of SOM substrates, and rhizosphere processes (Sulman
170 et al., 2014). Model development, simulation experiments, and figure generation were performed using *R* 4.2.0 (R
171 Core Team, 2022), using the packages *tidyverse* (Wickham et al., 2019), *doBy* (Højsgaard and Halekoh, 2022), and
172 *ggthemes* (Arnold, 2021). In the model, microbial biomass drives the decomposition of both C and N in SOM and
173 microbial biomass varies depending upon C use efficiency (CUE) and microbial turnover. SOM is classified into
174 three chemically distinct types, simple, complex, and microbial necromass, that are derived from different inputs,



175 vary in decomposition rates, and the rate that they are physically protected (Table 1). These chemically distinct
176 classes are further divided into unprotected and physically protected SOM pools. The physically protected SOM of
177 each chemical type cannot be decomposed because it is inaccessible to microorganisms, but there is a parameter that
178 controls the turnover of protected C back into the unprotected pool. The decomposition rates (D) of a given chemical
179 type (i) is determined using the following equation, from Sulman et al. (2014):

$$180 \quad D_i = V_{max,i}(T) * \left(\frac{\theta}{\theta_{sat}}\right)^3 * \left(1 - \frac{\theta}{\theta_{sat}}\right)^{2.5} * C_i \left(\frac{\frac{\sum M}{\sum C_i}}{\frac{\sum M}{\sum C_i + k_M}}\right) \quad (1)$$

181 where decomposition is a function of a temperature dependent maximum enzymatic decomposition rate (V_{max}), the
182 microbially accessible (unprotected) C pool (C), soil moisture (θ), and microbial biomass (M) as a function of the
183 ratio of microbial biomass to total unprotected C.

184 Complex SOM represents lignin and other complex C molecules that decompose at a slower rate owing to a
185 lower V_{max} and lower microbial uptake and CUE. Simple SOM represents labile compounds from litter and root
186 exudates that are quickly and efficiently degraded and assimilated by soil microbes (i.e., high V_{max} and CUE).
187 Microbial necromass has intermediate decomposition to both complex and simple SOM. Microbial necromass is
188 assumed to preferentially have the highest physical protection rate followed by the simple and complex SOM types.
189 Finally, the model also separates the soil into the bulk and rhizosphere compartments that have the same SOM pool
190 structure with all three chemical types separated into unprotected and protected fractions. The rhizosphere is
191 assumed to be 10% of the soil and the only difference from the bulk soil is that it receives inputs to the simple SOM
192 pool that are analogous to root exudates at a rate of $0.0676 \text{ kg C m}^{-2} \text{ year}^{-1}$.

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Model Parameter	Passive Consumers	2° Decomposers	1° Decomposers	1° Decomposers			
	Reference Simulation (no N fertilization)			N Fertilization Simulations			
				H1	H2	H3	H1 & H3
Relative Maximum Enzymatic Decomposition Rate (V_{max})							
Simple SOM	5.25	2.1	2.1	2.1	2.1	2.1	2.1
Complex SOM	0.06	0.06	0.15	0.15	0.15	0.06	0.06
Necromass	1.5	3.3	1.5	1.5	1.5	1.5	1.5
Enzyme Activation Energy (E_a)							
Simple SOM	5000	5000	5000	5000	5000	5000	5000
Complex SOM	30000	30000	30000	30000	30000	30000	30000
Necromass	3000	3000	3000	3000	3000	3000	3000
Microbial Lifetime at 20°C (years)	0.25	0.25	0.25	0.02	0.25	0.25	0.02
Carbon Uptake Efficiency (CUE)							
Simple SOM	0.63	0.54	0.54	0.54	0.54	0.54	0.54
Complex SOM	0.1	0.1	0.135	0.135	0.1	0.135	0.135
Necromass	0.5	0.6	0.5	0.5	0.5	0.5	0.5
Protected C Formation Rate (year^{-1})							
Simple SOM	0.525	0.525	0.525	0.525	0.525	0.525	0.525
Complex SOM	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Necromass	1.5	1.5	1.5	1.5	1.5	1.5	1.5

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195 *Table 1: Model parameters for microbial functional groups that differ across soil organic matter (SOM) types and*
196 *microbial functional groups in the reference simulation, and 1° decomposer functional group parameters that were*
197 *modified across the four N fertilization simulations. H1 = Reduced 1° decomposer abundance; H2 = reduced 1°*
198 *decomposer complex CUE; H3 = reduced 1° decomposer functional ability.*

199 2.2 Integrating CAES into functional group CORPSE

200 To integrate CAES into CORPSE, we modified the model to include three microbial functional groups (1°
201 decomposers, 2° decomposers, and passive consumers) that vary in their ability to decompose and assimilate the
202 three SOM chemical types (Figure 1). In the modified model, functional group CORPSE (FG-CORPSE), 1°
203 decomposers, 2° decomposers, and passive consumers specialize in degrading and using complex SOM, microbial
204 necromass, and simple SOM, respectively. Every functional group can decompose and utilize every SOM type, but
205 each group can more rapidly degrade and more efficiently take up C from their preferred SOM type than the other
206 functional groups. This specialization is represented by changing parameters that control maximum enzymatic
207 decomposition rates ($V_{\max}$) and CUE. The $V_{\max}$ and CUE of complex SOM by 1° decomposers is 150% and 35%
208 higher, respectively, than the other groups. $V_{\max}$ and CUE of simple SOM by passive consumers is 150% and 17%
209 higher, respectively. $V_{\max}$ and CUE of necromass by 2° decomposers is 120% and 20% higher, respectively (Table
210 1). FG-CORPSE parameters were calibrated to match model predictions to soil C measurements from a temperate
211 forested watershed at the Fernow Experimental Forest. Parameter space was constrained by choosing parameters for
212 each functional group that approximate the overall decomposer capacity of the microbial biomass pool in the
213 original CORPSE model. The core assumption of this parameterization is that $V_{\max}$ and CUE for each specialized
214 functional group are directly linked to the theory of the CAES framework where each group has a competitive
215 advantage for their preferred substrate class (Table 1).

216 In the CORPSE model, the availability of SOM for decomposition is limited by protection, rendering it inaccessible,
217 and by a saturation formula that limits microbial access when the unprotected pool is small and microbial biomass is
218 high, but all microbial functional groups are assumed to have equal access to unprotected SOM of every chemical
219 type. This model structure assumes that there is no competition for a given SOM chemical type across the functional
220 groups and that the growth of each functional group is independent of the growth of the other functional groups.
221 Decomposition and assimilation of each SOM chemical type is calculated at each time step using the biomass and
222 physiological traits of each individual functional group acting on a given SOM chemical type and then aggregating
223 the results across all three functional groups. As such, the microbial biomass and the distribution of the three
224 functional groups is an emergent property of the model.

225 2.3 FG-CORPSE spin up, parameterization data, and validation data

226 Model predictions of SOM pools were validated against soil C and N measurements from a long-term, whole
227 watershed N fertilization experiment in the Fernow Experimental Forest, WV, USA. The long-term N fertilization
228 experiment is composed of two adjacent watersheds of comparable size and age in a broadleaf deciduous forest. One
229 watershed (+N WS3; 34 ha) received 35 kg N Ha⁻¹ yr⁻¹ from January 1989 to October 2019 in the form of
230 ammonium sulfate ((NH₄)₂SO₄) in three annual applications (Adams et al., 2012; Eastman et al., 2021). The adjacent
231 watershed (control WS7; 24 ha) served as a no N input control. Forests in both watersheds were 18-19 years old at
232 the start of the N fertilization experiment in 1989, having been previously forested (Adams et al., 2012). Although
233 relative abundance differs slightly, the two watersheds have similar tree species, including *Prunus serotina*,
234 *Liriodendron tulipifera*, *Betula lenta*, *Acer rubrum*, and *Quercus rubra* (Eastman et al., 2021). Mean annual
235 precipitation is 1460 mm, and mean annual temperature is 9.3° C (Young et al., 2019; Eastman et al., 2021).

236 FG-CORPSE was spun up until predictions of SOM and microbial biomass reached equilibrium in simulation using
237 environmental and primary productivity/input data from the Fernow experiment site, about 250 years. Estimates of
238 total soil C pools in the top 45 cm of soil at equilibrium were validated against soil C stocks calculated for the
239 control WS7 from measurements of SOM in the top 45 cm of the soil taken in 2016 (Eastman et al., 2021). Protected
240 and unprotected C pool estimates were compared to measurements of light and heavy POM and mineral associated
241 organic matter (MAOM) taken in 2017 of the top 10 cm of soil in the control WS7 using density fractionation of
242 SOM (Eastman et al., 2022). SOM pools and microbial biomass at the end of the spin-up were used as initial pool
243 values in subsequent experiments.

244 2.4 Response of functional groups to shifts in litter chemistry



Microbial functional group response to C input composition was evaluated in three simulated experiments by altering the proportion of leaf and root litter inputs allocated to simple or complex SOM relative to the parameters for the reference model at spin up. The first experiment simulated increased complexity of litter inputs by increasing the proportion of leaf and root litter as complex SOM from 0.4 and 0.86 to 0.7 and 0.93, respectively. To simulate decreased litter complexity, we reduced the proportion of leaf and root litter as complex SOM from 0.4 and 0.86 to 0.1 and 0.79, respectively. We also simulated decreased complexity of root litter alone, reducing the proportion allocated to complex SOM from 0.86 to 0.4 to match the allocation of SOM classes for the leaf litter inputs. Each experiment was run for 30 years to determine the change in biomass of each functional group in response to varying inputs.

2.5 Evaluating hypotheses governing SOM responses to N fertilization

Three hypothesized mechanisms of microbial control of decomposition and SOM formation under increased N deposition were tested in four simulation experiments. Our aim was to determine which mechanism, or combination of mechanisms, most accurately captures the SOM responses quantified for +N WS3 soils. Enhanced N fertilization for almost 30 years in +N WS3 increased total soil C stocks by approximately 10.9% relative to control WS7, from ~8.8 to ~9.8 kg C m⁻² (Eastman et al., 2021). This increase in total C was driven by an increased proportion of soil C as POM (Eastman et al., 2022). The effect of N deposition was modeled by increasing the N input parameter (0.005 to 0.0085 kg N m⁻² year⁻¹) and reducing root exudation to the rhizosphere (from 0.0676 to 0 kg C m⁻² year⁻¹), representing reduced C partitioning belowground in response to N fertilization. To determine if changes in C and N availability alone could capture observed soil C responses, we ran a N deposition simulation in FG-CORPSE without modifying parameters for the microbial functional groups. To simulate our hypothesized mechanisms of microbial functional response to N deposition, we changed parameters for the 1° decomposer group in four simulation experiments (Table 1).

Reduced 1° decomposer abundance (H1): N deposition causes a shift in microbial community structure due to decreased 1° decomposer abundance in response to reduced belowground C allocation from trees. Reduced 1° decomposer abundance was simulated in our model by reducing 1° decomposer turnover time from 3 months to approximately 1 week, while turnover time for 2° decomposers and passive consumers remained the same.

Reduced 1° decomposer CUE (H2): N deposition reduces the CUE of complex substrates for 1° decomposers. This was modeled by reducing CUE from complex SOM for 1° decomposers to match the complex SOM CUE of the other two functional groups (Complex SOM CUE for all functional groups = 0.1).

Decreased 1° decomposer function (H3): The maximum decomposition rate of complex SOM by 1° decomposers is reduced as a result of declining enzyme production from decreased belowground C allocation under N deposition. This was modeled by decreasing the complex SOM decomposition rate (V_{max}) for 1° decomposers to match the rate of the other two groups (V_{max} of complex SOM for all functional groups = 0.06).

Combined decreased 1° decomposer abundance and function (H1 and H3): Decomposition of complex SOM by 1° decomposers is reduced, and abundance of 1° decomposers decreases under N deposition. This model combined reduced 1° decomposer turnover time and reduced complex SOM V_{max} by 1° decomposers.

To test these hypotheses, we modeled decomposition and SOM storage in the top 45 cm of a forest soil over 30 years of enhanced N deposition. Predictions of total C, protected C, and unprotected C pools following N deposition were compared to soil C stocks and SOM fraction measurements from the +N WS3 after almost 30 years of N fertilization. Estimates of total soil C were validated against soil C stocks calculated for +N WS3 from SOM measured in the top 45 cm of the soil sampled in 2016 (Eastman et al., 2021). Estimates of protected and unprotected C pools were compared to light and heavy particulate organic matter (light and heavy POM) and mineral associated organic matter (MAOM) SOM density fractionation measurements taken in 2017 for the top 10 cm of +N WS3 soils (Eastman et al., 2022).

2.6 Forward simulations of the recovery of decomposition from N fertilization

To project the fate of the N-induced increase in SOM in the future, SOM decomposition and storage was modeled for 30 years following the cessation of N fertilization in 2019. Recovery from N deposition was simulated following the reference simulation (representing no N deposition) and the four N deposition experiments. Final SOM and biomass pool values at the end of the 30-year N deposition simulations were used as initial values for the 30 year



recovery simulations, which were run using the reference N deposition and root exudation parameters ($0.05 \text{ kg N m}^{-2} \text{ year}^{-1}$ and $0.0676 \text{ kg C m}^{-2} \text{ year}^{-1}$, respectively).

3 Results

3.1 Model spin up and validation

The model reached equilibrium after spin-up using the Fernow Experimental Forest input data, and predictions of soil C pools at equilibrium were close to soil C measurements in the control WS7. Total soil C was predicted to be 8.40 kg m^{-2} , comparable to 8.84 kg C m^{-2} calculated from SOM measurements of the top 45 cm of soil in the control WS7 (Eastman et al., 2021). Approximately 67% of soil C was in protected pools (5.64 kg m^{-2}) and 33% in unprotected pools (2.76 kg m^{-2}). For comparison, density fractionation of SOM from the top 10 cm of soil from control WS7 found the majority of soil C to be protected by mineral association or occlusion, with 54% of soil C as MAOM, and 24% and 22% as heavy and light POM, respectively (Eastman et al., 2022). Total microbial biomass was approximately $0.043 \text{ kg C m}^{-2}$ after spin-up (Figure 3). The most abundant microbial functional group was passive consumers, comprising 51.1% of microbial biomass, followed by 2° decomposers then 1° decomposers, representing 25.7% and 23.2% of microbial biomass (Figure 3).

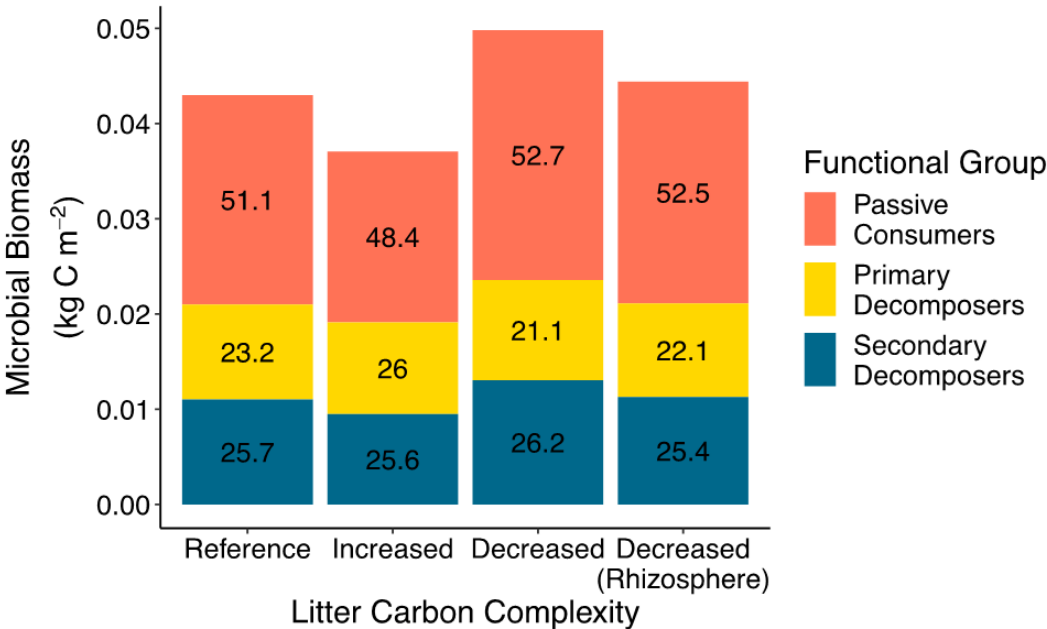


Figure 3: Total microbial biomass C and functional group relative abundance (% values in columns) after 30 years of differing litter C complexity.

3.2 Microbial response to varying litter input composition

Microbial biomass was sensitive to C input composition, decreasing ~14% from 0.043 to $0.037 \text{ kg C m}^{-2}$ with more complex litter C inputs and increasing ~16%, to 0.05 kg C m^{-2} , with less complex leaf and root litter and ~3%, to $0.044 \text{ kg C m}^{-2}$, with less complex root litter inputs (Figure 3). Microbial community composition also changed with input complexity. More complex litter input increased relative abundance of 1° decomposers from 23.2% to 26% and decreased relative abundance of passive consumers from 51.1% to 48.4%. Less complex litter C decreased the relative abundance of 1° decomposers from 23.2% to 21.1% and increased the abundance of passive consumers from 51.1% to 52.7% and 2° decomposers from 25.7% to 26.2%. Less complex root litter C also decreased relative



abundance of 1° decomposers to 22.1% and increased the abundance of passive consumers to 52.5% but had little effect on 2° decomposers.

3.3 Predicted soil C storage under N deposition

Predicted soil C stocks decreased slightly when N deposition was simulated by only increasing inorganic N inputs and reducing rhizosphere C inputs (Fig. A1), demonstrating the need for the model to account for shifts in microbial function in response to N deposition. Total soil C increased with N deposition in three of the four simulation experiments and decreased in one experiment, relative to predictions from a reference model of the control WS7 without enhanced N fertilization. Total soil C increases predicted by FG-CORPSE were highest in the combined decreased 1° decomposer abundance and function experiment, which estimated total C to be 9.28 kg C m⁻² after 30 years of simulated N deposition, 10.5% (0.88 kg C m⁻²) higher than the reference model (Figure 4). Total soil C was estimated to be 9.07 kg C m⁻², 8% (0.67 kg C m⁻²) higher than the reference, in the decreased function experiment, and 8.84 kg C m⁻², 5.2% (0.44 kg C m⁻²) higher, in the reduced 1° decomposer abundance experiment. Total C decreased by 1.2% (0.1 kg C m⁻²) to 8.3 kg C m⁻² in the reduced CUE experiment. For comparison, empirical measurements of total soil C stocks in the +N WS3 were calculated to be 9.8 kg C m⁻² after nearly 30 years of N fertilization, an increase of 10.9% (0.963 kg C m⁻²) relative to the control WS7 soils (Eastman et al., 2021). The predicted soil C increase from the combined decreased 1° decomposer abundance and function experiment (10.5%) was closest to the measured difference between the experimental watersheds (10.9%; dashed line in figure 4C).

In all N deposition experiments protected C decreased and unprotected C increased (Figure 5). Losses of protected C were similar for all experiments, ranging from 6% (0.34 kg C m⁻²) in the combined decreased 1° decomposer abundance and function experiment to 8.5% (0.48 kg C m⁻²) in the reduced CUE experiment (Figure 4). Unprotected C increased by 44.3% (1.22 kg C m⁻²) in the combined decreased 1° decomposer abundance and function experiment, 39.6% (1.09 kg C m⁻²) in the decreased function experiment, 29.1% (0.8 kg C m⁻²) in the reduced 1° decomposer abundance experiment, and 14% (0.38 kg C m⁻²) in the reduced CUE experiment (Figure 4). Empirical measurements comparing soil C pools in the +N WS3 relative to the control WS7 found light POM fractions increased from ~22% to ~29%, the proportion in heavy POM fractions decreased from ~24% to ~16%, and the proportion in MAOM remained about the same at ~55% (Eastman et al., 2022).

3.4 Predicted microbial community response to N deposition

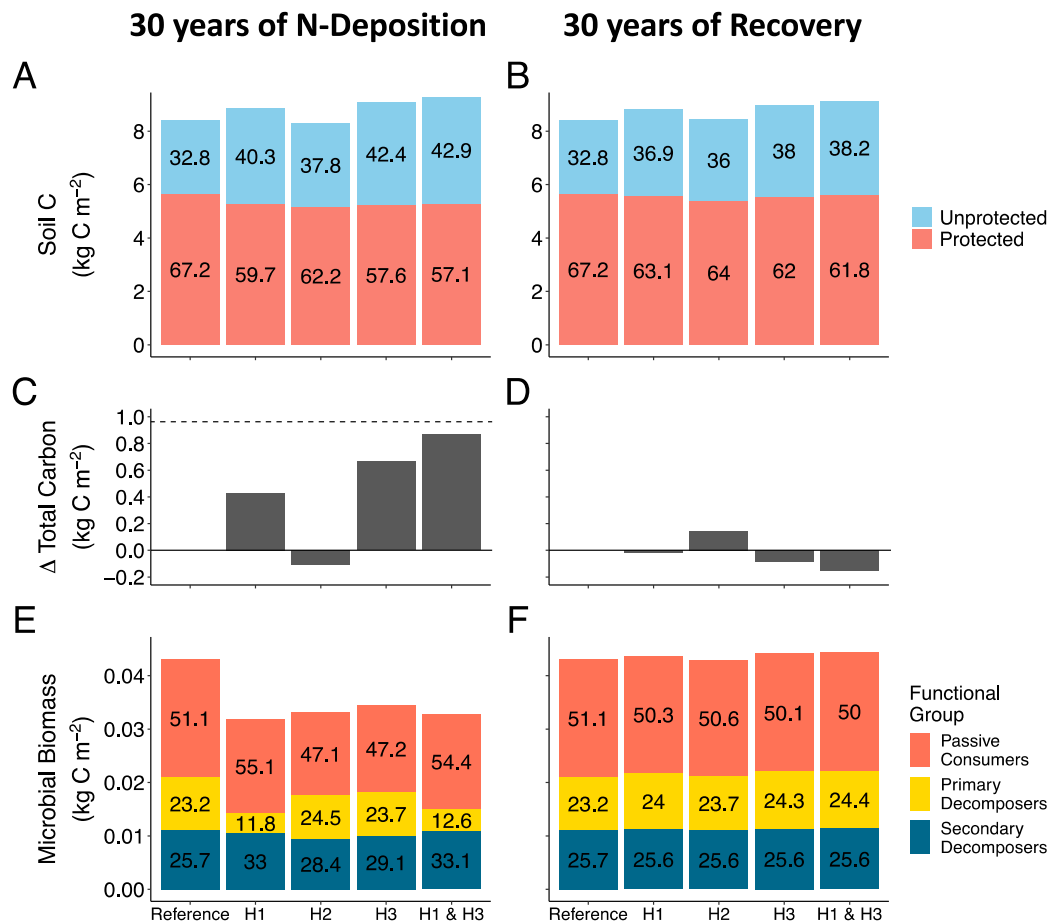
Microbial biomass was reduced under every N deposition modeling experiment relative to the reference, and microbial community composition was sensitive to the different N modeling experiments (Figure 4). Microbial biomass was estimated at 31.8 g MBC m⁻², 26.1% (11.2 g MBC m⁻²) lower than the reference, in the reduced 1° decomposer abundance experiment, 32.8 g MBC m⁻², 23.7% (10.2 g MBC m⁻²) lower, in the combined decreased 1° decomposer abundance and function experiment, 33.1 g MBC m⁻², 23% (9.9 g MBC m⁻²) lower, in the reduced CUE experiment, and 34.4 g MBC m⁻², 20% (8.6 g MBC m⁻²) lower, in the decreased function experiment. The relative abundance of 1° decomposers decreased in the reduced 1° decomposer abundance experiment and the combined decreased 1° decomposer abundance and function experiment from 23.2% to 11.8% and 12.6%, respectively. The relative abundance of passive consumers increased, from 51.1% to 55.1% and 54.4%, respectively, and 2° decomposers increased, from 25.7% to 33% and 33.1%, respectively. In the reduced CUE experiment the relative abundance of 1° decomposers increased to 24.2% and 2° decomposers to 28.4%, and passive consumer abundance decreased to 47.1%. Decreased function did not change 1° decomposer relative abundance, decreased passive consumer abundance to 47.2%, and increased 2° decomposer abundance to 29.1%.

3.5 Predicted recovery following the cessation of N deposition

Modeling projections of ecosystem recovery following the cessation of elevated N deposition found soil C to remain higher than the reference model in all N deposition experiments after 30 years (Figure 4; Figure 5). Recovery of microbial biomass and community structure was very similar for all experiments, mirroring the reference simulation (Figure 4F). Predicted C losses during recovery from N deposition were 12.5% in the combined decreased 1° decomposer abundance and function experiment, 11.2% in the decreased function experiment, 8.7% in the reduced 1° decomposer abundance experiment, and 3.5% in the reduced CUE experiment (Figure 4). These losses were slightly mitigated by smaller increases to protected C, which seemed to recover slowly but remained on an upward trajectory (Figure 5). Unprotected C losses ranged from 31% in the combined decreased 1° decomposer abundance



368 and function experiment, 29.9% in the decreased function experiment, 28.1% in the reduced 1° decomposer
369 abundance experiment, 24% in the reduced CUE experiment, and 22% for reference parameters (Figure 4).



370 Figure 4: Soil C stocks (A-B), change in total soil C (C-D), and microbial biomass C (E-F) after 30 years of N
371 deposition followed by 30 years of recovery without N deposition. The proportion of total soil C stocks (%) in
372 protected and unprotected pools is indicated by the numbers in columns in (A) and (B). In three of the four N
373 deposition scenarios, unprotected C increased and protected C decreased, resulting in an overall increase in total C
374 relative to the reference. Recovery without N deposition under ambient temperature led to decreased unprotected C
375 offset by increased protected C, resulting in little change in total C over the 30 years of recovery. Numbers in
376 columns in (E) and (F) indicate functional group % relative abundance. Dashed line in (C) represents the difference
377 in total soil C between the N-fertilized watershed (+N WS3) and the control watershed (WS7), reported by Eastman
378 et al. (2021). Ref = no N deposition reference; H1 = reduced 1° decomposer abundance; H2 = reduced 1°
379 decomposer complex C use efficiency; H3 = decreased 1° decomposer functional ability.

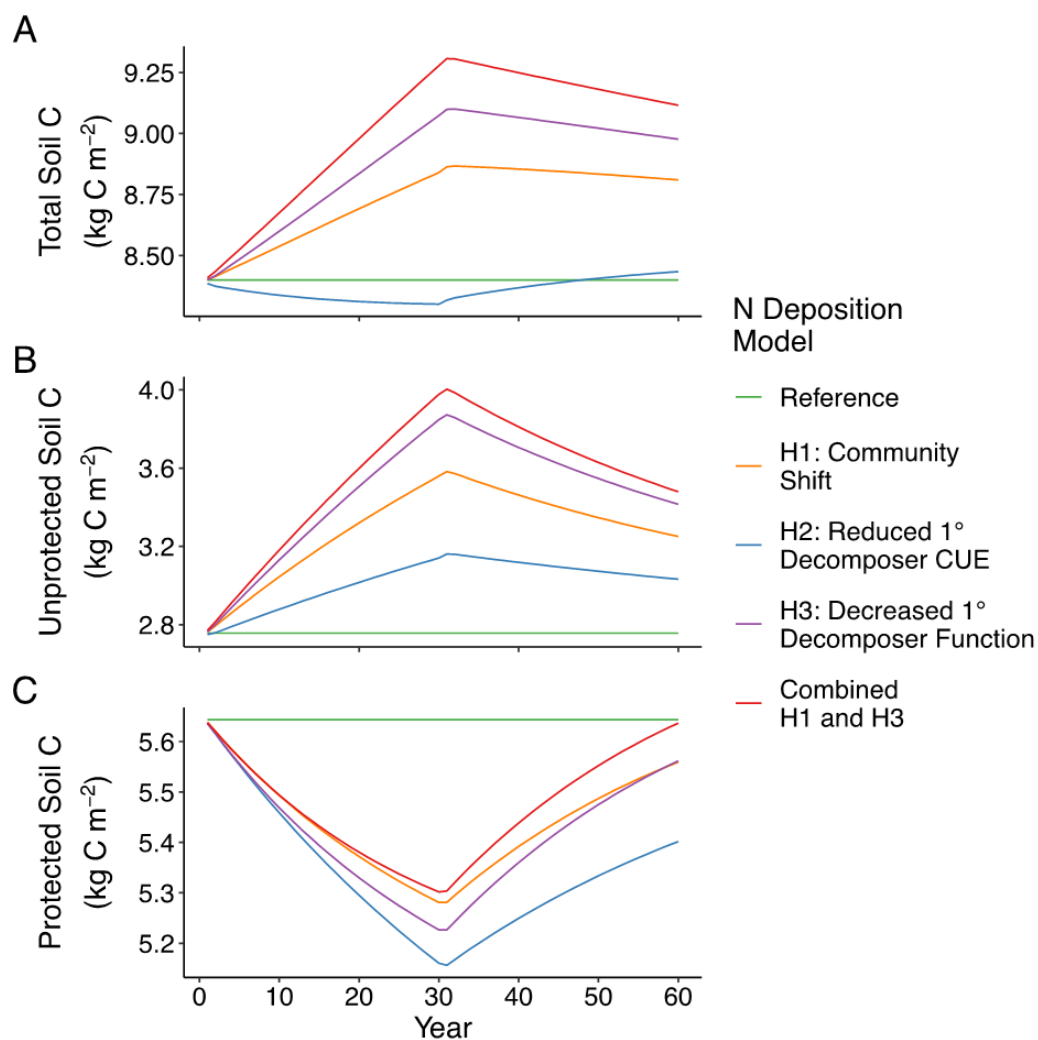


Figure 5: Change in predictions of (A) total soil C, (B) unprotected soil C, and (C) protected soil C over 30 years of simulated N deposition followed by 30 years of recovery without N deposition.



394 4 Discussion

395 The objective of this study was to determine if FG-CORPSE, a SOM decomposition model that represents microbial
396 functional groups derived from the CAES framework, could reproduce empirically observed patterns of C storage in
397 a forest soil under N fertilization, and test leading hypotheses on C accumulation under N fertilization. First, the
398 model was validated showing that functional groups are linked to specific substrates in that complex C and primary
399 decomposer relative abundance were positively correlated (Figure 3). Second, we showed that by explicitly
400 modelling microbial mechanistic responses to N deposition (e.g., decreased primary consumer abundance and
401 function) we were able to capture the accrual of C under N fertilization. Additionally we found that total microbial
402 biomass was reduced under all N fertilization model simulations, echoing empirical results showing that C
403 accumulation following N deposition may be driven by a decline in microbial biomass. Importantly, when we ran
404 the model forward, we found that the most model simulations showed that the majority of the N induced soil C gains
405 persisted due to a transfer of POM to more stable MAOM pools.

406 4.1 Modeled functional group responses to litter input complexity reflect CAES substrate preferences

407 FG-CORPSE predicted shifts in microbial community composition with changes in plant litter input chemistry that
408 reflect the substrate specialization of the functional groups (Figure 3). The respective changes in 1° decomposer
409 relative abundance and total microbial abundance from changing C availability mirror empirical observations from
410 litter manipulation experiments (Fanin et al., 2014; Zechmeister-Boltenstern et al., 2015; Soares & Rousk, 2019).
411 Sensitivity of microbial functional groups to variability in the amount and complexity of SOM inputs is an emergent
412 property of FG-CORPSE that allows decomposition to be controlled by microbial community composition and
413 functional diversity in addition to microbial biomass pool size. This agreement between the modeling results and
414 past empirical studies suggests representing microbial diversity in models based upon substrate preference can
415 capture empirical responses of microbial community composition and biomass to shifts in substrate complexity.
416 Given that the functional group representation in FG-CORPSE can capture these empirical shifts in microbial
417 abundance and biomass, our incorporation of the CAES framework into CORPSE may provide a tractable
418 foundation for modeling the impacts of microbial diversity on soil decomposition.

419 4.2 FG-CORPSE simulates microbial dynamics and their consequences for C cycling

420 Shifts in microbial functional diversity appear to be integral to capturing soil C responses to N fertilization at the
421 Fernow. At the ecosystem-scale, long-term research at the Fernow implicates that a reduction in plant C allocation
422 belowground leads to decreases in decomposition and a subsequent increase in soil C stocks (Eastman et al., 2021;
423 2022). However, when N deposition was simulated by only increasing N inputs to the soil and reducing plant C
424 inputs to the rhizosphere, FG-CORPSE predicted a slight decrease in total soil C (Supplementary Figure S1). Plant-
425 centric model mechanisms fail to capture known shifts in the composition of fungi and bacteria as well as the ability
426 of plant C investment to shape these communities. At the Fernow and across other long term N fertilization
427 experiments, chronic N amendments have been shown to reduce heterotrophic respiration, microbial biomass, and
428 enzyme production, and to change microbial community composition (Janssens et al., 2010; Frey et al., 2014;
429 Carrara et al., 2018; Freedman et al., 2016). These results suggest that changes in microbial diversity and their
430 decomposition functions are central to driving soil C responses to N availability. Thus, this inability of FG-CORPSE
431 to capture these patterns using a plant-centric mechanism alone supports the use of our functional group framework
432 to test our hypotheses of how shifts in functional diversity drive the response of soil C to enhanced N availability.

433 4.3 Reduced decomposer abundance and function drives predicted soil C increases under N deposition

434 While both the reduced 1° decomposer abundance hypothesis and the decreased 1° decomposer functional ability
435 hypothesis captured the broad patterns of an increase in soil C and a reduction in microbial biomass with N
436 fertilization, predictions from the model simulation that combined both mechanisms were the most similar to the
437 observed soil C increase between the +N WS3 and control WS7 in the Fernow (Figure 4). These results support the
438 hypothesis that reductions in SOM decomposition reflect an interaction between decreased plant C allocation
439 belowground and functional shifts in microbial communities (Carrara et al. 2018). FG-CORPSE results further
440 refine this hypothesis by showing the potential for a complimentary effect whereby a reduction in the abundance of
441 1° decomposers as well as a loss of functional capacity of the 1° decomposer group drive declines in soil
442 decomposition. In support, the simulation that only represented a decline in the abundance of 1° decomposers had
443 limited decomposition declines; whereas the one that reduced only their functional capacity didn't capture known
444 microbial community shifts (Figure 4). Only the model simulation that combined both was able to capture patterns



observed in the Fernow and other N fertilization experiments that show a reduced abundance of complex C degrading microbes and reduced complex C degrading enzyme activity (Frey et al., 2014; Carrara et al., 2018; Eastman et al., 2021). Thus, the soil C consequences of enhanced N availability in forest soils was only simulated by a model representation that included reductions in plant C allocation that led to shifts in microbial functional diversity and capacity.

FG-CORPSE results also suggest that shifting CUE alone is not sufficient to drive soil C increases that result from N fertilization. The model simulating reduced CUE for 1° decomposers did not project a net increase in soil C even though there was a decline in total microbial biomass and an increase in unprotected soil C. This model result was surprising, given that CUE is considered a keystone microbial trait that drives soil C responses to global change (Sinsabaugh et al., 2013; Schimel, 2023). As a parameter in microbial-explicit models, CUE determines the proportion of assimilated C allocated to microbial biomass growth or lost through respiration. Thus, reduced CUE is expected to result in declines in microbial biomass, enzyme production, and decomposition. However, developing views on the emergent nature of CUE have demonstrated the potential for shifts in CUE to have varying effects on soil C storage (Allison, 2025; He et al., 2024; Hagerty et al., 2018). Theoretical modeling work has shown that SOC pools can increase when microbial CUE is reduced due to less efficient C assimilation or decrease when increased enzyme production reduces CUE (Hagerty et al., 2018). By pegging a strict decline in CUE and only focusing on growth and respiratory losses, FG-CORPSE failed to capture the dynamics of a low CUE ecological strategies that could lead to increased decomposition, such as increased investment in resource acquisition. As such, the experiments that modeled microbial response as reduced enzyme production captured the observed decomposition dynamics better.

4.4 Enhanced soil C storage predicted to persist after cessation of N deposition

FG-CORPSE simulations of recovery suggest that additional C that accumulated as a result of N deposition may not be highly vulnerable to loss after N deposition declines. Predicted total C in all the N deposition simulations was higher than the reference simulation after 30 years of recovery under ambient conditions (Figure 5). This is consistent with the results of a recent study on decomposition in soils from the Fernow forest four years after the end of the 30-year N fertilization experiment (Kangi et al., 2025). In their study, Kangi et al. found that microbial respiration and SOM decomposition were lower in +N WS3 soils than control WS7 soils, suggesting that the effects of N deposition on soil microbial functioning can persist long-term. In FG-CORPSE, recovery from decades of enhanced N fertilization stimulated the rapid recovery of microbial groups, which targeted excess unprotected C pools, stimulating loss in total C but also an increase in protected C pools that buffered the decline in total C. As N deposition is switched off, belowground C allocation from trees is turned on, and this input of simple SOM stimulates microbial growth. Despite rapid recovery of microbial biomass, predicted soil C losses are not as rapid as the accumulation of soil C under N deposition. In our N deposition experiment simulations, long-term soil C storage results from gradual decreases in unprotected C that are accompanied by increases in protected C pools, which are driven by the rapid recovery of microbial biomass and subsequent protection of larger pools of microbial necromass.

4.5 Future development and applications of FG-CORPSE

Key areas for the future development and application of the FG-CORPSE model are the further testing of hypothesized mechanisms of microbial community response to environmental factors and the parameterization of microbial community composition and functional traits from empirical data. In FG-CORPSE, we found that reduced abundance and decomposition capacity of 1° decomposers best captured the empirical response of soil C to N fertilization (Figure 4). While this simulation supported our hypothesis, there are likely also covarying microbial traits that could accompany these shifts. Empirical examinations of whether reduced decomposer abundance and enzyme production are accompanied by changes in CUE, turnover, or the necromass chemistry of 1° decomposers could inform how these parameters change in the model and alter predictions. Additionally, our simulations do not include any changes to microbial function due to environmental factors other than N deposition. Warming and moisture availability can influence CUE, microbial turnover, and enzyme activity (Allison et al., 2010; Wieder et al., 2013; Daou et al., 2016; Wang et al., 2023). Simulating the effects of temperature and moisture, as well as their interactions with N deposition, on microbial functional diversity in the context of the CAES framework is an important direction for future research.

In addition to using soil C measurements to support model predictions, microbial community composition and trait data should be used to parameterize and validate simulated microbial communities in future studies. The



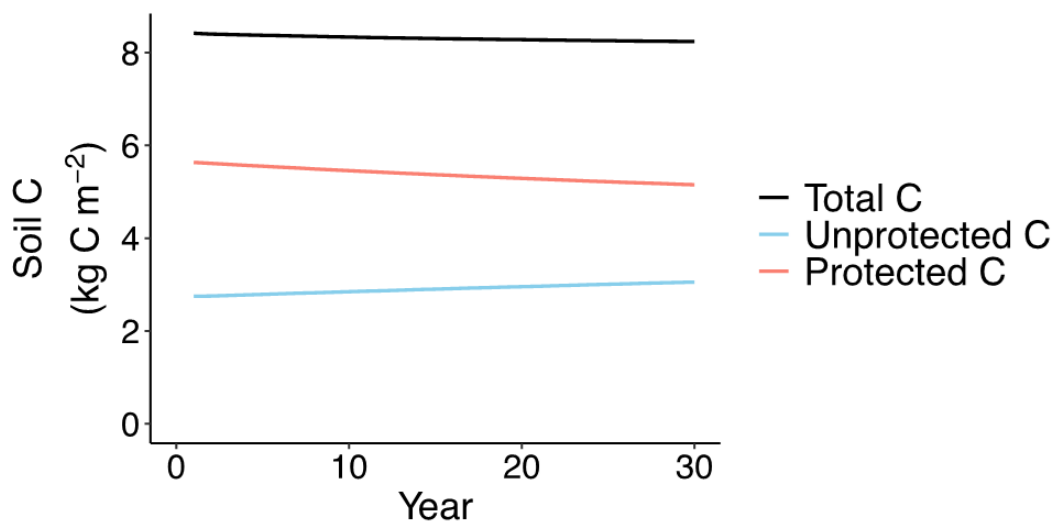
496 characterization of functional traits of soil microorganisms can be challenging, but there is a clear need to develop
497 pipelines to simplify these data for use in predictive modeling. The C acquisition strategies of microbial taxa can be
498 characterized based on their targeted degradation and assimilation of C substrates, which can be measured using
499 methods like quantitative stable isotope probing or inferred from genomic analysis (Morrissey et al., 2023).
500 Quantitative microbial traits that are emphasized by the CAES framework, like substrate assimilation, can be scaled
501 by microbial biomass and taxon abundance to link microbial diversity to ecosystem level processes (Walkup &
502 Morrissey, 2025). As such, the characterization of microbial traits that conceptually and quantitatively relate to pools
503 and fluxes in soil C models is necessary to parameterize the next generation of microbial-explicit models.

504 **4.6 Conclusions**

505 FG-CORPSE advances the field of theoretical trait-based ecosystem modeling by integrating microbial functional
506 diversity into a microbial-explicit SOM decomposition model through functional groups derived from the CAES
507 framework. FG-CORPSE demonstrates how C acquisition strategies can influence plant-microbial interactions that
508 determine ecosystem response to N deposition. When simulating reductions in 1° decomposer abundance and
509 complex SOM degrading enzyme production, C storage predictions by FG-CORPSE for a forest soil under
510 enhanced N deposition were in line with empirical observations. Because N deposition influences SOM
511 decomposition through the interaction of plant and microbial responses, capturing the patterns of soil C storage and
512 decomposition observed in N fertilization experiments was only possible when modeling the response of a
513 functionally diverse decomposer community to reduced belowground C allocation. Classifying microorganisms
514 according to C acquisition ecological strategies directly associates functional groups to C pools and fluxes. FG-
515 CORPSE enables the representation of functional diversity that influences growth and decomposition, providing a
516 platform to test microbe-centric hypotheses and identify key parameters or processes that can provide priorities or
517 hypotheses for new experiments or observations.

518

519 **Appendix A**



520 *Figure A1: Predicted soil carbon (C) stocks over 30 years of nitrogen (N) deposition, modeled without microbial*
521 *mechanisms of functional response to N deposition. The effect of N deposition was modeled by increasing the*
522 *inorganic N input parameter (0.005 to 0.0085 kg N m⁻² year⁻¹) and reducing root exudation to the rhizosphere (from*
523 *0.0676 to 0 kg C m⁻² year⁻¹), representing reduced C partitioning belowground by plants in response to N*
524 *fertilization. Total soil C decreased 2.1%, from 8.42 to 8.24 kg C m⁻², protected C decreased 8.6%, from 5.64 to 5.15*
525 *kg C m⁻², and unprotected C increased 11.2%, from 2.75 to 3.06 kg C m⁻².*

526



527

528

529 **Code and data availability**

530 The R code for the FG-CORPSE model and the input data used to run the simulations presented in this paper are
531 available on Zenodo at <https://zenodo.org/records/15440033> and on GitHub at [https://github.com/jgwakeup/FG-](https://github.com/jgwakeup/FG-CORPSE)
532 CORPSE.

533 **Author contributions statement**

534 E.B. and E.M. developed the concept and secured funding for the study. J.W. and E.B. developed the methodology.
535 J.W. performed the formal analysis and created the data visualizations. C.D. created the conceptual visualizations.
536 J.W. and E.B. wrote the first draft of the manuscript and all authors edited and contributed to subsequent drafts.

537 **Competing interests**

538 The authors declare that they have no conflict of interest.

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