

# Soil microorganisms regulate extracellular enzyme production to maximize their growth rate

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## Abstract

Soil carbon cycling and ecosystem functioning can strongly depend on how microbial communities regulate their metabolism and adapt to changing environmental conditions to improve their fitness. Investing in extracellular enzymes is an important strategy for the acquisition of resources, but the principle behind the trade-offs between enzyme production and growth is not entirely clear. Here we show that the enzyme production rate per unit biomass may be regulated in order to maximize the biomass specific growth rate. Based on this optimality hypothesis, we derive mathematical expressions for the biomass specific enzyme production rate and the microbial carbon use efficiency, and verify them with experimental observations. As a result of this analysis, we also find that the optimal enzyme production rate decays hyperbolically with the soil organic carbon content. We then show that integrating the optimal extracellular enzyme production into microbial models may change considerably soil carbon projections under global warming, underscoring the need to improve parameterization of microbial processes.



## Abstract

Soil carbon cycling and ecosystem functioning can strongly depend on how microbial communities regulate their metabolism and adapt to changing environmental conditions to improve their fitness. Investing in extracellular enzymes is an important strategy for the acquisition of resources, but the principle behind the trade-offs between enzyme production and growth is not entirely clear. Here we show that the enzyme production rate per unit biomass may be regulated in order to maximize the biomass specific growth rate. Based on this optimality hypothesis, we derive mathematical expressions for the biomass specific enzyme production rate and the microbial carbon use efficiency, and verify them with experimental observations. As a result of this analysis, we also find that the optimal enzyme production rate decays hyperbolically with the soil organic carbon content. We then show that integrating the optimal extracellular enzyme production into microbial models may change considerably soil carbon projections under global warming, underscoring the need to improve parameterization of microbial processes.

## Plain Language Summary

Understanding how soil microbial communities function and cycle carbon and nutrients is detrimental to accurately project future soil carbon stocks and global warming and to properly manage soils. In order to assimilate carbon and energy, microbial communities produce extracellular enzymes that degrade soil organic matter to then be able to uptake it. However, the enzyme production process requires microbes to invest resources at the expense of their own growth, giving rise to a complex trade-off that has been difficult to predict. Here we leverage a microbial model and recent experimental data on soil microbial communities across the UK to show that soil microbial communities regulate the investment in resource acquisition to maximize their growth rate. This finding provides an underlying principle that describes how soil microbes may regulate physiological trade-offs and results in a mathematical formulation that can be integrated into microbial carbon models to improve predictions of soil carbon cycling.

## 1 Introduction

Soil microbial communities play a major role in the biogeochemical cycles of carbon (C) and nutrients in the biosphere (Falkowski et al., 2008; Paul, 2014; Naylor et al., 2020) and their functioning is essential not only to soil health and fertility (Brady & Weil, 2016), but also to soil carbon sequestration and, in turn, global warming (Singh et al., 2010). Microbially-explicit C models are increasingly being used to account for how microbial processes control soil C decomposition (Allison et al., 2010; J. Schimel, 2001; German et al., 2012; Davidson et al., 2014; Sulman et al., 2018). An important aspect of these models is that through the microbial carbon use efficiency (CUE) —the ratio of C used for growth to C acquired— they account for how microbes are allocating C between anabolism, catabolism, and production of extracellular enzymes (Manzoni et al., 2012; R. L. Sinsabaugh et al., 2013; Geyer et al., 2016; Manzoni et al., 2018). However, how microbes regulate their enzyme production in response to soil environmental conditions is not clear and, as a result, microbial models are formulated assuming that the enzyme production rate is simply proportional to the microbial biomass.

The production of extracellular enzymes is an essential step in the decomposition of soil organic carbon (SOC) (Conant et al., 2011). Soil microorganisms release extracellular enzymes in order to catalyze the depolymerization of complex soil organic matter compounds and produce soluble, low molecular weights compounds that can be readily assimilated (R. Sinsabaugh & Moorhead, 1994). To this end, microbes need to allocate available resources, in the form of carbon and energy, to the synthesis of these enzymes, hence necessarily facing a trade-off (Koch, 1985; Ramin & Allison, 2019; Malik et al., 2019). In fact, while synthesizing extracellular enzymes comes at the expense of

63 growth and carbon use efficiency, higher extracellular enzyme concentrations result in  
 64 faster depolymerization and, in turn, potentially higher C assimilation by microbes. Char-  
 65 acterizing this trade-off and how it is affected by environmental conditions is crucial to  
 66 quantify SOC decomposition dynamics, especially in response to perturbations (Sihi et  
 67 al., 2016; Sulman et al., 2018).

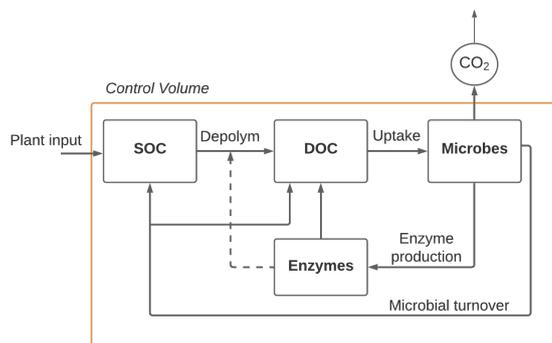
68 Recent developments in microbial C modeling incorporate extracellular enzyme pro-  
 69 duction and activities (Allison et al., 2010; Allison, 2014; Sihi et al., 2016; Sulman et al.,  
 70 2018; Abramoff et al., 2018). Contrarily to traditional C models which adopted a con-  
 71 stant CUE value or directly estimated C decomposition as a first-order reaction not ex-  
 72 plicitly controlled by microbial activity (e.g., (Parton et al., 1987; C. Li, 1996; Manzoni  
 73 et al., 2009)), microbial models with explicit physiology couple C decomposition to mi-  
 74 crobial and extracellular enzyme activities (R. Sinsabaugh & Moorhead, 1994; J. Schimel,  
 75 2001; Allison et al., 2010; Sihi et al., 2016). These new models have the potential to im-  
 76 prove the prediction of soil C dynamics by taking into account the microbial response  
 77 to different biological, physical, chemical conditions across environmental gradients (Wieder  
 78 et al., 2013, 2015). However, they include more equations, state variables, and param-  
 79 eters that make them difficult to calibrate. In-depth analyses of microbial processes and  
 80 trade-offs in resource allocation can improve parameterization of such models and con-  
 81 tribute to reducing the structural and parameter uncertainty in SOC projections.

82 Here, we hypothesize that microbial communities regulate the trade-off between  
 83 growth and production of extra-cellular enzymes in order to maximize the biomass spe-  
 84 cific growth rate. We provide explicit mathematical expressions for the biomass specific  
 85 enzyme production rate and for the microbial CUE, and show that the trade-off between  
 86 growth and enzyme production is controlled by the SOC content. After testing our pre-  
 87 dictions against experimental observations, we show that including this optimal regu-  
 88 lation of enzyme production in microbial models provides a considerably different response  
 89 to warming, especially in terms SOC content.

## 90 2 Theory: optimal regulation of extracellular enzymes production

91 We begin by deriving an  
 92 expression for the production  
 93 of extracellular enzymes based  
 94 on the optimality hypothesis  
 95 that soil microbes regulate the  
 96 production of enzymes to max-  
 97 imize the biomass specific growth  
 98 rate. We will then test our re-  
 99 sults against experimental ob-  
 100 servations in Sec. 3.

101 We leverage the core for-  
 102 mulation of microbial carbon  
 103 models, which reflect our state-  
 104 of-the-art understanding of mi-  
 105 crobial processes in soils. Soil  
 106 microbial processes are gener-  
 107 ally described considering four  
 108 carbon pools, as showed in Fig.  
 109 1 (Allison et al., 2010; Sihi et  
 110 al., 2016). The soil organic car-  
 111 bon (SOC), supplied by plant  
 112 input, is broken down into soluble organic carbon (DOC) via the depolymerization of  
 113 complex SOC molecules, catalyzed by extracellular enzymes (the enzyme pool,  $E$ ) pro-



**Figure 1.** Schematic representation of carbon pools and their interactions in microbial carbon models.

duced by soil microbes ( $B$ ) (J. P. Schimel & Weintraub, 2003; Allison et al., 2010). The microbial biomass uptakes DOC, partly converting it to  $\text{CO}_2$  to obtain energy (i.e., catabolism) and using the rest for synthesizing new biomass and producing extracellular enzymes (Figure 2A). The soil microbial community is thus treated as a single species with average physiological parameters that are meant to represent the large variability of the soil environment.

These microbial processes lead to the following expression for the biomass growth rate (Manzoni et al., 2012; Hagerty et al., 2018),

$$G = U - P_E - R_U - R_B - R_E, \quad (1)$$

where  $U$  is the DOC uptake rate,  $P_E$  is the production rate of extracellular enzymes, and  $R_s$  are the carbon losses via respiration. These include respiration linked to C uptake ( $R_U$ ), to the production of enzymes ( $R_E$ ), and maintenance respiration  $R_B$ . The formulation of  $G$  in Eq. 1 is particularly suitable to variational approaches, as it explicitly distinguishes the terms contributing positively to growth ( $U$ ) from those contributing negatively ( $R_s$  and  $P_E$ ). Eq. 1 in fact contains the trade-off between growth and production of extracellular enzymes, as it accounts for the C costs of producing enzymes ( $P_E$  and  $R_E$ ), which necessarily reduce growth by reducing the microbial CUE (Koch, 1985; Ramin & Allison, 2019) (Figure 2).

The uptake rate  $U$  is expressed as a function of the dissolved organic carbon (DOC) and microbial biomass ( $B$ ), according to the Michaelis-Menten expression

$$U = U_{max} B \frac{\text{DOC}}{\text{DOC} + K_U}, \quad (2)$$

where  $U_{max}$  is the biomass specific uptake rate under non-limiting DOC concentrations and  $K_U$  is the half-saturation constant. We assume that the concentration of DOC is proportional to product of SOC and enzyme concentration  $E$ . Under quasi-steady state conditions for the enzyme pool, the enzyme concentration  $E = P_E/\delta_E$ , so that  $\text{DOC} = \beta \text{SOC} \cdot P_E/\delta_E$  ( $\beta$  being a proportionality coefficient) and the uptake rate

$$U = U_{max} B \frac{\text{SOC} \cdot P_E}{\text{SOC} \cdot P_E + K'_U}, \quad (3)$$

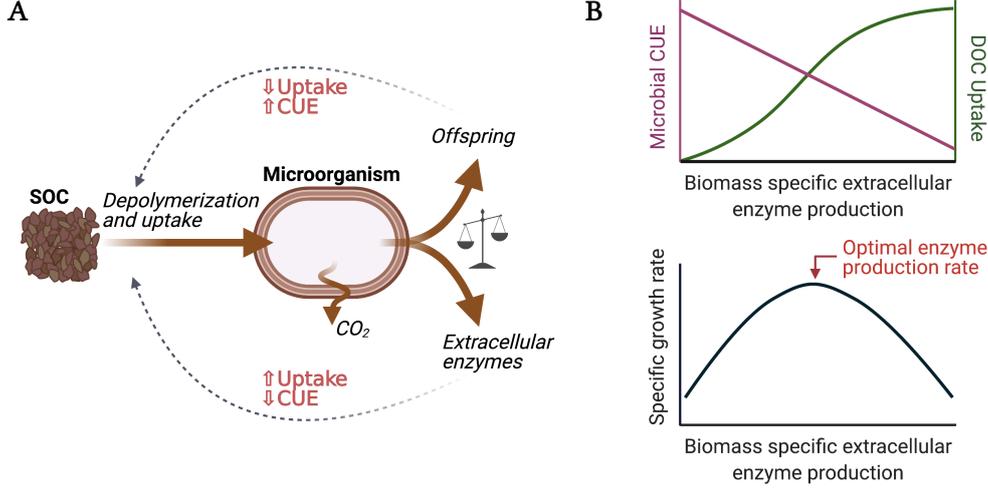
where  $K'_U = \delta_E \cdot K_U/\beta$ ,  $P_E$  is the enzymes production rate, and  $\delta_E$  is the enzymes turnover rate. Expressing, as in microbial models, the respiration fluxes as  $R_U = r_U \cdot U$ ,  $R_B = r_B \cdot B$ ,  $R_E = r_E \cdot P_E$  (where  $r_s$  are respiration coefficients) and the production of enzymes as  $P_E = k_E \cdot B$ , ( $k_E$  is the biomass specific enzyme production rate) the biomass specific growth rate ( $\mu = G/B$ ) can be written as

$$\mu = U_{max} \frac{\text{SOC} \cdot k_E \cdot B}{\text{SOC} \cdot k_E \cdot B + K'_U} (1 - r_U) - r_B - k_E(1 + r_E). \quad (4)$$

Based on the observation that  $B = \alpha \text{SOC}$ , where  $\alpha$  is a constant (see Fig. 2D in (Malik et al., 2018)), Eq. 4 can be re-expressed as

$$\mu = U_{max} \frac{k_E}{k_E + K_U^*} (1 - r_U) - r_B - k_E(1 + r_E), \quad (5)$$

where  $k_U^* = K'_U/(\alpha \text{SOC}^2)$ . Eq. 5 expresses the growth rate as a function of only SOC (which is inside  $k_U^*$ ) and the biomass specific rate of extracellular enzyme production,  $k_E$ . Note also that  $k_E$  appears both in the uptake rate, a term which positively affect  $\mu$ , and in last term ( $k_E(1+r_E)$ ), which negatively affects  $\mu$ . In fact, under given resource availability, higher  $k_e$  result in faster depolymerization of SOC and in turn higher C uptake, but carries higher C costs, hence reducing the CUE (see Figure 2A). On the contrary, for lower  $k_e$  microorganisms can invest more in growth and increase their CUE,



**Figure 2.** **A** Schematic representation of C flow through a microorganism facing a trade-off in C allocation between growth and production of extracellular enzymes. **B** Conceptual plot showing the effect of enzyme production rate on microbial CUE, DOC uptake rate, and specific growth rate. For given resource availability, higher enzyme production rates promote depolymerization and uptake, but the C costs increase, causing the microbial CUE to decrease. As a result the growth rate ( $\mu = \text{CUE} \cdot U/B$ ) has a maximum at an intermediate (“optimal”) enzyme production rate.

152 but this results in lower resource acquisition (uptake). In this trade-off, there is an ideal  
 153 balance between CUE and production of enzymes that maximizes  $\mu$  (Figure 2B).

154 To obtain the expression for the value of  $k_E$  that satisfies our optimality hypoth-  
 155 esis, we compute the derivative of Eq. 5 with respect to  $k_E$ ,

$$\frac{d\mu}{dk_E} = \frac{U^* \cdot k_U^*}{(k_U^* + k_E)^2} - (1 + k_E), \quad (6)$$

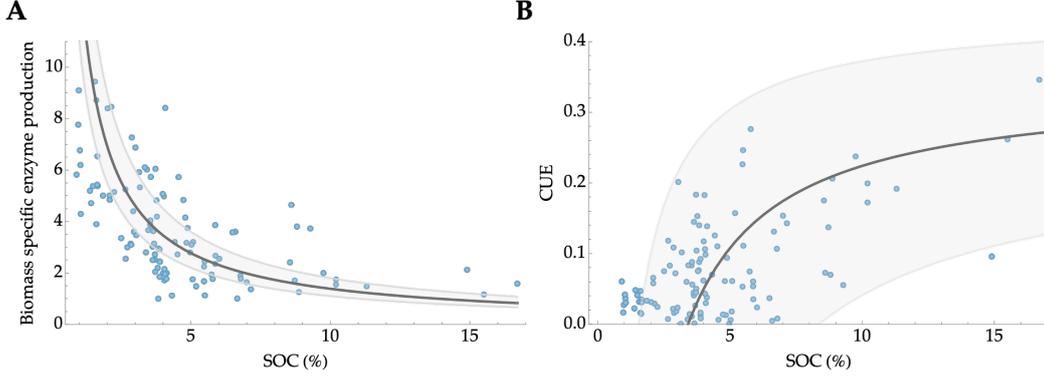
156 where  $U^* = U_{max}(1 - r_U)$  was introduced for conciseness. Setting  $d\mu/dk_E = 0$  and  
 157 solving for  $k_E$  then yields the “optimal” value of  $k_E$  that maximises  $\mu$ ,

$$k_E^{opt} = \sqrt{\frac{U^* k_U^*}{1 + r_E}} - k_U^* \approx \sqrt{\frac{U^* k_U^*}{1 + r_E}}, \quad (7)$$

158 where the last equality derives from the fact that  $k_U^*$  is very small ( $k_U^* \ll 1$ ) and it is  
 159 negligible compared to the first term where it is inside the square root. Lastly, we can  
 160 group all the constants—which encode key information on microbial physiology—to  
 161 obtain a relation between the optimal biomass specific production of enzymes and SOC,

$$k_E^{opt} = \gamma \cdot \frac{1}{\text{SOC}}, \quad (8)$$

162 where  $\gamma = \sqrt{U^* K'_U / (\alpha + \alpha r_E)}$ . According to Eq. 8, which is a mathematical formu-  
 163 lation of the optimality hypothesis,  $k_E^{opt}$  is not a constant, but a function of the SOC  
 164 content, an important indicator of resources availability. Soil microorganisms thus adjust  
 165 their biomass specific enzyme production rate  $k_E^{opt}$  to maximize the growth rate  $\mu$  by “sens-  
 166 ing” the availability of resources (i.e., “SOC”) in their environment. From this optimal-  
 167 ity hypothesis, we can also predict how the microbial CUE and the growth rate change



**Figure 3.** **A** Relationship between biomass specific enzyme production and SOC. The continuous line is computed from Eq. 8, fitted by means of a Major Axis Regression ( $p < 0.001$ ). **B** Relationship between microbial CUE and SOC. The continuous line is computed from Eq. 9, also fitted by means of a Major Axis Regression ( $p < 0.01$ ). Gray shaded areas indicate the confidence bands. The experimental observations of biomass specific enzyme production rate, SOC, and CUE are from Malik et al. (2019).

168 with respect to SOC. Substituting Eq. 8 in Eq. 5 and dividing by  $U/B$  yields a relation-  
 169 ship of the form

$$\text{CUE} = \text{CUE}_0 - \frac{b}{\text{SOC}}, \quad (9)$$

170 where  $\text{CUE}_0 = 1 - r_u - r_B/(U_{max}\eta)$ ,  $b = (\gamma\sqrt{U^*(1+r_E)})/(U_{max}\eta)$ , and  $\eta = \gamma/(\gamma +$   
 171  $k'_U/\alpha)$ . Combining Eqs. 8 and 9 then yields the relationship between CUE and  $k_E^{opt}$ ,

$$\text{CUE} = \text{CUE}_0 - \frac{b}{\gamma} k_E^{opt}, \quad (10)$$

172 Finally, from Eq. 8 and the fact that  $\text{CUE} = B\mu/U$ , the specific growth rate  $\mu = U_{max}\eta\text{CUE}$ .

### 173 3 Experimental verification

174 To test if soil microorganisms regulate their investment in extracellular enzymes  
 175 so as to maximize their specific growth rate, we checked whether the observations of  $k_E$   
 176 and CUE vary with SOC as predicted by Eqs. 8 and 9, respectively. To this regard, we  
 177 retrieved experimental data from Malik et al. (2019), which measured biomass specific  
 178 enzyme production and carbon use efficiency in circumneutral pH soils from 38 sites across  
 179 the United Kingdom. Sites have variable land uses, including pristine and intensive grass-  
 180 lands and arable soils. Soils also had different SOC content, allowing us to specifically  
 181 test our expressions. We refer to Malik et al. (2018) and Malik et al. (2019) for more de-  
 182 tails on soil sampling and analyses. The measurements do not provide directly  $k_E$ , but  
 183 a proxy for it. Therefore, here we focused on how  $k_E$  decays with the SOC content and  
 184 not on the specific values of  $\gamma$  or the coefficient relating  $k_E$  to its proxy. We then sim-  
 185 ply fitted the value of  $\gamma$  through a standard least squares method and tested whether  
 186 Eqs. 8, 9, and 10 match the observations. By doing so, we also avoided introducing un-  
 187 certainty in the choice of physiological parameter values.

188 Figure 3A readily shows that Eq. 7 matches the observations throughout the enti-  
 189 re range of measured SOC, lending support to our hypothesis that microorganisms opti-  
 190 mize their biomass specific production of enzymes to maximize the specific growth rate,  
 191  $\mu$ , for given SOC content. Our theory thus provides a principle according to which soil

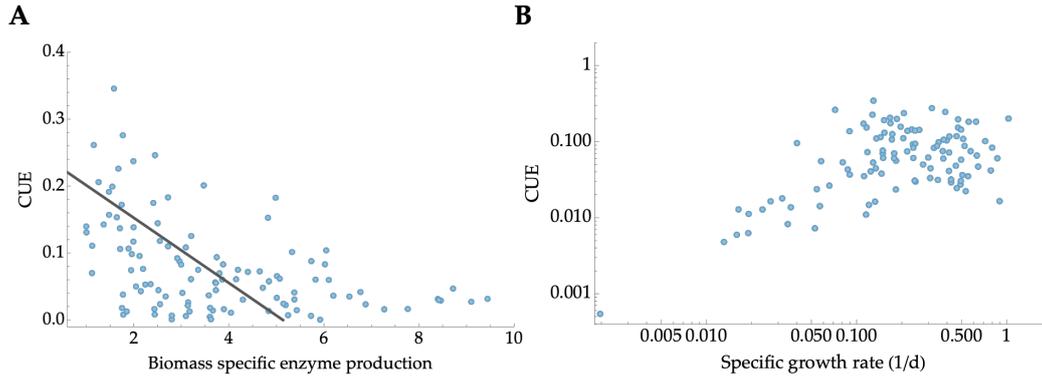
192 microorganisms regulate the extracellular enzymes-growth trade-off. At low SOC con-  
 193 tents microorganisms find it convenient (or are forced) to invest highly in enzymes so  
 194 as to accelerate the depolymerization of the available SOC content, even if this is low,  
 195 and obtain enough DOC to uptake. On the contrary, at high SOC contents depolymer-  
 196 ization can proceed at high rates even with lower enzyme concentrations. Accordingly,  
 197 rather than producing excess enzymes with limited benefits, microbes can “afford it” to  
 198 invest more heavily in the biosynthesis of new biomass. The scatter around the predicted  
 199 curve is expected, as these measurements include soils with different land use and land  
 200 cover, soil pH, and microbial diversity (Malik et al., 2018), in addition of course to pos-  
 201 sible measurement error and uncertainty.

202 Figure 3B shows that our predictions also match the relationship between CUE and  
 203 SOC, independently supporting our optimality hypothesis. Eq. 9 provides a mathemat-  
 204 ical formulation for microbial regulation of the community CUE under optimal regula-  
 205 tion of C allocation between growth and production of extracellular enzymes. As we have  
 206 seen above, at low SOC soil microorganisms need to produce more enzymes to obtain  
 207 sufficient labile substrate, and as a result the CUE (fraction of C allocated to biosyn-  
 208 thesis of new biomass) declines. As the SOC increases, microbes invest less in enzyme  
 209 production and more into growth, hence increasing their CUE up to their physiological  
 210 maximum,  $CUE_0$ . This trade-off becomes clearly evident in the negative relationship be-  
 211 tween CUE and  $k_E^{opt}$  expressed by Eq. 10 (Figure 4A).

## 212 4 Discussion and implication

213 Soil microorganisms are biological machines able to evolve and adapt to local en-  
 214 vironmental conditions to improve their fitness (von Stockar, 2013; Falkowski et al., 2008;  
 215 Roach et al., 2018; Noda-Garcia et al., 2018; Naylor et al., 2020). To provide a more phys-  
 216 ical foundation to the concepts of evolution and adaptation, there have been a number  
 217 of studies trying to identify quantitative measures of fitness for microbial communities,  
 218 such as the growth rate (Westerhoff et al., 1983; Manzoni et al., 2017), the growth yield  
 219 (Bachmann et al., 2013), or the energy efficiency (Maitra & Dill, 2015), from which a  
 220 variety of optimality principles were formulated. According to which one of these prin-  
 221 ciples microorganisms evolve remains an open question, whose answer may depend on  
 222 the environmental conditions (Lipson, 2015) and the timescale of observation (Roach et  
 223 al., 2018), but also on the specific independent variables that are being explored, those  
 224 that are being kept fixed, and what is included as mathematical constraints (Martyushev  
 225 & Seleznev, 2006). Here we hypothesized that soil microorganisms regulate the produc-  
 226 tion of extracellular enzymes in order to maximize the biomass specific growth rate, while  
 227 all other physiological parameters (e.g., maximum uptake rate  $U_{max}$ , biomass specific  
 228 maintenance rate  $r_B$ ) are considered constant, or better that they do not vary with  $k_E$ .  
 229 The hypothesis is supported for circumneutral pH soils by experimental observations.

230 To better understand this optimal regulation of enzyme production from a phys-  
 231 ical point of view, we can further analyze Eq. 7, focusing in particular on  $k_U^*$  ( $= (K_U \cdot$   
 232  $\delta_E)/(\alpha \text{ SOC}^2)$ ). As a half-saturation constant for the functional response of microbial  
 233 uptake to  $k_E$  (see Eq. 5),  $k_U^*$  measures how effective an increase in  $k_E$  would be in in-  
 234 creasing microbial C uptake. A high value of  $k_U^*$  indicates that external conditions are  
 235 not favorable to the action of enzymes and the formation of DOC (i.e., lower SOC) and  
 236 that there is a relatively low number microorganisms available to produce enzymes. In  
 237 turn, when  $k_U^*$  is high a microorganism needs to invest more in enzymes to catalyze the  
 238 formation DOC and uptake it. By contrast, a low value of  $k_U^*$  is indicative of favorable  
 239 environmental conditions and higher microbial biomass, such that a microorganism can  
 240 reallocate more resources to growth. Interestingly, inside the square root,  $k_U^*$  is then mul-  
 241 tiplied by the C gains (in terms of growth,  $U^* = U_{max}(1 - r_U)$ ) and divided by the C  
 242 costs ( $1 + r_E$ ) of producing more enzymes. This emphasizes the fact that microorgan-



**Figure 4.** **A** Trade-off relationship between CUE and biomass specific enzyme production. The continuous line is computed from Eq. 8. **B** Derived relationship between microbial CUE and specific growth rate, reflecting a resource acquisition strategy (Lipson, 2015). The experimental observations of biomass specific enzyme production rate and CUE are from (Malik et al., 2019).

isms both evaluate whether producing more enzymes is actually necessary to increase uptake (through  $k_U^*$ ) and weigh C benefits and costs (through the ratio  $U^*/(1+r_E)$ ).

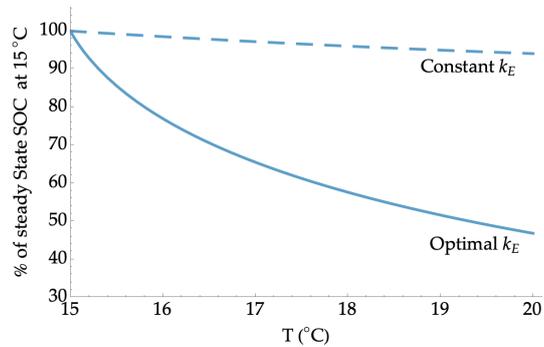
Resource allocation strategies have the potential to greatly impact SOC sequestration and ecosystem functioning, for instance by affecting the relation between growth rate and yield (here CUE) (Lipson, 2015; Ferenci, 2016). Interestingly, our optimality hypothesis (investment in extracellular enzymes to maximize the growth) leads to a positive relationship between the CUE and the specific growth rate (i.e.,  $\mu = U_{max}\eta CUE$ ), in agreement with what hypothesised by Lipson (2015) for a resource acquisition strategy. This is also in line with what was observed in these soils through metaproteomics (Malik et al., 2018), which indicated higher investment in growth and in resource acquisition in higher SOC and lower SOC soils, respectively. The observations follow the predicted trend (Figure 4B), at least up to a growth rate of  $\approx 0.3$  ( $d^{-1}$ ), after which the CUE tends to decrease. However, the regulation of enzyme production is only one of the multiple factors determining the CUE vs. growth rate relationship. Across the sampled soils, there might be multiple strategies occurring simultaneously and, while resource acquisition may be more important at lower growth rates and CUE (i.e., the increasing branch of the curve in Fig. 4B), fast and energy-wasting growth might affect the CUE at higher growth rates (i.e., the decreasing branch of the curve). Nevertheless, observations span a variety of soils with their own specific properties (e.g., microbial diversity, carbon sources, etc.) and each measurement averages out multiple microbial species and their different strategies within a given soil sample. These factors make it difficult to fully interpret the relationship between CUE and growth rate shown in Figure 4B.

Due to the nonlinear, intertwined interactions between microbial processes and SOC dynamics, the effects of microbial regulation of extracellular enzymes production can also propagate in scale and impact the long-term SOC dynamics (Wieder et al., 2015). Indeed, microbial carbon models have been developed to account for the importance of microbial extracellular enzyme production and depolymerization of SOC, a possible rate-limiting step in SOC decomposition (Bengtson & Bengtsson, 2007; Conant et al., 2011), and improve SOC projections in response to global warming (Allison et al., 2010; Wieder et al., 2015). Here we explored whether improving the representation of enzyme production using our optimality hypothesis gives rise to a different response to warming. To this end, we considered the temperature dependence of the depolymerization and uptake rates, the maintenance respiration, and the half-saturation constants, and analyzed

the steady state SOC content as a function of a temperature increase. The results readily show that the SOC depletion upon warming is substantially more pronounced when using  $k_E^{opt}$  than with a constant  $k_E$  (see Figure 5), suggesting that the representation of enzyme production can considerably alter SOC projections. The optimality hypothesis in fact introduces a positive feedback on SOC depletion. As the SOC content decreases, microbial communities produce more extracellular enzymes and accelerate the depolymerization, further enhancing SOC depletion.

While these results highlight the importance of integrating microbial regulation of extracellular enzyme production in microbial models, Figure 5 should still be interpreted with caution since additional processes affect SOC projections. First, the interaction between biomass (e.g., necromass) and mineral surfaces may contribute to SOC stabilization and regulate its depletion under higher temperatures. This may be particularly important in situations (e.g., regeneration of degraded soils) in which microbial communities can switch strategy and start investing more in growth (higher CUE), which will eventually lead to more production of microbial necromass that can be stabilized on mineral surfaces. Second, while temperature does not affect how  $k_E^{opt}$  decays with SOC, it may affect the coefficient  $\gamma$ , which here was calibrated so as to obtain the same steady state SOC as “traditional” microbial models with constant  $k_E$  at 15 °C. This assumption may thus have affected the depletion in SOC content at higher temperatures. There are other possible mechanisms –often not taken into account– that can alter the response to warming, such as the fact that there might be a limited number of SOC-enzymes reaction sites or that microbial communities may feed on free enzymes (Sihi et al., 2016). Microbial communities may also adapt to changes in temperature and regulate their CUE (Allison, 2014), a strategy that may have an impact on resource acquisition investments such as on extracellular enzymes production. Experiments on the effects of soil warming on microbial traits (CUE,  $k_E$ ,  $\mu$ ) (J. Li et al., 2019; Nottingham et al., 2020) and microbes-mineral interactions (Kleber et al., 2021) may provide additional clues to understand the impact of warming on microbial processes more holistically.

Lastly, integrating resource allocation strategies in microbial carbon models (such as Eqs. 8 and 9) may improve their prediction of SOC dynamics, not only in response to warming but also when soils experience temporal variability in resources, such as agricultural ecosystems (Wei et al., 2014; Smith et al., 2016; Sanderman et al., 2017). In these systems the continuous regulation of extracellular enzyme production as resource availability vary might not be captured by a constant  $k_E$  value, likely introducing uncertainty. Improving the parameterization of microbial processes in these cases might help better understand the effect of regeneration practices as well as design suitable agricultural practices for preserving soil health (Kallenbach et al., 2019).



**Figure 5.** Depletion in steady state soil SOC pool as a function of temperature, highlighting the important role that enzyme production regulation plays in SOC projections. We used default parameters provided by (Hagerty et al., 2018),  $Q_{10}$  values from (Sihi et al., 2016), and temperature dependence of half-saturation constants from (Allison et al., 2010).

## 5 Conclusions

There are still large uncertainties in the representation of microbial processes in microbial models, causing them to diverge in their C projections under warming (e.g., (Sihi et al., 2016; Sulman et al., 2018)). Here we focused on the production of extracellular enzymes for the depolymerization of SOC and showed, by interpreting experimental observations, that soil microbes regulate enzyme production so as to maximize their specific growth rate for given SOC content. While microbes invest largely in resource acquisition (i.e., extracellular enzymes) at low SOC contents, they adjust their resource allocation to invest more on biosynthesis of new biomass at higher SOC contents, leading to higher microbial CUE. Our analysis also provided a mathematical expressions for including the regulation of extracellular enzyme production into microbial models and showed that this optimal regulation may result in considerably different SOC projections under warming from those of models assuming a constant biomass specific production rate. Future investigations will explore how to improve parameterization of extracellular enzyme production in state-of-the-art soil microbial and ecosystem models, especially considering mineral-microbes interactions.

## Data Availability Statement

The original data used in this paper can be found in Malik et al. (2018) and Malik et al. (2019). Processed data and the Mathematica code used to draw the Figures is available at 10.4211/hs.7ada4439903f4156be3143e36a9693c7 under a Creative Common Attribution CC-BY.

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