Soil Biology & Biochemistry 53 (2012) 133–141

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition

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ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form
10 May 2012
Accepted 13 May 2012
Available online 2 June 2012

Keywords:
Decomposition
Extracellular enzymes
Model
Ecological stoichiometry
Threshold element ratio (TER)
Overflow metabolism
Carbon use efficiency

ABSTRACT

We developed an Extracellular EnZYme model (EEZY) of decomposition that produces two separate pools of C- and N-acquiring enzymes, that in turn hydrolyze two qualitatively different substrates, one containing only C (e.g., cellulose) and the other containing both C and N (e.g., chitin or protein). Hence, this model approximates the actions of commonly measured indicator enzymes ß-1,4-glucosidase and ß-1,4-N-acetylglucosaminidase (or leucine aminopeptidase) as they hydrolyze cellulose and chitin (or protein), respectively. EEZY provides an analytical solution to the allocation of these two enzymes, which in turn release C and N from the two substrates to maximize microbial growth. Model behaviors were both qualitatively and quantitatively consistent with patterns of litter decay generated by other decomposition models. However, EEZY demonstrated greater sensitivity to the C:N of individual substrate pools in addition to responding to factors directly affecting enzyme activity. Output approximated field observations of extracellular enzyme activities from studies of terrestrial soils, aquatic sediments, freshwater biofilm and plankton communities. Although EEZY is largely a theoretical model, simulated C- and N-acquiring enzyme activities approximated a 1:1 ratio, consistent with the bulk of these field observations, only when the N-containing substrate had a C:N ratio similar to commonly occurring substrates (e.g., proteins or chitin). This result supported the emerging view of the stoichiometry of extracellular enzyme activities from an environmental context, which suggests that a relatively narrow range of microbial C:N, carbon use efficiency and soil/sediment organic matter C:N across ecosystems explains the tendency towards this 1:1 ratio of enzyme activities associated with C- and N-acquisition. Sensitivity analyses indicated that simulated extracellular enzyme activity was most responsive to variations in carbon use efficiency of microorganisms, although kinetic characteristics of enzymes also had significant impacts. Thus EEZY provides a quantitative framework in which to interpret mechanisms underlying empirical patterns of extracellular enzyme activity.

1. Introduction

Decomposer microorganisms obtain their energy and nutrients from dead organic matter by producing hydrolytic and oxidative enzymes that catalyze the extracellular degradation of complex organic molecules (Burns, 1978; Burns and Dick, 2002). It is difficult to quantify the mass of enzymes in environmental samples, but their potential activities can be readily assayed (Sinsabaugh et al., 1997; Marx et al., 2001) and compared to rates and patterns of litter decay (Sinsabaugh, 1994). Recent syntheses indicate that the relative balance in activities of extracellular enzymes responsible for C, N and P acquisition reflect the stoichiometric (balance of elements) and metabolic (energy) needs of microorganisms (Sinsabaugh et al., 2008, 2009, 2010, 2011). Moreover, Sinsabaugh et al. (2009) suggest that a convergence in microbial characteristics (C:N, carbon use efficiency), organic matter chemistry (C:N), and patterns in extracellular enzyme activities reveal a global pattern of the stoichiometry of extracellular enzyme activity across ecosystems. However, few mathematical models have sufficient mechanistic resolution to simulate these patterns (Allison, 2005; Davidson et al., 2012).

The most widely measured enzyme activities generate consumable products from the hydrolysis of the most common pools of detrital organic matter. Cellulose is the largest product of plant production and ß-1,4-glucosidase (ßG), which hydrolyzes glucose from cellobiose and other celloligosaccharides, is the most commonly measured cellulose-degrading enzyme. The largest

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doi:10.1016/j.soilbio.2012.05.011

organic N sources are proteins and chitins. For protein, leucine aminopeptidase (LAP) hydrolyzes the most abundant protein amino acid from the ends of polypeptides and is the most commonly measured indicator enzyme. For chitin, the most commonly measured indicator enzyme is ß-1,4-*N*-acetylglucosaminidase (NAG), which hydrolyzes *N*-acetylglucosamine from chitobiose and other chito-oligosaccharides (Sinsabaugh, 1994; Sinsabaugh et al., 1997). Although the degradation of polymeric compounds into assimilable substrates usually requires the interactions of many enzymes, the activities of hydrolytic enzymes that target the same classes of compounds are strongly correlated (Sinsabaugh et al., 2011). Because the activities of extracellular enzymes link microbial metabolism to decomposition processes, they provide a uniquely mechanistic insight to biogeochemical cycles (Sinsabaugh et al., 2008, 2010, 2011; Sinsabaugh and Shah, 2011; Marklein and Houlton, 2011). Not surprisingly, more studies of environmental enzymes, decomposition and soil organic matter dynamics couple C and N biogeochemical cycles than any other elements.

Manzoni and Porporato (2009) recently reviewed 250 biogeochemical models and found that few of them described decomposition as a direct product of microbial activity, and even fewer included the activities of extracellular enzymes. The best-known exception was the model developed by Schimel and Weintraub (2003), which includes one pool of extracellular enzymes that hydrolyzes one pool of soil organic matter for use by one pool of microorganisms. It is based on a general, mechanistic model developed by Parnas (1976) that linked microbial C:N stoichiometry (CN_M) to soil organic matter C:N content (CN_S) by carbon use efficiency (CUE; fraction of carbon released from organic matter that is incorporated in biomass), such that decomposition exactly meets microbial C and N demands for growth when $CN_M = CN_S/CUE$. However, Parnas (1976) calculated decay rate with a typical Michaelis–Menten equation of substrate-saturation, i.e., substrate pool size regulated the rate. In contrast, Schimel and Weintraub (2003) used a Michaelis–Menten equation of enzyme-saturation, which regulated decay rate by the amount of extracellular enzyme present. This was a substantial improvement over an earlier, possibly the first, enzyme-based decomposition model (Sinsabaugh and Moorhead, 1995), which calculated decay rate as a first-order function of C-acquiring enzyme activity. However, neither of these two enzyme-based models included more than one pool of enzymes, so that model output could not be compared to empirical observations of relative C- versus N-acquiring enzyme activities (Sinsabaugh, 1994; Sinsabaugh and Moorhead, 1994).

Our primary objective in the present study was to develop a decomposition model that used two specific enzymes to gain C and N from organic matter to meet microbial energy and nutrient requirements. This is the minimum model complexity that can be used to explore the factors responsible for balancing the activities of these enzymes reported from experimental observations. We also wanted a parsimonious approach that was yet sufficient to capture these relationships. To this end, we integrated key methods used by Parnas (1976), Sinsabaugh and Moorhead (1994, 1995) and Schimel and Weintraub (2003) to model the growth and metabolism of a microbial community, and the production of two distinct kinds of enzymes, one that degrades organic molecules containing both C and N (e.g., chitin or protein) and another that degrades molecules containing C but not N (e.g., cellulose). These are the two most common elements included in decomposition models (Manzoni and Porporato, 2009) and enzymes associated with their acquisition are among the most commonly assayed in field studies (Sinsabaugh et al., 2008, 2010, 2011). We then used this model of Extracellular EnZYme (EEZY) activities to generate patterns of enzyme production and activity in response to variations in soil organic matter chemistry, to compare with recent syntheses of field data (Sinsabaugh et al., 2009, 2010, 2011; Sinsabaugh and Shah, 2011). Finally, we conducted a sensitivity analysis to determine the relative importance of variation in key model parameters that represent key features of microbial C and N demands, and kinetics of enzymes responsible for C and N acquisition.

2. Modeling approach

EEZY included six pools of organic carbon: (1) a carbon- + nitrogen substrate (C_1), hydrolyzed by (2) an extracellular pool of enzymes (E_{C1}), (3) a carbon-only substrate (C_2), hydrolyzed by (4) another pool of enzymes (E_{C2}), which together produce a pool of (5) dissolved organic carbon (DOC) that was consumed by (6) microorganisms (B_C) (Fig. 1). Although the model equations are provided in Appendix 1, we present herein those that define the novel feature of EEZY. At the core of our model is the "reverse" Michaelis–Menten equation described by Schimel and Weintraub (2003). They coined this term because this equation estimates

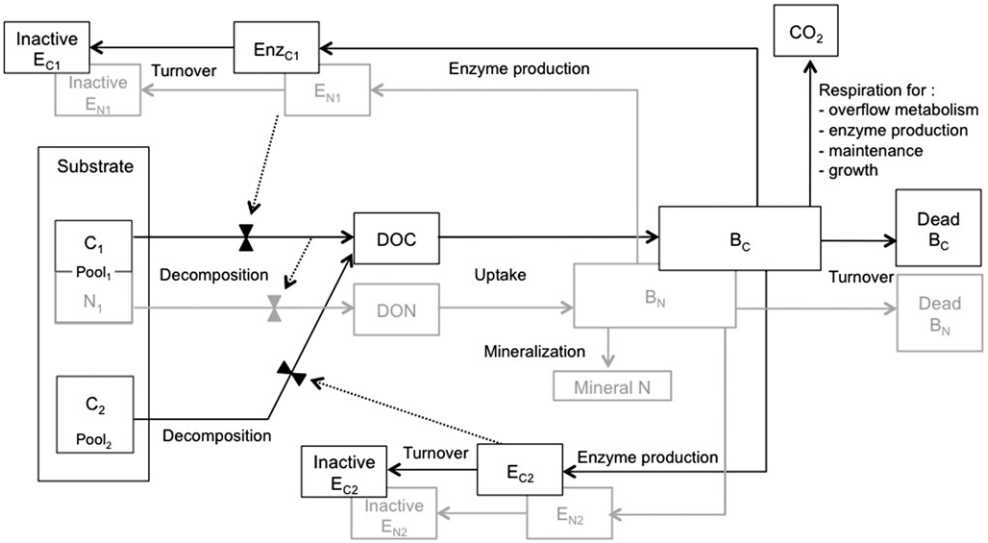

Fig. 1. Carbon (black lines) and nitrogen (gray lines) flow diagram for the EcoEnZYme model (EEZY).