Soil Organic Matter Does Not Break Itself Down: The Implications of Exoenzyme Activity on C Flow and Microbial Carbon and Nitrogen Limitation in Soil

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Introduction

There are several contradictions apparent in our understanding of soil microbial processes. The first is that microbes often appear carbon limited, even in soils with plentiful biodegradable C, such as peat. The conclusion of C limitation comes from results such as those of Waksman and Stevens (1929), who stated, "The fact that the addition of available nitrogen, phosphorus, and potassium did not bring about any appreciable increase in the evolution of CO_2 points definitely to the fact that nitrogen is not a limiting factor in the activities of microorganisms in peat but that the available carbon compounds are." The second contradiction is that while C-based studies almost always conclude microbial C limitation, N-based studies often conclude microbial N-limitation (Nadelhoffer et al. 1984, Giblin et al. 1991, Polglase et al. 1992, Wagener and Schimel 1998, Jackson et al. 1989, Hart et al. 1994, Chen and Stark 2000, Schimel and Firestone 1989). Even in a soil that is actively immobilizing N (suggesting microbial N limitation), adding C almost always enhances respiration (suggesting C limitation).

We believe that at least a partial resolution to both these apparent contradictions may lie in the nature of organic matter processing and our conceptual and mathematical models to describe it. Most soil organic matter models use simple first order kinetics:

$$dC/dt = K * M_d * T_d * C \qquad \text{(Parton et al. 1987)}$$

In this equation, C is the size of a soil carbon pool, K is a first order rate constant, and M_d and T_d are reducing functions based on temperature and moisture. Each soil organic matter (SOM) pool has a single K value that defines its quality. This approach to SOM dynamics is at the heart of almost all SOM models (e.g., van Veen et al. 1984, Parton et al. 1987, Chertov and Kumarov 1996, Li 1996) and is found in every soil biology textbook we examined (Alexander 1977, Killham 1994, Coleman and Crossley 1996, Paul and Clark 1996, Sylvia et al. 1998). The argument that grows out of this approach is that a low enough K induces C and energy limitation, since the decay of SOM is slow enough that it only provides adequate C to support the maintenance requirements of the soil biomass. However, the simple argument that a low K value induces C limitation is flawed in a fundamental assumption. The flaw is that that, biochemically, SOM decomposition is *not* simply first order on SOM. Rather, SOM breakdown is catalyzed by extracellular enzymes that are produced by microorganisms. To accurately describe the kinetics of catalyzed reactions, the concentration of the catalyst *must* be part of the rate equation (Roberts 1977), producing at least second order or likely even more complex kinetic relationships. The most familiar such rate equation is the Michaelis-Menton equation:

$$dC/dt = K * E * C/(K_m+C) \qquad \text{(Roberts 1977)}$$

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where K is the decomposition constant, E is catalyst concentration, and K_m is the half-saturation constant. The equation is commonly simplified by assuming that E is constant, and thus can be combined with K into a "V_{max}" term (the maximum reaction rate, defined as K*E). Under some conditions, this relationship can be effectively simplified to a pseudo first order equation, but even in that case, catalyst concentration remains part of the rate equation (Schimel 2001, Schimel and Weintraub 2003). At the microbial scale, considering SOM breakdown kinetics and C supply to microbes requires considering the dynamics of the catalyst to accurately model processes (Vetter et al. 1998, Schimel 2001). A number of authors have discussed the role of exoenzymes in controlling decomposition kinetics (e.g., Sinsabaugh and Moorhead 1994, Foreman et al. 1998, Moorhead and Sinsabaugh 2000). However, we argue further – to understand the basis of microbial C and N limitation in soil, it is necessary to consider the nature of catalysis and the mechanisms of C flow at the microbial scale.

The argument that a low K value alone should induce C limited microbes fails in a model that includes exoenzymes because if microbes increase their investment in exoenzymes, they should be able to accelerate the breakdown of SOM and increase the flow of C back to the microbes, thus alleviating C limitation, regardless of the fundamental K value for organic matter breakdown. Thus, recalcitrance, in terms of a low K value can not, by itself, induce C limitation. The key control of C limitation becomes the "return on investment" microbes get in producing exoenzymes. That return is partially dependent on K, but also on the allocation of resources to enzymes, the functional kinetics of enzyme activity, and the lifetime of the enzymes.

To test these ideas theoretically we developed two simple theoretical models of C flow from soil organic matter through the microbial system (*fig. 1*; Schimel and Weintraub, 2003); the first treats a C-limited system, while the second develops a simple system with linked flows in which either C or N can limit microbial growth (whichever is in limiting supply). The fundamental model structures are simple. There are,

Figure 1a. C-only model structure

Figure 1b. Linked C & N model structure

however, several major differences from traditional SOM models. The core difference is that the model separates the "biochemical" processes of depolymerization from the "biological" processes of uptake and metabolism, allowing disconnects between them. Thus C flow to microbes may be controlled by somewhat different mechanisms than actual use of that C. Microbes growing on N-poor material may find themselves C saturated, i.e., getting more C than they can use for growth, and having to use the rest in "overflow metabolisms" (Tempest and Neijssel 1992). The model is described in detail in Schimel and Weintraub (2003). There were a number of important conclusions that arose from that modeling exercise:
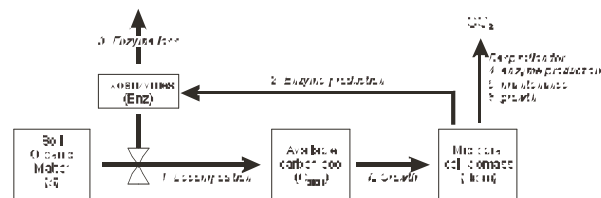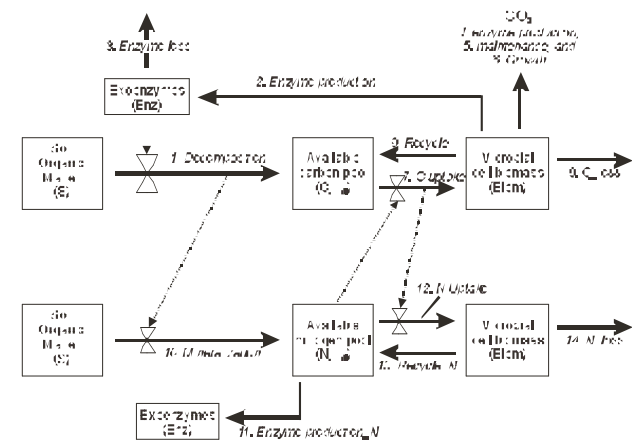
1. Microbial C limitation is regulated by the return on investment in exoenzymes – only if this value is zero or less will microbes be C limited. As long as microbes receive more C and energy back from decomposition than they "spent" synthesizing the enzymes responsible, they will be able to grow and produce more enzymes.
2. Enzyme kinetics must be non-linear, with a decreasing rate of activity with increasing enzyme concentration. Linear kinetics make the system unstable, since a constant, positive return on investment leads to runaway enzyme synthesis and decomposition rates. Non-linear kinetics, however, ultimately lead to C-limited microbes even in the presence of potentially available C.
3. Because the kinetics of the exoenzyme system stabilize C flow, microbial growth may be limited by N without altering the overall flow of C in the system. Carbon that isn't used to support growth is still consumed through one of several "overflow metabolism" processes. At very low available N levels, altered allocation to exoenzyme synthesis may reduce actual C flow to microbes as well.

By changing the way we view the most basic elements of decomposition, and by adding the mechanism of decomposition, this model can explain some of the apparent contradictions in soil biology. A small investment of C and N in exoenzymes is capable of maintaining the maximal rate of polymer breakdown and a flow of C from detritus and soil organic matter to microbes. The non-linearity of enzyme function limits C flow, and can induce C limitation of respiration, even while microbial growth may be N limited. This can also explain why C flow and soil respiration have often appeared insensitive to N addition, and so suggest C limitation, even in soils where N immobilization and NO_3^- assimilation data strongly suggest microbial N limitation. Though the results of the model are consistent with, and can explain, many field and lab studies, the model is still purely theoretical. However, some of the model predictions can be framed as testable hypotheses, and we did so to guide our research in our first Kearney proposal:

1. Microbes become C limited regardless of the potential availability of polymeric C through non-linear exoenzyme kinetics.
2. Polymer breakdown and microbial monomer use become disconnected following stress events.
3. As substrate C/N ratio increases, waste generation of DOC/DON will occur.
4. Adding C will always increase microbial respiration, but when microbes are N limited it will not increase microbial biomass.
5. Adding N will not increase soil respiration, rather when microbes are N limited it may reduce respiration by diverting C flow from overflow respiration to microbial growth.

To test those hypotheses, we have been working with three soils to establish a gradient of relative C and N availability. These soils include the following:

*   **Annual grassland from Sedgwick Reserve.** This soil appears to be strongly C limited.
*   **Conifer forest floor from Sequoia National Park.** This soil appears to be intermediate in terms of C vs. N limitation.
*   **Arctic tussock tundra.** This soil has shown evidence of being extremely N limited.

Currently our work is still underway. In our original proposal we made it clear that a limited two-year project could fully test the ideas developed here, but we have also faced some unanticipated challenges and the learning curve for some of the work has been steep. Thus, we have not accomplished all that we set forth in our first Kearney proposal. The major obstacle we have faced was preparing the isotopically labeled polymers. Unfortunately, labeled starting monomers for synthesizing "artificial" lignin were no longer available commercially. We have therefore had to synthesize our own from scratch. That has been a substantial task in synthetic organic chemistry. To accomplish it we established a collaboration with Dr. Dan Little in the Department of Chemistry & Biochemistry. He had a talented undergraduate working up the synthesis as an independent research program. As a result of this, however, we have not accomplished the work we had proposed analyzing C flow from specific polymers. We hope to finish this work in a second phase of funding from the Kearney Foundation. Here we describe some of the important progress we have made on our work so far. We focus on the work with cellulase as this is the most complete data set we have.

Hypothesis 1. Carbon flow to microbes become C limited regardless of the potential availability of polymeric C through non-linear exoenzyme kinetics.

The work under this hypothesis is critical in determining the nature of exoenzyme kinetics in soils. Do enzymes show saturation kinetics on substrate? We have experimented with adding cellulase at a range of concentrations in all three soils (Sedgwick, Sierra, and Tussock) and found a kinetic response that showed saturation of enzymes on substrate (*fig. 2*), even in a soil that contain 30% α-cellulose and almost 20% hemicellulose.

Fig. 2. Kinetic response to added cellulase. A) Sedgwick soil. B) Tussock soil.

The second aspect of this hypothesis is estimating the functional lifetime of enzymes in soil, since this is an important component of an organism's return on investment in enzymes, and hence in controlling the extent of C limitation. We did this by incubating soils under $CHCl_3$ and periodically assessing cellulase activity. We found that there was as much as a 50% decrease over about three days, but potentials then remained stable for at least a week (*fig. 3*). Thus, there appear to be two cellulase pools – one that turns over rapidly and may be associated with organisms and thus vulnerable to proteases, and a more stable pool with turnover time of weeks. The model assumes 1st order loss term for enzymes (half life of ca. two weeks), which

Fig. 3. Potential Cellulase Activity of Soils Stored in Gas Phase Chloroform
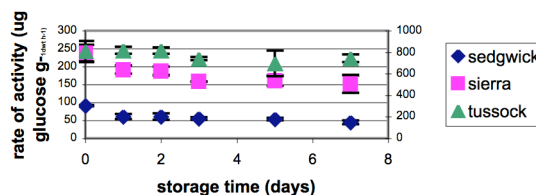
appears to be an oversimplification but at least the right order of magnitude.

Hypothesis 2. Polymer breakdown and microbial monomer use become disconnected following stress events.

Testing this hypothesis requires isotopically labeled substrates. Work is still underway.

Hypothesis 3. As substrate C/N ratio increases, waste generation of DOC/DON will occur.

As an initial test of this hypothesis, we incubated the three soils with differing amounts of added cellulose, and periodically leached them to measure the amount of dissolved organic C (DOC) generated. We then incubated the leached DOC to estimate its biodegradability. The results are at least broadly consistent with model predictions (*fig. 4*). The C limited Sedgwick soil showed no increase in DOC with added cellulose (*fig. 4*), though cellulase activity increased (*fig. 5*). DOC generation from Sierra increased substantially with added C. DOC from tussock soils increased slightly, but that was coincident with a slight decrease in cellulase (*fig. 5*). Interestingly, the "quality" of the DOC appeared to be unaffected by adding cellulose to the soils.


Fig. 4. Effects of added C (as cellulose) on DOC generation
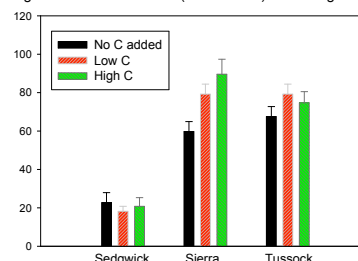
Hypothesis 4. Adding C will always increase microbial respiration, but when microbes are N limited it will not increase microbial biomass.

The responses to C additions were somewhat consistent with the predictions of the model. Relative increases in respiration declined with both increasing N limitation (Sedgwick , Sierra, Tussock) and with increasing C addition (*fig. 5*). Even though we added cellulose as a C source, cellulase activity only increased with C addition in Sedgwick soil. In the tussock tundra soil, cellulase actually declined slightly with cellulose additions. Substrate use efficiency (SUE) decreased slightly with C additions in the two soils on the C limited side of the spectrum. This is what would be expected as substrates shift from limiting to saturating- C should be used less efficiently. However, SUE was highest in the C saturated soil (tussock) and did not change with C additions. Interestingly, microbial biomass (as measured by $CHCl_3$ fumigation-extraction [CFE]) was relatively unchanged with C additions, although the model predicts that it should increase in the C-limited soils. That lack of fit may simply result because CFE is somewhat insensitive to moderate changes in biomass and increases in microbial mortality, and turnover could mask an increase in growth. Further research needs to focus on better understanding the flow of C into microbial biomass, microbial growth dynamics, and the fate of C compounds.

Fig. 5. Effects of added C on soil processes
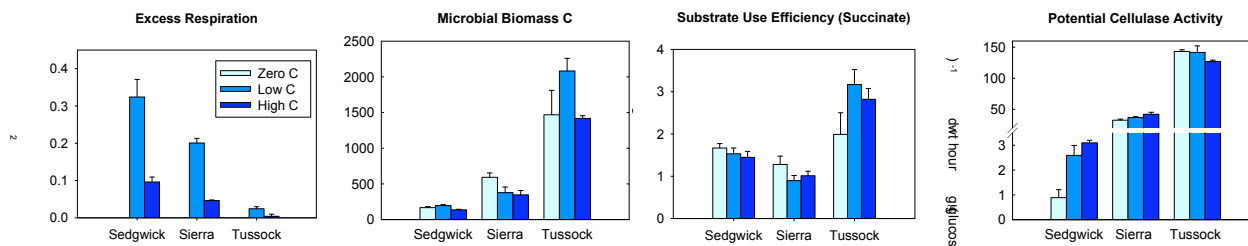
Hypothesis 5. Adding N will not increase soil respiration, rather when microbes are N limited it may reduce respiration by diverting C flow from overflow respiration to microbial growth.

The responses to N additions in the three soils were consistently in line with model predictions. Sedwick soil (C limited) showed very limited responses to N additions – respiration rates declined slightly, biomass was unchanged, SUE increased slightly but only in the highest N addition, and cellulase activity was unchanged. The intermediate Sierra soil showed exactly the pattern predicted from the model in which N availability was adequate to support enzyme synthesis: respiration rates declined slightly but SUE increased, suggesting more efficient use of C for synthesizing microbial biomass. The Tussock tundra soils show the behavior predicted by the model when soils are extremely N limited. At very low N availability microbes should reduce exoenzyme synthesis because they are so N rich it becomes a drain on biomass production. Thus, adding N should increase enzyme synthesis, C flow, and biomass (Schimel and Weintraub 2003, *fig. 6*). This is what occurred in tussock soil: adding N up-shifted all metabolic processes, including respiration, biomass, SUE, and enzyme activity.

Fig. 6. Effect of added N on soil processes
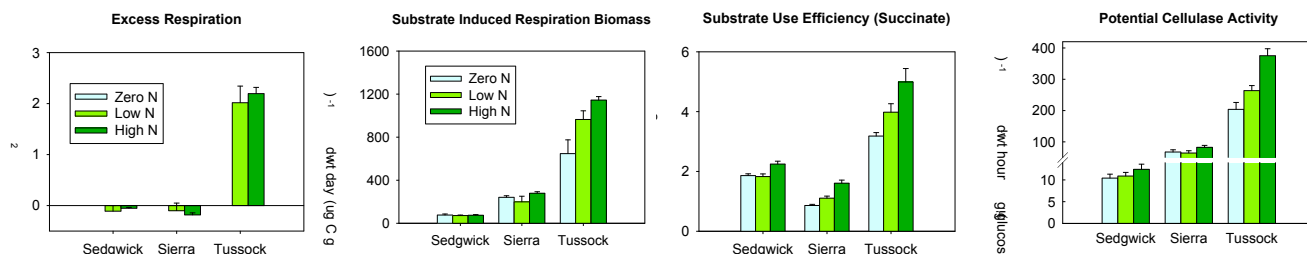
Assessment of research

The work we have done to date suggests that many of the specific predictions that grew from the exoenzyme model are correct, to at least some degree. That is encouraging. However, a number of patterns and behaviors are more complex than the model predicted. That is no surprise, as the model is extremely simplistic (deliberately so). In reality, the interactions between N availability and C flow patterns are complex. These patterns do appear to involve a) altered C flow by altered allocation of N to exoenzyme synthesis, and b) the potential for diverting C between waste metabolism and biomass synthesis as suggested by the data for the Sierra soil, in which respiration rates decline with N addition but substrate use efficiency increases. However, the work that will provide truly critical tests for some of these hypotheses is still ongoing. We hope to finish it with a second phase of funding from the Kearney Foundation.

Products of research

Training

To date, this project has partially supported one Ph.D. student's research (Mike Weintraub), two undergraduate independent study projects (ecology – work on exoenzyme dynamics and chemistry – synthesis of lignin starting materials), and two high school interns.

Publications

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