



2nd International Enzymes in the Environment Workshop
Incorporating Enzymes and Microbial Physiology into Biogeochemical Models
Fort Collins, CO May 15-18 2012

WELCOME

Welcome to the 2nd International Enzymes in the Environment RCN Workshop on “Incorporating Enzymes and Microbial Physiology into Biogeochemical Models”.

The goal of this workshop is to assess the challenges and opportunities to integrate enzymes and microbial physiology into biogeochemical models. By bringing together scientists with expertise spanning scales and ecosystems, we hope that our collective experience will spur advances on this topic. In particular, we expect our expert microbial ecologists to express what they think is missing from current models; and we hope to learn about the constraints and trade-offs involved in increasing model complexity from our expert modelers. We hope to find some middle ground- clarifying specific areas of uncertainty and data needs to move this field forward.

The most important outcome of this meeting will be new ideas and new collaborations, and the RCN has committed funds to support follow-up research visits. In addition, we expect to develop at least one synthesis review paper highlighting current needs and challenges of integrating enzymes and microbial physiology into biogeochemical models.

In this program you will find:

- Table of contents
- Introduction to World Café and the ‘Art’ of Harvesting Information
- Agenda of activities
- Participant contact list
- Abstracts for talks
- Abstracts for posters
- Participant submitted model schematics
- Future travel support RFP

Thank you for all your hard work to help make this conference a success. We sincerely hope that you enjoy your stay with us here in Fort Collins CO.

Mary Stromberger and Matthew Wallenstein, Chair
Colin Bell, Program Director



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SPONSORS

This workshop was made possible by funding from the following sponsors:



GETTING AROUND IN FORT COLLINS, CO

For your convenience, a map of Colorado State University (featured on the next page) and a map of Fort Collins, CO has been provided in your welcome bag. All meeting locations and times can be referenced in the Agenda of Activities section of this workshop program. Please don't hesitate to ask if you need assistance finding your way around while attending this workshop!

A word about Fort Collins, CO:

Fort Collins is conveniently located at the foot of the Rocky Mountains, offering exciting recreational and cultural offerings. Live music and great local dining can be found throughout the historic downtown area. For more information regarding recreational or evening activities, we have listed a few sites that you can visit:

<http://www.downtownfortcollins.com>

<http://www.coloradoan.com/section/entertainment>

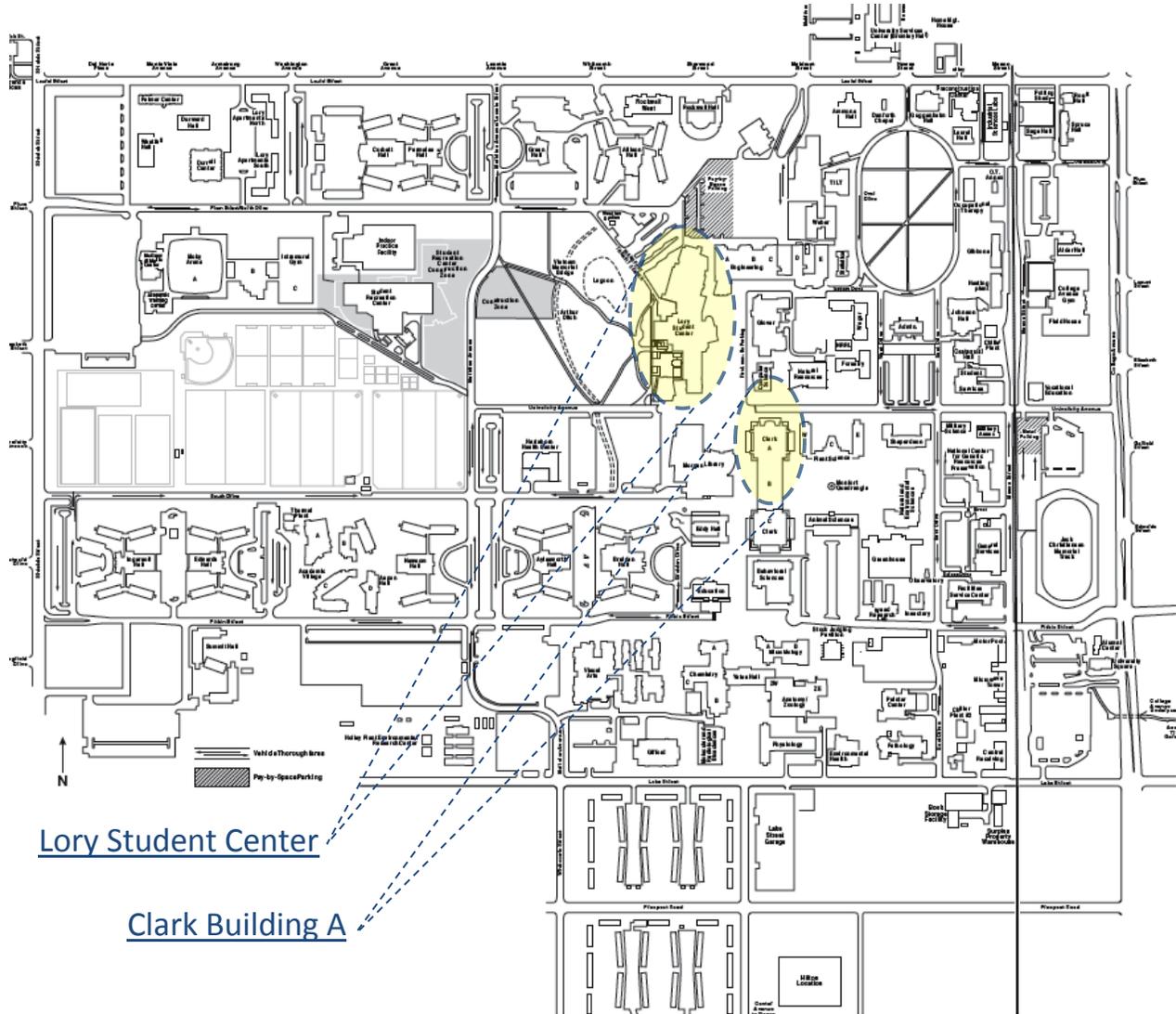
<http://www.fcbikelibrary.org>

<http://www.coloradoinfo.com/fortcollins/food-fun>



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MAP OF THE CSU CAMPUS



Lory Student Center

Clark Building A



THE WORLD CAFÉ

We will implement a “World Café” format for the Wednesday and Thursday morning breakout sessions. We feel this is a great way to engage all workshop participants and efficiently synthesize multiple perspectives on our focal topics.

Drawing on integrated design principles, the World Café methodology is a simple, effective, and flexible format for fostering group dialogue. We will distribute participants into tables of about five people each. The session leader will introduce the general topic, and each table will have a ‘host’ to moderate the discussion and work through a set of 3 – 4 topic questions designed for the specific working group session. The discussion then will proceed with the first of two twenty minute rounds of conversation for the small group seated around a table. The same questions will be used for all small round– table groups. At the end of the twenty minutes, members from each small group will move to a different table. The “table host” will remain at the table for the next round, welcoming the next group and briefly describing what happened in the previous round. After the first of two twenty minute rounds of small group conversation, the table hosts are invited to share insights or other results from their table - conversations to the large group. For more information regarding World Café format, please visit: (<http://www.theworldcafe.com>).

THE ‘ART’ OF HARVESTING INFORMATION

We have hired a ‘graphic facilitator’ (Karina Mullen) who will help to visually document the oral presentations, poster presentations, and discussions that emerge from the World Café roundtables. Her primary role is to help us to integrate our ideas into a conceptual framework as the workshop progresses. Karina may also take an active role in facilitating our discussions by encouraging us to clarify our ideas. At the end of the workshop, we will have a graphic representation of our discussions that will be a product we can share with the broader community, as well as fodder for synthesis papers and other collaborations that we expect to emerge from the workshop. You can learn more about Karina’s work by visiting her website: (<http://www.naturalvisionfacilitation.com>).

Photo (right): Karina Mullen graphically recording discussions at a recent Education in Natural Resources conference hosted at Colorado State University in March 2012





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AGENDA OF ACTIVITIES

Day	Time	Activity
TUESDAY, MAY 15		
Afternoon	4:00 **	Workshop Social (be sure to pick up your name tags and programs) (<i>El Monte Mexican Grill</i>)
	4:45	Welcome from Enzymes in the Environment RCN 2nd International Workshop at Colorado State University Introduction by Matt Wallenstein Keynote talk by Josh Schimel (University of California Santa Barbara) Make everything as simple as possible, but not simpler
Evening	6:15 to 9:30 pm	Banquet Dinner at El Monte Mexican Grill
WEDNESDAY, MAY 16		
Morning	8:30 to 10:10	Speaker Session 1A: Incorporating enzymes in biogeochemical models: New paradigms (30 min each; <i>Clark A 101</i>) Carol Arnosti (University North Carolina) Microbial enzymes as selective gateways into the marine carbon cycle Klaus Butterbach-Bahl (Karlsruhe Institute of Technology) Representation of N cycling processes in the biogeochemical model Landscape DNDC Daryl Moorhead (University of Toledo, Ohio) A theoretical model balancing C- and N-acquiring exoenzyme activities driving decomposition
	10:10 - 10:30	Break (drinks and snacks) (<i>outside Clark A 101</i>)
	10:30 - Noon	Speaker session 1B: Current applications (20 min each; <i>Clark A 101</i>) Tina Kaiser (International Institute for Applied System Analysis) From individuals to the community: interactions between microbial functional group dynamics and C and N flows in a spatially explicit soil decomposition model Ellen Kandeler (University of Hohenheim, Germany) Modeling Carbon Dynamics in Small-Scale Microbial Ecology of Soils Seeta Sistla (University of California Santa Barbara) Exploring the effects of long-term warming on tundra plant-soil feedbacks through changes in community structure and extracellular enzyme activity using a modeling approach



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Day	Time	Activity
WEDNESDAY, MAY 16 (continued)		
	10:30 - Noon	Kathe Todd-Brown (University of California Irvine) Starting small: an extracellular-enzyme driven model of a microbial microcosm
Afternoon	Noon to 1:30	Lunch and informal discussion (<i>LSC North Ballroom</i>)
	1:30 to 2:30	Resource Interactions: World Café Format Working Group Session 1(<i>LSC North Ballroom</i>) WGS 1A: Priming Effects: introduced by Matt Wallenstein (Colorado State University)
	2:30 - 3:00	Break (drinks and snacks; <i>LSC North Ballroom</i>)
	3:00 - 4:00	WGS 1B: The Role of Soil Nitrogen: introduced by Mike Weintraub (University of Toledo)
	4:30 to 6:00 **	Poster Session 1: Resource Interactions and Incorporating Biology into Models (<i>LSC North Ballroom</i>)
THURSDAY, MAY 17		
Morning	8:30 to 9:45	Speaker Session 2: Enzymes in biogeochemical models: Challenges and knowledge gaps (30 min each; <i>Clark A 101</i>) Recent Technological Advancements and Application by Mike Weintraub (University of Toledo) Recent advances in incorporating enzymes into decomposition models Applying Enzymes from the Modeling perspective by Steve Del Grosso (USDA-ARS Fort Collins) Applying Enzymes and Microbial Processes from the Modeling Perspective
	9:45 - 10:15	Break (drinks and snacks; <i>LSC North Ballroom</i>)
	10:15- 10:50	Incorporating Enzymes into Models World Café Format Working Group Session 2 (<i>LSC North Ballroom</i>) WGS 2A: Trade-offs: introduced by Klaus Butterbach-Bahl (Karlsruhe Institute of Technology)
	10:50- 11:25	WGS 2B: Scale: introduced by Josh Schimel (University of California Santa Barbara)
	11:25- Noon	WGS 2C: Defining Useful Data: introduced by Gwenaëlle Lashermes (INRA, France)
Afternoon	Noon to 1:30	Lunch and informal discussion (<i>LSC North Ballroom</i>)
	1:30 to 3:30	Working Group Session 2: Incorporating Enzymes into Models (continued) (<i>LSC small rooms</i>) WGS 2A: Trade-offs: led by Daryl Moorhead (University Toledo) (<i>LSC 220-222</i>) WGS 2B: Scale: led by Ellen Kandeler (University of Hohenheim, Germany) (<i>LSC 224</i>) WGS 2C: Defining Useful Data: led by Stephen Del Grosso (USDA-ARS Fort Collins) (<i>LSC 226</i>)
	3:30 to 4:00	Break (drinks and snacks) (<i>LSC North Ballroom</i>)
	4:00 - 4:30	Working Group Session 2: Group Reports and discussion (<i>LSC North Ballroom</i>)



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Day	Time	Activity
FRIDAY, MAY 18		
Morning	8:30 to 9:50	Speaker Session 3: Moving Forward Synthesis and Goals (<i>Clark A 101</i>) Jinyun Tang (Lawrence Berkeley National Lab) Incorporating enzyme and microbial dynamics into the global land model CLM4: Plans, progress, and preliminary results Synthesis and reflection - Led by Matthew Wallenstein, Mary Stromberger, and Colin Bell (Colorado State University)
	9:50 - 10:30	Break (drinks and snacks; <i>outside Clark A 101</i>)
	10:30 to Noon	Reports from Working Groups (summarize what we learned and define future actions) (<i>Clark A 101</i>)
	Noon	Adjourn - Safe trip home!

** To poster presenters: Please put up your posters on Wednesday morning (05/16) (in the Lory Student Center NORTH BALLROOM). Please take down your posters at the end of the day on Thursday (05/17)

** Cash bar will be available

LOGISTICS

Off campus meeting locations (date):

(TUESDAY, MAY 15)

El Monte Mexican Grill

1611 S College Ave., Suite 100,

Fort Collins, CO 80525

Ph: 970-372-18

On Campus meeting locations (date(s)):

(WEDNESDAY, MAY 16 – FRIDAY, MAY 18)

Colorado State University

- Clark A 101 is located in the Clark building
- LSC = Lory Student Center
 - (please refer to CSU campus map)



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Organized Alphabetically by Participant's Last Name

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SPEAKER ABSTRACTS

Organized by session then alphabetically by Presenting Author's Last Name

KEYNOTE TALK – TUESDAY 05/15/2012

Make everything as simple as possible, but not simpler

Josh Schimel

Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA, USA

In modeling there is a constant tension between simplicity and “reality.” There is always pressure to add processes to a model because they are “our” processes and we think they are important. Yet, every added process requires additional parameters that must be measured or estimated, which is notably difficult in enzyme-based models as some are somewhere between extraordinarily difficult and flat-out impossible to measure, such as the functional decay rate of enzymes. Adding processes also increases the complexity of the model. Not only does this make it more challenging to run for any given system, but it potentially reduces a model’s utility as an intellectual tool—above a certain level of complexity, we may only be able to comprehend the outputs, rather than the dynamics of a model. So as we move away from simple 1st order decomposition models to agent-based models, the critical question remains how simple can we make them, while still capturing essential dynamics? What are those essential dynamics? What are the most useful approaches? Where are the chief constraints?



SPEAKER SESSION 1A - WEDNESDAY 05/16/2012

Microbial enzymes as selective gateways into the marine carbon cycle

Carol Arnosti

Department of Marine Sciences, University North Carolina, Chapel Hill, NC, USA

The activities of extracellular enzymes initiate remineralization of a major portion of marine organic carbon, since heterotrophic microbial communities cycle an estimated half of total marine primary productivity (Azam 1998). These enzymes serve as 'gateways' into the marine carbon cycle by hydrolyzing substrates to sizes sufficiently small to cross microbial membranes for further metabolism. Despite their importance in carbon and nutrient cycling, little is known of enzyme diversity, substrate specificities, and activities in marine systems. This gap in our knowledge is due in part to the vast (and largely uncharacterized) diversity of marine microbial communities, as well as to a paucity of methods suitable to characterize specific enzyme activities in seawater and marine sediments. Most measurements of microbial enzyme activities in marine systems have been made using simple substrate proxies, typically glucose or leucine labeled with MUF or MCA fluorophores (Hoppe 1991). While these substrate proxies facilitate inter-comparisons among studies, they yield little information about the diversity of enzymes produced to hydrolyze complex high molecular weight substrates. We have used an alternative approach to measure microbial enzyme activities, by fluorescently labeling polysaccharides, plankton extracts, and plankton-derived DOC (dissolved organic carbon), and using the change in substrate molecular weight with incubation time to measure hydrolysis rates (Arnosti 2003). This approach has revealed substantial differences in enzymatic capabilities of marine microbial communities between sediments and bottom waters, along latitudinal gradients in the surface ocean, in depth transects in the water column, and in comparisons of coastal and offshore environments. On the basis of these data, we hypothesize that the enzymatic gateway to the marine carbon cycle—the initial step in remineralization of high molecular weight organic matter—varies in a manner that shows broad trends in the ocean. Surface water communities at temperate latitudes have a wider gateway than at high latitudes, the width of the gateway decreases with depth in the water column, coastal microbial communities have wider enzymatic gateways than offshore communities, and sedimentary communities at all locations have wide enzymatic gateways. Recent evidence of broad biogeographical patterns in microbial community composition (e.g. Zinger et al. 2011) may be linked to functional patterns such as those we observe.



Representation of N cycling processes in the biogeochemical model Landscape DNDC

Klaus Butterbach-Bahl¹, Edwin Haas¹, Ralf Kiese¹

¹Institute for Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology, Kreuzeckbahnstr. 19, 82467 Garmisch-Partenkirchen, Germany

For estimating soil N₂O emissions at various temporal and spatial scales, to assess different mitigation options and to understand and predict feedbacks of global changes modeling approaches of different complexity are needed. Specifically for assessing mitigation options at site to regional scales, for identifying hot spots and hot moments of N₂O fluxes and to predict global change feedbacks biogeochemical models capable to simulate N cycling and exchange processes across biosphere-atmosphere-hydrosphere boundaries are increasingly used. However, due to the close coupling of microbial N₂O production and consumption processes to ecosystem N as well as C cycling – with N₂O exchange being a tiny flux as compared to other N fluxes - and in view of the multitude of involved microbial, plant and physico-chemical processes, modeling of N₂O emission from terrestrial ecosystems is a very complex and challenging research task. Moreover, little is still known about specific rates of e.g. N₂O losses during nitrification and denitrification – often due to short comes in measuring techniques - so that simplified assumptions are often used or parameters describing different processes are lumped. Thus, parameterization of N cycling processes and the assessment of parametric uncertainty is an important and often not addressed issue.

In Landscape DNDC, a biogeochemical model which can be used to simulate N fluxes at site and regional scale, we explicitly simulate the most important N cycling processes, with e.g. having a focus to represent gross rather than net rates of microbial N turnover processes. However, from rigorous testing of the model at site scale we learned that our understanding of environmental controls of microbial N turnover at a given site is still limited and that used parameters for describing selected processes such as nitrification or denitrification, or the splitting of soil in aerobic and anaerobic zones, e.g. during freeze-thaw events with observed high pulses of N₂O emissions, are highly variable.

Can we do better and what is needed for doing so? It becomes increasingly obvious that a further integration of knowledge is needed and that targeted field and laboratory experiments not only of fluxes but also of underlying microbial dynamics are needed to further improve process understanding and, thus, model parameterization. However, it still remains unexplored if a further increase in model complexity and implementation of further process details will finally lead to improved simulation results, specifically with regard to simulate N₂O emissions from soils.



A theoretical model balancing C- and N-acquiring exoenzyme activities driving decomposition

Daryl Moorhead¹, Gwenaëlle Lashermes² and Robert Sinsabaugh³

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²UMR Fractionnement des AgroRessources et Environnement (FARE), INRA, Reims, France; ³Department of Biology, University of New Mexico, Albuquerque, NM, USA

We extended the enzyme-based decomposition model of Schimel and Weintraub (2003), which simulates the dynamics of one enzyme that degrades one substrate, to two enzymes that degrade two qualitatively different substrate pools: a carbon-only pool (e.g. cellulose) and a carbon + nitrogen pool (e.g. chitin or protein). Substrate hydrolysis was estimated as a “reverse” Michaelis-Menten function of enzyme saturation. The allocation of extracellular enzyme activities (EEA) to simultaneously meet the energetic and stoichiometric demands of decomposer microorganisms varied with both C:N content of C+N substrate pool (CN1) and availability of a second, C-only pool. The addition of a C-only pool reduced N-mineralization and increased microbial biomass and respiration when CN1 was less than the quotient of the microbial C:N ratio (CNM) divided by the C-utilization efficiency of substrate (SUE); this quotient is commonly known as the threshold element ratio (i.e., $TER \approx CNM/SUE$) at which growth limitation switches from one element to another. In every set of simulations, maximum microbial biomass and respiration corresponded with maximum total enzyme pool size, which in turn corresponded to a balanced allocation of enzymes between pools (i.e., $EEA \approx 1:1$). This optimal EEA occurred when $CN1 = CNM$. However, this threshold varied with key model parameters (SUE, enzyme half-saturation coefficients, and maximum rates of substrate hydrolysis). Sensitivity analysis showed that variations in these parameters explained over 90% of variation in subsequent model behaviors, despite the non-linear relationships between enzyme pool sizes, biomass, respiration and enzyme activity. Model results also showed that variations in TER with respect to gross litter chemistry could be explained by finer scale mechanisms of specific enzymes hydrolyzing specific substrates in response to microbial requirements for multiple resources and the qualities of substrates providing these resources.



SPEAKER SESSION 1B - WEDNESDAY 05/16/2012

From individuals to the community: Interactions between microbial functional group dynamics and C and N flows

Christina Kaiser¹, Andreas Richter², Oskar Franklin³, Ulf Dieckmann¹

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Microbial decomposition of terrestrial organic material releases about 60 Gt carbon per year to the atmosphere. Despite its importance, mechanisms driving decomposition processes remain mostly unclear and are only poorly represented in traditional biogeochemical models.

Recent work suggests that not only microbial decomposition processes, but also the composition of the soil microbial community is highly sensitive to changing environmental parameters, such as temperature, moisture or the input of labile C and N. Since decomposition of litter or soil organic matter requires the concerted action of an array of microbes with different functions and abilities, it is likely that such changes in microbial community composition will in turn affect rates of soil organic matter decomposition, and subsequently feed-back on carbon and nitrogen release from terrestrial ecosystems. Such feedbacks could not, however, be predicted by traditional stock and flow models, as they do not include microbial community dynamics.

To investigate this link between functional microbial community dynamics and soil carbon and nitrogen cycling, we have developed a novel biogeochemical model, which is based on interactions between individual microbes belonging to different functional groups in a spatial environment. Functional groups of microbes in the model are defined by traits and functions in the categories of (1) growth and turnover rates, (2) production of different extracellular enzymes and (3) stoichiometry and internal structure. Specific values for these parameters determine the success of a functional group in the presence of competing functional groups with different traits in a certain environmental scenario (i.e. for a specific C and N availability). The selection of a combination of functional groups determines community dynamics and thereby overall decomposition rates under certain C:N scenarios, and vice versa, the variation of C and N availability shifts community composition and thus affects decomposition rates in the model. Based on the link between stoichiometric of C and N fluxes, microbial community composition and decomposition processes, which is intrinsic to the model, we are able to simulate microbial community dynamics in response to varying C and N availabilities, as well as C and N flows responding to changing community composition.

By comparing the outcome of model scenarios based on different levels of microbial functional diversity with a comprehensive experimental data-set for litter decomposition, we are able to test the potential role of microbial community dynamics and microbial functional community composition for organic matter decomposition models.



Modeling Carbon Dynamics in Small-Scale Microbial Ecology of Soils

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An important challenge for future research is to combine processes operative at the cellular, organism and community scales with phenomena at the microhabitat or plot scale of soils. For a couple of years we have performed various microcosm experiments to improve our understanding of carbon dynamics at the soil-litter interface. We were able to simulate the β -glucosidase activity observed in the detritusphere using a convective-diffusive transport model with first order decay (Poll et al. 2006). The results indicated that the spatial dimension of the detritusphere is governed by the ratio between decay rate of available substrates and transport rate. In a second step, we traced the fate of litter carbon in the detritusphere to develop a new 1D dynamic mechanistic model (Ingwersen et al. 2008). The 1D model simulated both the total carbon and the ¹³C carbon pools and fluxes. The model operated with two decomposer populations; the first one was assumed to be dominated by bacteria (initial-stage decomposer) and the second one by fungi (late-stage decomposer). In a third series of experiments, we focus on the effect of carbon transport on metabolic and co-metabolic degradation of the model substance MCPA (2-methyl-4-chlorophenoxyacetic acid) in the detritusphere (Poll et al. 2010). We studied microbial degradation, microbial abundance of degraders, adsorption, desorption and transport of MCPA along a gradient of decreasing availability of dissolved organic matter. Isotopic data (¹⁴CO₂, ¹⁴C_{mic}, ¹⁴C-DOC, ¹⁴C_{org}, ¹⁴C-MCPA) and molecular data (tfdA, 16S rDNA and 18S rDNA sequence copy numbers) were used as input variables for a mechanistic model. Potential laccase and peroxidase activities will account for fungal co-metabolic degradation of MCPA at the soil litter interface. Our study shows that the combination of experimental work and mathematical modeling is a powerful approach to gain a comprehensive insight into the small-scale carbon turnover in soil and that it can provide an explanation of a soil phenomenon frequently observed (i.e. priming).



Exploring the effects of long-term warming on tundra plant-soil feedbacks through changes in community structure and extracellular enzyme activity using a modeling approach

Sistla, SA¹, Rastetter, E.B.², Schimel, JP¹

¹Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA, USA;

²Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, USA

Arctic soils are among the largest stores of terrestrial organic carbon (C) globally. Climate models unambiguously predict that the Arctic will continue to warm over the next century; therefore, there is substantial interest in developing mechanistic descriptions of Arctic systems' responses to warming, in particular C storage potential. Previous studies have suggested that nitrogen (N)-limitation regulates both plant and microbial growth in tundra soils in what may be a seasonally-dependent pattern; however, there is a little information on the impact of the seasonality of warming on long-term soil biogeochemical dynamics or plant-soil feedbacks. We are using a mechanistic modeling approach to address this knowledge gap, by exploring the consequences of long-term warming on tundra soil microbial dynamics, nutrient cycling and net C storage.

The model includes three classes of soil organic matter (SOM), microbially-synthesized extracellular enzymes specific to these SOM pools, a microbial biomass with a variable C:N ratio, and a plant biomass that adjusts its allocation to wood, root and leaf growth. The plant biomass dynamically allocates growth effort to wood, root and leaf biomass, based on N-uptake. The plant biomass pools provide inputs to the SOM pools at a higher C:N than their standing biomass (via N retranslocation). The microbial community acclimates between a more bacterial-like (lower C:N, faster turnover) and fungal-like (higher C:N, slower turnover) community, depending upon the SOM environment and inorganic nutrient availability they see at a given time step. This stoichiometric flexibility allows for the microbial C and N use efficiency to vary, feeding back into system decomposition and productivity dynamics. These feedbacks scale from the microbial to ecosystem level, including changes in the relative allocation to oxidative and hydrolytic extracellular enzyme synthesis, nutrient turnover rates, and plant growth.

This research highlights the potential for the *seasonal* nature of warming to be a highly significant factor in regulating microbial activity and thus the potential magnitude of tundra soils' decomposition with climatic warming. In particular, our model system reveals that winter warming has a greater net effect on ecosystem C loss than summer warming, due to changes in microbial nutrient-limitation status.



Starting small: an extracellular-enzyme driven model of a microbial microcosm

Kathe Todd-Brown

Department of Earth System Science, University of California at Irvine, Irvine, CA, USA

Extracellular enzymes produced by microorganisms play a key role in carbon mineralization. These enzymes break down complex substrates into simpler compounds that microbes can utilize for metabolism. However, little is known about key parameters, including the rate and cost of enzyme production by microbial communities.

We used mathematical modeling and laboratory experiments to determine the rate and cost of enzyme production and the consequences for growth of two *Pseudomonas fluorescens* strains, a wild-type that produces a casein-digesting protease and a mutant that did not produce the protease. These two strains were grown individually and in competition over several days in three well-mixed media types: glucose, glucose + casamino acids, and glucose + casein. During the experiment, measurements were taken of CO₂ respiration rates, protease activity, and biomass. The mathematical models were optimized against the data collected using a modified Monte Carlo Markov Chain routine with a weighted log-likelihood measure and parameter ranges from the literature and separate laboratory experiments.

We found that $0.74 \pm 0.03\%$ of the carbon taken up by the wild-type was allocated to enzyme production with an additional metabolic cost of $1.06 \pm 0.21\%$ of the biomass up-take. The optimized model matches the data well with a relatively high log-likelihood and good qualitative match with the data. These costs result in a growth disadvantage for the wild-type strain when in competition with the protease-negative mutant.



SPEAKER SESSION 2 - THURSDAY 05/17/2012

Applying Enzymes and Microbial Processes from the Modeling Perspective

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Key microbial processes, such as enzyme activity, are represented only implicitly in many widely used soil organic matter cycling models. For example, the DayCent and CENTURY biogeochemical models contain surface and soil microbial biomass pools and the portions of these pools assumed to be made up of fungi increase as substrate quality, in terms of C/N and lignin content, decreases. In addition to microbial pools, the models also include pools to represent soil organic matter with intermediate (slow pool) and long (passive pool) turnover times. Fungi and bacteria have different microbial growth efficiencies but the models do not explicitly represent the microbial processes that are responsible for differences in growth efficiencies. In addition to different growth efficiencies, fungal products from decomposition of high lignin material increase SOM in the slow pool. In contrast, the size of the passive pool is driven not by lignin or by differences in the stability of microbial products, but by the clay content of soil. Modeling different pools for bacterial and fungal products could improve model performance but this would increase model complexity and evidence regarding the stability of microbial products is not consistent. Theoretically, fungal products are expected to be more stable but evidence from the LIDET experiment are not consistent with this because decomposition of litter with high C/N ratio litter occurs more slowly at first but faster after 6-8 years than material with lower C/N ratio. Opportunities to improve model performance by including priming of decomposition by inputs of labile carbon will also be explored using results from the Duke FACE experiment.



Enzymes in biogeochemical models: application, recent advancements, challenges, and knowledge gaps

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It is well understood that extracellular enzymes produced by microorganisms largely drive decomposition, but traditional decomposition models have included neither microbes nor their enzymes as explicit drivers of decomposition. To date, patterns of decomposition have largely been approximated by empirical relationships that do not include driving mechanisms and thus lack the generality to adequately address perturbations such as N deposition. Most predictive models of decomposition are driven by initial litter chemistry and environmental conditions, rather than microbial dynamics, and fail to capture impacts arising from “bottom up” changes in microbial community composition or function, that play a critical role in the stabilization of organic matter. When enzymes and the groups of microorganisms that produce them, with enzyme activities and affinities for substrates varying by group, are added to decomposition models, several phenomena emerge that qualitatively change the behavior of the models and the conclusions that can be drawn from them. The accuracy of such models is currently limited, however, by the availability of data on microbial enzyme production, enzyme stabilization and loss, as well as reaction product formation and substrate availability. Recent advances in genomics and proteomics have the potential to make a significant contribution toward the creation of decomposition models that include different microbial groups and the enzymes they produce, which in turn will improve our ability to predict decomposition and elucidate the mechanisms controlling C sequestration and loss.



SPEAKER SESSION 3 – FRIDAY 5/18/2012

Incorporating enzyme and microbial dynamics into the global land model CLM4: Plans, progress, and preliminary results

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Processes such as enzymatic degradation, thermal degradation, and photo-degradation drive organic matter decomposition. Existing global land biogeochemistry models lump these processes into a few simple parameterizations that depend on organic matter quality, soil moisture, soil temperature, and sometimes the soil oxygen content. These simplifications raise concerns when the lumped models are used to predict organic matter decomposition and resulting greenhouse gas fluxes in a changing climate. For example, are there shifts in organic matter decomposition due to changes in microbial community structure, which are closely coupled to the organic matter quality and quantity? Will these shifts affect prediction of ecosystem gas fluxes and soil carbon storage? To address these questions, we developed an enzyme-driven belowground biogeochemistry model by leveraging the vertical transport capability for multiphase tracers from the CLM4-BeTR framework. This new belowground biogeochemistry model includes a spectrum of organic matter decomposition processes, from exoenzyme-driven macromolecule degradation to selective uptake of small monomers by different microbial species. We considered microbial interactions between microbial functional groups, including aerobic heterotrophs, autotrophic nitrifiers, and facultative denitrifiers. All substrates are allowed to undergo physical transport and conversions inside the soil. Genomic ecophysiological data was used to describe traits and requirements for the microbes during competition for resources. The model was parameterized with data from a literature review, and preliminary tests indicate the model accurately predicted microbial community shifts during competition for various substrates under a range of physical and chemical environments. Future model development includes addition of methane dynamics, anaerobic decomposition by fermenters, and abiotic aqueous chemistry. The overall goal of the model is to provide a numerical tool that can analyze interactions between microbial processes, terrestrial ecosystem dynamics, soil organic matter decomposition and stabilization, and climate change.



POSTER SESSION ABSTRACTS

Abstracts organized alphabetically by Presenting Author's Last Name

Enzyme turnover as function of enzyme pool size: enzyme turnover presents an upper limit on soil organic matter turnover rate

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Our current focus on enzymes as the proximate drivers of soil organic matter (SOM) decomposition emphasizes that SOM turnover should be modeled as a function of enzymes, not merely a decay constant, k . Enzyme pool size is then modeled as the difference between enzyme production, a function of microbial resource availability, and enzyme turnover, a function of the enzyme pool size and its own decay constant, k . Enzymes are also SOM however, and in principle are subject to enzymatic degradation as well. Therefore, modeling enzyme turnover using a decay constant is inappropriate. Enzyme turnover should be modeled similarly to SOM turnover, as a function of enzyme pool size. I explore mathematically how enzyme self-catalyzation may limit maximum sustainable enzyme pool size in soil by describing enzyme turnover using Michaelis-Menten enzyme kinetics and then inserting this formulation into a return on investment microbial ecology model. Based on my analysis I suggest (1) enzyme specific turnover rate should increase with enzyme pool size. (2) Self-catalyzed enzyme turnover represents an upper theoretical limit on maximum sustainable enzyme pool size, and in turn sets an upper limit on the maximum rate of SOM turnover. This limit is independent of microbial energy or nutrient limitation. Finally, (3) the greater the cost to produce an enzyme or the lower the K_m value of an enzyme, the smaller the maximum sustainable enzyme pool size when substrates are not limiting.



Predicting soil carbon feedbacks as plant-mycorrhizal interactions shift

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Interactions between plants and their symbiotic mycorrhizal fungi are at the interface of above and belowground systems and their interactions can influence soil carbon (C) dynamics. Plants allocate a portion of their C to mycorrhizae, where it becomes hyphal biomass; in return, mycorrhizal fungi increase plant water and nutrient uptake.

However, when plants become stressed the symbiosis can breakdown. When plants are stressed they reduce their C allocation to mycorrhizae; mycorrhizae then shift to gain C via soil organic matter (SOM) degradation. This can cause a soil C shift where the plant-mycorrhizal interaction becomes a source of C to the atmosphere instead of a sink. The magnitude of the effect of ectomycorrhizal C acquisition, whether from plants or SOM, is crucial to predicting soil C efflux and storage. We developed a theoretical model that predicts soil C dynamics following a shift in the plant-mycorrhizal C interaction.

Following a reduction in C allocation to roots, our model predicts changes over time in five C pools: aboveground biomass, roots, litter, fungal biomass, and soil. Analysis of model dynamics allowed us to identify the range of parameter values required for the interaction between plant allocated C, ectomycorrhizal metabolism, and soil C for the system to switch to a C source. Preliminary parameter sensitivity analysis shows that decreases in C allocation to roots and mycorrhizal fungi significantly alter all of the C pools represented in the model.



Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils

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The mobilization of nitrogen (N) from soil organic matter in temperate forest soils is controlled by the microbial production and activity of extracellular enzymes. The exudation of carbon (C) by tree roots into the rhizosphere may subsidize the microbial production of extracellular enzymes and increase the access of roots to N. The objective of this research was to investigate whether the stimulation of extracellular enzyme activity in the rhizosphere (i.e., rhizosphere effect) differs between tree species that form associations with ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) fungi. This research was conducted at two temperate forest sites, the Harvard Forest (HF) in Central MA and the Morgan Monroe State Forest (MMSF) in Southern IN. At the HF, we measured the rhizosphere effects on enzyme activity, N cycling, and C mineralization in ECM and AM soils. At the MMSF, we girdled AM and ECM dominated plots in July 2011 to examine whether ECM and AM trees differ in the impact of severing belowground C allocation on rhizosphere processes.

At both sites, the rhizosphere effect on proteolytic, chitinolytic and ligninolytic enzyme activities was greater in ECM soils than in AM soils. Higher rates of proteolytic enzyme activity increased the availability of amino acid-N in ECM rhizospheres relative to the bulk soils. Further, this stimulation of enzyme activity was directly correlated with higher rates of C mineralization in the rhizosphere. At the MMSF, experimental girdling led to a larger decline in enzyme activity in the rhizosphere and bulk soil of ECM trees than AM trees. In both ECM and AM soils, however, there has yet to be a response of soil respiration to girdling. The results of this study contribute to the growing evidence that temperate forest tree roots, in particular ECM roots, can enhance soil-N cycling and extracellular enzyme activity through the allocation of C to the rhizosphere. Given the magnitude of the rhizosphere effects on enzyme production and activity, rhizosphere processes should be incorporated into future mechanistic and ecosystem models.



A full annual course of gross N turnover as a prerequisite to test and improve the biogeochemical model Landscape DNDC

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The gross rates of ammonification and nitrification are catalyzed by key enzymes of the nitrogen (N) cycle. So far, almost all studies reporting actual gross process rates of N turnover were extremely restricted in their temporal resolution, i. e. single or few measurements were conducted only. Based on year round sampling in winter-grazed and ungrazed steppe soils of Inner Mongolia we compiled the first full annual dataset of gross ammonification and nitrification with sub monthly temporal resolution. Four different seasons with characteristic functional patterns of N turnover were identified: (1) Growing season dynamics as characterized by drying/rewetting cycles and negatively correlated temporal courses of net microbial growth and periods with intensive gross ammonification, contributing 40 - 52% and 29 - 32% to cumulative annual gross ammonification and nitrification, respectively. (2) Microbial N dynamics during the autumn freeze-thaw period was characterized by a sharp decline in microbial biomass in conjunction with a peak of gross nitrification contributing 19 - 36% to cumulative annual fluxes. (3) During winter at constantly frozen soil, a net build-up of microbial biomass was observed, whereas gross N turnover rates were low, contributing 7 - 10% and 6 - 11% to cumulative annual gross ammonification or gross nitrification, respectively. (4) The spring freeze-thaw period showed extremely dynamic changes in gross N turnover. This period contributed 34 - 44% and 21 - 46% to cumulative annual gross ammonification and nitrification, respectively.

The observed pronounced dynamics of N turnover within and between seasons emphasizes the necessity for high resolution sampling of gross N turnover and corresponding enzyme activities as a prerequisite to infer functioning and annual budgets of ecosystem N cycling. The full annual course of N turnover is representing a hitherto not available prerequisite to test and improve biogeochemical models. Simulations of the observed pronounced dynamics of microbial N turnover using the process-oriented biogeochemical ecosystem model Landscape DNDC are presented and potential improvements discussed.



The effect of temperature and soil properties on microbial growth yield efficiency

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Microbial growth yield efficiency (GYE), or the amount of carbon assimilated relative to carbon dioxide (CO₂) produced, is an important parameter in ecosystem models because it affects the amount of carbon stored or released from soils. GYE is often an empirically derived constant in ecosystem models, but a mounting body of evidence shows that GYE is, in fact, variable with temperature. GYE typically decreases with increasing temperature in temperate systems. However, in cold-adapted systems, microbial communities may be so well-adapted to the temperature regime that they utilize carbon most efficiently at near-native temperatures, thus complicating the effect of temperature on GYE. We aim to determine what factors affect GYE in a cold-adapted system, and to explore how GYE can explain carbon flux.

We collected soils from under three types of vegetation during June and October in Thule, Greenland giving us six microbial communities. In the lab, ¹³C-labeled glucose was added to trace the relative proportion of carbon respired versus assimilated during decomposition. Soils were incubated at +4°C and -2°C for 7 and 17 days, respectively. Headspace CO₂ and microbial biomass carbon (MBC) were analyzed for ¹³C enrichment, and GYE was calculated.

We expected that soils incubated at temperatures near their native temperatures (e.g. October samples incubated below and June samples incubated above freezing) would have higher GYE than soils incubated at non-native temperatures. Rather, all of the samples trended towards having a higher GYE at +4°C than -2°C. Therefore, our prediction of an “adaptation effect” to temperature was not confirmed. June soils had GYE's more than 1.5 times greater than two of the three October soil types, but the trend was reversed for the third soil type, indicating that there are abiotic or biological differences in the soil between the seasons. To test how soil factors affect GYE, we built a multiple linear regression model and found that temperature, total-C and MBC were significant predictors of GYE ($p=0.005$, 0.0024 and 0.0172 , respectively). In a second modeling exercise, we found that including GYE in predictions of CO₂ flux improved the model fit (AIC; 242.8 with GYE and 251.7 without). Incorporating microbial physiology, specifically GYE, may aid estimates of carbon flux and storage in soils, however this endeavor may be complicated by the fact that GYE is not only affected by temperature, but also by parameters that are more heterogeneous within ecosystems, such as soil-C and microbial biomass.



Does moisture niche partitioning drive changes in microbial community composition under long-term drought?

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Drought is likely to become more frequent and more severe under future climate regimes. Although precipitation is a strong driver of community composition in other organisms like plants, it is unclear whether climate is the primary driver of microbial community dynamics. We have observed changes in both microbial and plant community composition under an 11-year drought manipulation in the shortgrass steppe, and were interested in whether microbial community composition is driven directly by precipitation changes induced by these treatments, or by indirect effects of drought. We examined which microbial species grew in response to different moisture levels and compared them to communities that emerged under long-term drought to address whether the shifts in microbial communities we observed were a result of different microbial species responding to different moisture conditions. Although we did document microbial moisture niche partitioning in soil in laboratory incubations, the taxa that grew in response to drier conditions were not the same taxa that emerged under drought. A history of drought did cause a short-term shift in respiration moisture-response, but long-term respiration rates were not affected by drought history. These results suggest that although microbial communities, like other organisms, do display niche partitioning in response to moisture, other drivers such as changes in soil chemistry or plant community composition may drive community composition in the long-term. These results have implications for predicting the response of microbial communities, and the biogeochemical processes they control, to new precipitation patterns expected under future climate regimes.



How much and which extra-cellular enzymes should a microbe optimally produce?

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Integrating microbial physiology and biomass stoichiometry opens far-reaching possibilities for linking microbial dynamics to ecosystem processes. For example, the growth-rate hypothesis (GRH) predicts positive correlations among growth rate, RNA content, and biomass phosphorus (P) content. A recently developed model (Franklin et al. 2011) suggests that the GRH is the outcome of a trade-off among cellular components that maximizes growth under certain resource conditions, such as P limitation. Other resource conditions, such as N limitation, lead to contrasting relationships between growth and biomass stoichiometry. This optimization based model, supported by empirical data, provides a basis for understanding the mechanisms of variability in microbial biomass stoichiometry and element recycling under variable resource C:N:P ratios. However, so far no individual-level model has explained mechanistically and quantitatively the production of extracellular enzymes (exoenzymes) by microbes in response to resource stoichiometry. We added the capability for exoenzyme production to the Franklin et al. (2011) optimization model in order to address the response of enzyme production to resource stoichiometry. The new model predicts the amounts produced of the major C, N and P acquiring exoenzyme classes: e.g. cellulases, proteases and phosphatases. The production of each exoenzyme depends on C:N:P availabilities of labile and complex substrate and the efficiency and capacity of the enzymes, which are functions of environmental factors, such as temperature and diffusion rates.



The Effect of Saltwater Intrusion on Extracellular Enzyme Activity in a Tidal Freshwater Marsh: Correlating Changes in Microbial Community Function with Ecosystem-Scale Gas Flux Measurements

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Sea level rise is a climatic stressor that has a unique impact on tidal freshwater wetlands. It causes saltwater intrusion into environments historically dominated by freshwater flows, where even modest increases in salinity can adversely affect plant community composition and productivity, and potentially change ecosystem-scale carbon dynamics. In addition, microbial activity in the wetland soils may also be affected because the influx of sulfate offers a new substrate for anaerobic microbial respiration, shifting redox conditions and changing pathways of carbon mineralization. The objective of this research was to determine microbial community responses to elevated salinity associated with a long-term field study in a tidal freshwater marsh in South Carolina, where *in situ* manipulation consistently raised pore- water salinities from freshwater to oligohaline levels (~2-5 ppt).

At the end of the three-year field manipulation, soil cores were collected and extracellular enzyme assays (EEA) were performed for several labile (β -1,4-glucosidase and 1,4- β -cellobiosidase) and recalcitrant (β -D-xylosidase, phenol oxidase, and peroxidase) components of the soil carbon pool. Saltwater addition did not have a consistent effect on EEA of the labile substrates, but activity decreased dramatically for the more recalcitrant substrates. For example, the activity of phenol-oxidase and peroxidase enzymes in the saltwater-added plots were 10-20% of the activity in the control plots. These changes in microbial activity were correlated with rate potential measurements for methanogenesis, methanotrophy, and anaerobic CO₂ production, which were all greater under freshwater conditions. Further, whole-core incubations showed a similar pattern in that soil O₂ demand was 4-6 times higher in fresh water versus salinized soils. Combined, the results demonstrate the value in understanding enzyme activity as a possible predictor of the greenhouse gas fluxes, and reveal a community response to global change capable of affecting ecosystem-level processes. The change in community function due to elevated salinity was coincident with a shift in the population structure of sulfate reducers and methanogens as assessed using whole-community DNA fingerprinting (T-RFLP for each functional group), demonstrating that both microbial community structure and function can be altered by global change stimuli. This information is important for understanding the potential long-term effects on organic matter decomposition, which may play a role in wetland sediment accretion and net carbon storage



Incorporating Enzyme Kinetics in a Grassland Ecosystem Model

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Ecosystem models allow users to understand natural systems. Many of the current ecosystem models do not explicitly model microbial processes, but rather use empirical constants to estimate decomposition rates. Efforts to model microbial enzyme kinetics have increased, but have not been incorporated into ecosystem scale models. In this effort I developed an ecosystem model of C to examine the effect of incorporating enzyme kinetics on C turnover in a grassland system.

Using the Simlie 5.8, I constructed an ecosystem scale grassland model where enzyme kinetics drove microbial processing of C. The model consisted of four sub-models: Aboveground Plant, Water, Roots, and Carbon and Nitrogen. Plant growth depended on water, N, and temperature. Growing degree days regulated plant growth and were estimated based on switch grass (*Panicum virgatum*) for key plant development stages. Soil N availability depended on C decomposition rates of previous years plant biomass. Carbon and N availability regulated enzyme production, and enzymes were produced from DOC. I used an adapted Michaelis-Menten equation to incorporate temperature dependence on enzyme kinetics. Furthermore enzyme kinetics was affected directly by water availability, which was modeled in a separate sub-model. The model was parameterized with data from Konza biological station.

Overall incorporating greater complexity through enzyme kinetics destabilized the original model. The model correctly predicted seasonal dynamics in aboveground plant biomass. However, several limitations prevented accurate prediction of microbial biomass and decomposition. Multiple dependencies on available N caused microbial biomass C at times to be negative to maintain critical C/N ratios. More work to understand how to correctly regulate each of these pools is needed. Furthermore decomposition may have been overestimated due to enzyme turnover times, and assumption that all biomass was available for decomposition once it moved into the decomposing biomass pool. In future iterations a transport mechanism would be needed to regulate the rate at which enzymes are capable of acting on biomass. Overall the model does not perform well, but knowledge gained on the various inter-dependencies present from incorporating enzyme kinetics proved valuable. Future model constructions will require thorough testing of model stability.



Assessing organic nitrogen use by ectomycorrhizal fungi through natural abundance isotopic measurements

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Existing ecosystem models rely on uptake of inorganic nitrogen to fuel plant growth yet organic nitrogen probably also contributes. Plants may access organic nitrogen by supplying symbiotic (ectomycorrhizal) fungi with sugars fixed in photosynthesis and receiving nutrients in exchange. Some fungi have enzymatic capabilities extending the available nitrogen sources to include chitin and protein. In studies with ¹⁵N- and ¹³C-labeled amino acids added to soil, plants assimilate organic nitrogen. However, adding isotopically labeled compounds creates experimental artifacts hindering extrapolation to undisturbed settings. Measures of organic nitrogen use are accordingly needed that avoid experimental manipulation.

To test whether fungi assimilate organic nitrogen, we compared the ¹⁴C signal from 1950s and 1960s thermonuclear testing (expressed as $\Delta^{14}\text{C}$) in protein (potentially derived from soil organic nitrogen) and structural carbon (derived from recent photosynthate) of *Tuber oregonensis*, an ectomycorrhizal fungus specialized on mineral soil. As expected, $\Delta^{14}\text{C}$ signatures of both structural carbon and protein correlated highly with the $\Delta^{14}\text{C}$ of recent photosynthesis (adjusted $r^2 = 0.999$ and 0.998 , respectively); however, protein $\Delta^{14}\text{C}$ was 10-48% lower than structural carbon. About 10% of protein carbon derived from uptake of old organic nitrogen, with low $\Delta^{14}\text{C}$ values reflecting organic nitrogen up to hundreds of years old.

In samples from the 1970s, ¹⁴C patterns corresponded to enzymatic capabilities. In taxa with strong proteolytic capabilities (*Leccinum* and *Cortinarius*), protein had lower $\Delta^{14}\text{C}$ than structural carbon ($^{14}\text{C}_{\text{protein}} / ^{14}\text{C}_{\text{structural}} = 0.971 \pm 0.009$, $n = 4$), indicating incorporation of pre-bomb (pre-1950) organic N into fungal protein pools. In contrast, protein had higher $\Delta^{14}\text{C}$ than structural carbon in litter-inhabiting taxa (*Russula* and *Lactarius*, generally with weak proteolytic capabilities) ($^{14}\text{C}_{\text{protein}} / ^{14}\text{C}_{\text{structural}} = 1.038 \pm 0.027$, $n = 3$), indicating incorporation of post-bomb organic nitrogen.

Similar tests of organic nitrogen uptake used ectomycorrhizal fungi from the Duke Free Air CO₂ Enrichment experiments, where ¹³C labels were introduced via photosynthesis. Fungal protein was more ¹³C-enriched relative to structural C in elevated CO₂ plots than in control plots in three ectomycorrhizal taxa, but not in a saprotrophic taxon. We calculate that soil-derived organic N contributed up to 15% to fungal protein for the three ectomycorrhizal taxa. Our results prove organic nitrogen use in ectomycorrhizal fungi without requiring isotopic tracers.



Soil enzyme activities correlate with fungal abundance and abiotic conditions

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There is a growing need to incorporate mechanistic processes, such as enzyme activity, into soil biogeochemical models. The drivers of enzyme activity are not completely understood, making them difficult to model. Specifically the relative importance of microbial composition and abiotic factors on enzyme activity is not clear.

We compared relationships between enzyme activity and fungal community composition, fungal functional groups, and abiotic variables in 15 sites throughout Southern California. These sites include grasslands, forests, deserts, and shrublands over 1000km² from Santa Barbara to San Diego. For each sample we measured resin extractable nitrate, ammonium, and phosphate; moisture; total N; total C; and pH. In addition, elevation, mean annual temperature, mean annual precipitation, and fungal biomass were noted for each site. Fungal composition was measured via 454 pyrosequencing, and enzyme activities were measured fluorometrically for hydrolytic enzymes and colorimetrically for oxidative enzymes.

Enzyme activities were correlated with abiotic parameters, abundance of fungal functional groups, and fungal phyla, but not with fungal community composition. In particular, beta-glucosidase, acid phosphatase, and N-acetyl-glucosaminidase activities were significantly or marginally significantly associated with the abundance of Glomeromycota (BG: $R^2 = 0.20$, $P = 0.08$; AP: $R^2 = 0.28$, $P = 0.02$; NAG: $R^2 = 0.23$, $P = 0.04$). Alpha-glucosidase activity was marginally correlated with the abundance of Basidiomycota ($R^2 = 0.16$, $P = 0.07$), and beta-xylosidase with the ratio of saprotrophic fungi to arbuscular mycorrhizal fungi ($R^2 = 0.15$, $P = 0.09$). Two enzymes were mostly correlated with abiotic parameters: cellobiohydrolase with annual minimum temperature ($R^2 = 0.21$, $P = 0.001$), and phenol oxidase with mean annual precipitation ($R^2 = 0.18$, $P = 0.001$). None of these enzymes was significantly correlated with fungal community composition or with composition within functional groups (i.e., pathogens, saprotrophs, ectomycorrhizal fungi and arbuscular mycorrhizal fungi) ($P > 0.1$). These results suggest that including detailed metrics of fungal composition in models is not necessary. Instead, explicitly incorporating the abundance of fungal phyla and functional groups could explain a large portion of shifts in enzyme activity and, ultimately, nutrient and carbon cycling across ecosystems.



Enzyme activities altered by increased nutrient availability in Arctic tundra soils

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The Arctic tundra is a biome affected most by global warming predicted in the future. Such warming is expected to increase nutrient availability to soil microbes which, in turn, may accelerate soil organic matter decomposition. We investigated how soil enzyme activities were affected by increasing nutrient availability in an Arctic tundra ecosystem. Specifically, we measured potential activities of seven enzymes at three profiles (organic, organic/mineral interface, and mineral) of soils which had been fertilized in long- (23 years) and short-terms (six years), assayed at four temperatures. The long-term site had a high fertilization treatment ($10\text{g Nm}^{-2}\text{ year}^{-1}$ and $5\text{g Pm}^{-2}\text{ year}^{-1}$) and control, and the short-term site had a low fertilization treatment ($5\text{g Nm}^{-2}\text{ year}^{-1}$ and $2.5\text{g Pm}^{-2}\text{ year}^{-1}$) in addition to the high fertilization treatment and control. The fertilization treatments significantly altered most of the enzyme activities in both sites. The fertilization treatments increased activities of enzymes hydrolyzing products for C and nitrogen N sources, but decreased phosphatase activities. Such alterations were most pronounced in the organic soils. The fertilization treatments also increased ratios of total enzyme activities involved in hydrolysis for C products to those for N products. This result is consistent with an observation that long-term N and P fertilization decreased soil organic C in the same tundra ecosystem. Altered enzymatic stoichiometry with increased nutrient availability should be considered when modeling biogeochemical cycles in Arctic tundra ecosystems in response to warming predicted in the future.



Bioavailability of DOM in the York River Estuary: A stoichiometric and enzymatic approach

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The bioavailability of DOM in the York River estuary was determined in bioassays monitoring the stoichiometric decomposition of DOM along the fresh to saltwater axis of the estuary. Concurrent evaluations of ectoenzyme allocation and kinetics served as indicators of the types of labile compounds consumed in the DOM decomposition studies and provided an intimate link between bacterial production dynamics and the composition of the DOM pool supporting it. The York River estuary exhibits distinct disparities along the fresh to saltwater continuum with regard to both the source and lability of DOM. These differences are reflected in the bulk stoichiometry between the estuarine end members. Freshwater DOM is depleted in both N and P (C:N:P= 2150:92:1) relative to the mouth (C:N:P= 550:38:1). In addition bacterial decomposition studies indicate striking differences in the degradation rates both within the C, N and P containing constituents of the DOM as well as between York River end-members. The decomposition rates at the mouth relative to the freshwater end-member are elevated by 26% and 86% for DOC and DON, respectively. Approximately 46% of the DOP pool was demineralized at the mouth with no detectable DOP decomposition in freshwater. This diagenetic disparity suggests a variation in the sources and lability of DOC, DON and DOP supporting bacterial production between the two sites. Potential ectoenzymatic hydrolysis rates corroborate the conclusions from the stoichiometric data. The decline in potential maximum hydrolysis rates (V_{max}) of β -glucosidase and β -glucosidase from the freshwater end member to the mouth suggest bacteria rely on C-rich compounds to fuel production at the head of the estuary. The opposing trend in the V_{max} of leucine aminopeptidase implies a greater dependence on N-enriched compounds at the mouth. The high C:N:P ratio of the freshwater DOM coupled with increased glucosidase V_{max} values points to a larger terrestrial influence, while lower C:N:P ratios and a greater leucine aminopeptidase V_{max} suggest a more planktonic origin of the DOM at the mouth of the estuary. The elevated alkaline phosphatase V_{max} at the freshwater site implies seasonal P limitation. The spatial differences in bulk DOC, DON and DOP concentrations, the initial lability of these DOM pools, seasonal variations in inorganic nutrients availability, and implied enzymatic contrasts in the sources of DOM supporting bacterial production along the salinity gradient suggests spatial and temporal variations in the metabolism of DOM which may be a function of ionic (salinity) interactions or differences in bacterial community structure.



Terrestrial and aquatic microbial enzyme response to shifting nitrogen and phosphorus availability: experiments at the whole-watershed scale

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Human-driven nitrogen (N) enrichment of watersheds has a strong influence on microbial enzyme activity and the suite of ecosystem processes associated with those enzymes. This phenomenon occurs at the whole-watershed scale, yet rarely are terrestrial and aquatic components of watersheds examined in tandem. We examined microbial enzyme activity in soils and streams at a long-term, whole-watershed experiment site at the Bear Brook Watershed in Maine (BBWM). The BBWM is a paired watershed experiment comprised of two watersheds: one subjected to 22 years of N enrichment and acidification through ammonium sulphate fertilization and a contiguous, untreated reference watershed. Within these watersheds we nested terrestrial and stream phosphorus (P) enrichment experiments to test for enhanced P limitation under chronic N enrichment. Activity of enzymes associated with carbon (C), N and P acquisition (α -glucosidase, xylosidase, N-acetylglucosaminidase, leucine aminopeptidase and phosphatase) were measured in tandem with assays of decomposition rates and microbial respiration in soils, terrestrial and stream litter, and in stream water. Soils and streams displayed differential response to the chronic N and acute P enrichments. In soils and terrestrial detritus, activities of all enzymes were either lower in the treatment watershed or similar in the treated and reference watersheds. Experimental addition of P to terrestrial soil and litter typically reduced phosphatase activity, but yielded little change in the activity of other enzymes, rates of microbial respiration, or rates of decomposition. In streams, phosphatase activity was higher in the N enriched watershed with enzyme ratios suggesting elevated P limitation under chronic N enrichment and acidification. This was confirmed in P enrichment experiments that reduced phosphatase activity, elevated activity of enzymes associated with C and N, and accelerated microbial respiration and decomposition of organic matter. Our results show that long-term N enrichment of watersheds influences both terrestrial and aquatic systems, but in rather different ways. Differential results may be due to the roles of other constraints (e.g. moisture, temperature, enzyme stabilization) that are not equally expressed in terrestrial and aquatic environments. Such issues will need to be considered in modeling enzyme response to large-scale changes in nutrient availability.



Ecoenzymatic stoichiometry of soils, sediments and plankton

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The stoichiometry of ecoenzymatic activities is regulated by nutrient availability and microbial metabolism through a complex system of biogeochemical interactions and microbial signal transduction. Analyses of large data sets show that terrestrial soils, freshwater sediments and plankton systems show similar scaling relationships among the most commonly measured ecoenzymatic activities. For beta-glucosidase (BG) and phosphatase (AP), the slopes of ln-ln regressions are 1.16, 0.95 and 0.85 for soils, sediments and plankton, respectively, with R² values of 0.40, 0.43 and 0.72. The mean BG/AP ratios are 0.62, 1.64 and 0.26, respectively. For beta-glucosidase and the sum of leucine aminopeptidase (LAP) and beta-N-acetylglucosaminidase (NAG) activities, the regression slopes are approximately 1.1 for all three habitats, with R² values of 0.16, 0.62 and 0.50, respectively. The mean BG/ (LAP+NAG) ratios are 1.43, 1.83 and 0.12, respectively. The BG/AP relationships correlate with elemental stoichiometry data showing that (1) P availability is greatest in freshwater sediments and least in plankton systems; and (2) the C:P ratio of planktonic biomass is twice that of attached microbial communities. BG/ (LAP+NAG) relationships correlate with elemental stoichiometry data showing that C:N ratio of organic matter and biomass are similar across habitats. The low mean BG/ (LAP+NAG) ratio of plankton communities reflects (1) the low production of cellulose and (2) the strong microbial dependence on polypeptides as C sources. These ecoenzymatic data extend predictions of ecological theory and provide a basis for new models of microbial decomposition.



Methane uptake by grassland soils: Biogeochemistry, microbial ecology and integrative modeling

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Methane (CH₄) is a potent greenhouse gas, but controls on its past and future concentration remain highly uncertain. The uptake of CH₄ by methanotrophic bacteria in oxic, well-drained soils is a key part of the global CH₄ budget, and although global-scale models for present and future atmospheric CH₄ treat the soil sink as constant, numerous studies have documented the sensitivity of CH₄ uptake to climate, suggesting that interannual variation in sink strength may be significant, and that future climate change may further alter its magnitude. **The overall goal of this proposed work is to improve our mechanistic understanding of CH₄ uptake in oxic soils and to begin development of next-generation models that can better predict changes in the strength of this sink.** Our work focuses on temperate grasslands, a significant part of the soil sink.

Much of the climate sensitivity of CH₄ uptake hinges on the ecophysiological responses of local methanotroph communities to environmental variation. Our preliminary data from 3 grasslands (Konza, Sevilleta and Shortgrass Steppe LTER sites) indicate that both methanotroph community composition and ecophysiology differs among sites, suggesting that ecosystem simulation models may need to consider composition and biogeography to better predict uptake. Moreover, our prior work shows that CH₄ production can occur in well-drained soils with net CH₄ uptake, suggesting that this hidden or “occult CH₄ production” can also affect the net CH₄ uptake rate in ways that are poorly understood.

Efforts to achieve our overall goal are broken down into 3 objectives. First, we will collect data on CH₄ uptake and its controls from 29 study sites that cover the climatic diversity of the North American Great Plains grasslands. Second, we will determine the controls on CH₄ uptake by evaluating the hypotheses associated with the following 4 questions: Q1: What are the contributions of diffusivity, methanotroph activity and occult methanogenesis for structuring temporal and spatial patterns in CH₄ uptake? Q2: Which ecophysiological responses of the methanotroph community are most important for explaining temporal and spatial patterns in methanotroph activity? Q3: How are ecophysiological differences among sites related to methanotroph community composition? Q4: How well does the environmental regime of a site predict methanotroph community composition? These questions will be evaluated in a Bayesian multi-level model, using a model-selection context. Finally, the selected model will be used to improve predictions of CH₄ uptake in the DAYCENT ecosystem model.



Development of microbial-enzyme-mediated decomposition model parameters through analytical steady-state analysis and numerical simulation

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We developed a Microbial-ENzyme-mediated Decomposition (MEND) model, based on the Michaelis-Menten kinetics that describes the dynamics of physically defined pools of soil organic matter (SOC). These include particulate, mineral-associated, dissolved organic matter (POC, MOC, DOC, respectively), microbial biomass, and associated exoenzymes. The ranges and/or distributions of important parameters were determined by combining both analytical steady-state analysis and numerical simulations with SOC data from the literature. We used an improved multi-objective parameter sensitivity analysis (MOPSA) method to identify that the maintenance and turnover of microbial biomass, the turnover and production of enzymes, and the carbon use efficiency (CUE) were the most important parameters for the full model. As for the adsorption/desorption between MOC and DOC, the maximum sorption capacity was more important than the binding affinity of MOC. We applied the model to investigate the SOC responses to warming by further partitioning POC into two substrates, lignin and cellulose, decomposed by ligninase and cellulase, respectively. Under a scenario of +2°C (baseline temperature = 12°C) and constant CUE, we observed net C losses over 50 yr for all pools except DOC. With CUE that varies according to temperature, a temperature increase of 2 -5°C led to net losses of lignin and +1°C caused a slight net increase of lignin. Higher temperature with varied CUE might result in greater accumulation of C in both cellulose and MOC pools. Different dynamics in different SOC pools elucidated the catalytic functions of specific enzymes targeting specific substrates (e.g., ligninase and cellulase for lignin and cellulose, respectively) and the interactions between microbes, enzymes, and SOC. With the feasible parameter values estimated in this study, models incorporating fundamental principles of microbial-enzyme dynamics can lead to qualitatively different results for global change simulations.



Enzyme abundance drives leaf litter decomposition in a wet tropical forest

Bonnie Waring

Department of Ecology, Evolution, and Behavior, University of Texas, Austin, TX, USA

Rates of leaf litter decomposition are often modeled as a function of foliar concentrations of carbon (C), nitrogen (N), and phosphorus (P). However, microbial demand for these nutrients is actually the proximate control on rates of decay. Exoenzyme abundance reflects the difference between the stoichiometry of the microbial biomass and the availability of nutrients in the environment (Sinsabaugh et al. 2009), and is therefore often measured as a surrogate for microbial C, N, and P demand. To explore mechanistic links between enzyme activity and foliar decomposition, I used a litterbag experiment to test two hypotheses: 1) rates of foliar decay should be positively correlated with the abundance of exoenzymes on leaf litter, and 2) stoichiometry of enzymes involved in C, N, and P cycles should reflect the elemental composition of the foliar substrate.

In a wet lowland forest in Costa Rica, I used the litterbag method to examine the relationship between enzyme abundance and decomposition rates of five plant species that vary widely in their foliar chemistry (*Dipteryx panamensis*, *Geonoma cuneata*, *Hyeronima alchorneoides*, *Lecythis ampla*, and *Pentaclethra macroloba*). I periodically measured mass loss, fungal abundance, and activities of acid phosphatase (AP), beta-glucosidase (BG), cellobiohydrolase (CB), and glycine aminopeptidase (GAP) on the leaf litter. As expected, enzyme abundance was able to explain a majority of variance in mass loss rate for all plant species. However, enzyme stoichiometry did not vary with plant chemistry; instead, ratios of C-, N-, and P-degrading enzymes on leaf litter converged upon the 1:1:1 ratio observed across multiple ecosystems (Sinsabaugh et al. 2008). These enzyme ratios greatly diverge from those observed in soils at this tropical forest site, suggesting different patterns of nutrient limitation between leaf litter and soil microbial communities. Taken as a whole, these results suggest that microbial investment in exoenzymes can drive interspecific differences in rates of foliar decay, and that enzymatic controls on organic matter breakdown in forest ecosystems can differ between litter and mineral soil.



Extracellular enzyme activities and stoichiometry in a lowland tropical soil exposed to leaf litter and through - fall manipulation

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¹University of Colorado, INSTAAR and Ecology & Evolutionary Biology, Boulder, CO, USA;

²William R. Wieder, Division of Climate & Global Dynamics, National Center for Atmospheric Research, Boulder, CO, USA;

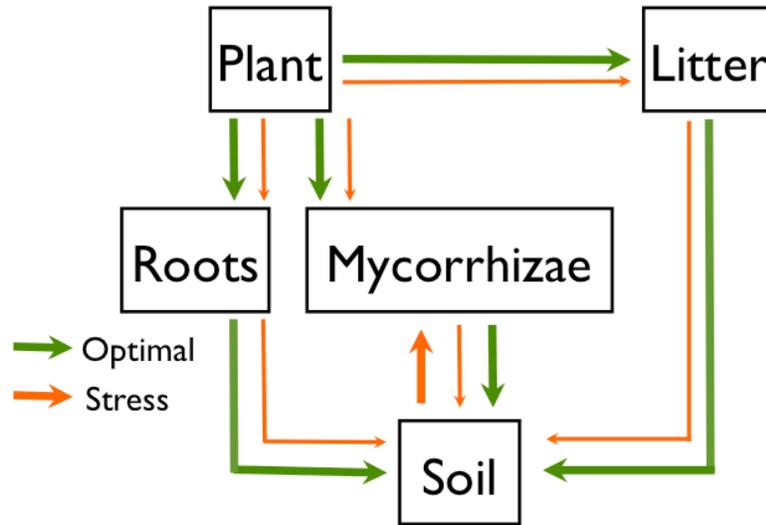
³Department of Ecosystem and Conservation Sciences, University of Montana, Missoula, MT, USA

The role of soil exo-enzymes in mediating organic matter (OM) mineralization in tropical forests is not well understood. Recent syntheses have revealed robust relationships between soil carbon (C) content and soil exo-enzyme activities and relative stoichiometric equilibrium between enzymes that acquire C, nitrogen (N) and phosphorus (P) at the global scale. Whether these patterns apply to wet tropical forests, which often have large litter-to-soil dissolved organic carbon (DOC) fluxes and highly weathered soils, has not been resolved. We used an in-situ manipulation of both rainfall and litterfall in a lowland tropical forest to explore hydrolytic extracellular enzyme activities (EEA) across a spectrum of OM inputs. In plots with double litter addition, soil C, C: N, and litter-to-soil DOC concentrations all increased. Soil EEA per gram soil was higher for one N and two C-mineralizing enzymes. In plots with no litter inputs, soil C, microbial biomass, DOC and soil moisture all declined. Five of six hydrolytic enzymes had lower activities per gram soil, yet acid phosphatase (aP) activities were elevated when normalized by soil C. When 50% of throughfall was blocked without alteration of leaf litter, DOC concentrations increased yet total soil and microbial C pools did not change, nor did enzyme activities. Exo-enzymes generally did not relate to DOC concentrations but did relate to soil C to differing degrees. β -1,4-N-acetylglucosaminidase (NAG) displayed tight coupling with C concentration ($R^2 = 0.60$), aP displayed weaker positive correlation ($R^2 = 0.34$) and C-mineralizing enzymes were intermediate. We used multiple regression to determine best predictors of soil EEA across all treatments; soil C: N, microbial biomass N and soil moisture in combination explained the most variance in enzyme activities. The mean β -1,4-glucosidase (BG): aP ratio in this tropical soil (0.20) was low compared to the global average and declined even further in zero litter plots. β -1,4-N-acetylglucosaminidase + leucine aminopeptidase (NAG+LAP): aP ratios were also low (0.16) yet they increased significantly in double litter plots. The former likely reflects elevated heterotrophic P limitation upon litter removal and the latter, increased relative N-demand with the addition of C-rich litter. Finally, while the regression slopes of BG: aP and BG: (NAG+LAP) were not different between our treatments, mean slopes (1.36 and 1.32 respectively) were significantly higher than global soil averages. Heterotrophic limitation by nutrients over C is thus likely in this wet tropical soil.



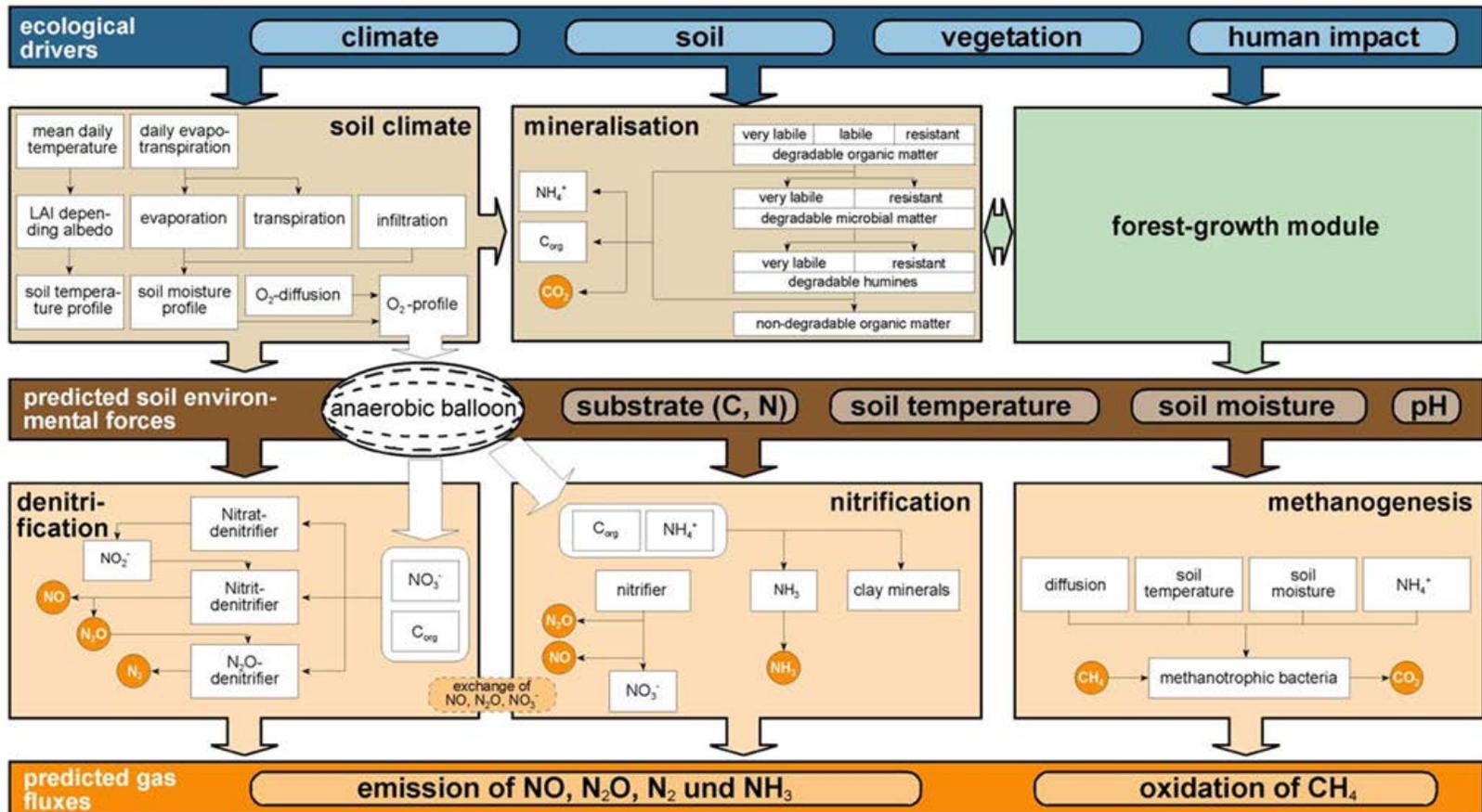
CONTRIBUTED MODEL SCHEMATICS

Organized Alphabetically by Contributing Participants Last Name



Carbon fluxes among pools in the plant-mycorrhizae-soil interaction. When plants are producing at optimal rates (green), soil carbon decreases in magnitude only through soil turnover. When plants produce less because of stress that limits production (orange), soil carbon decreases in magnitude due to reduced inputs (thinner arrows) and uptake of carbon by mycorrhizae.

Contributed by:
Jessica Bryant



Contributed by:
 Michael Dannenmann
 and Klaus Butterbach-Bahl

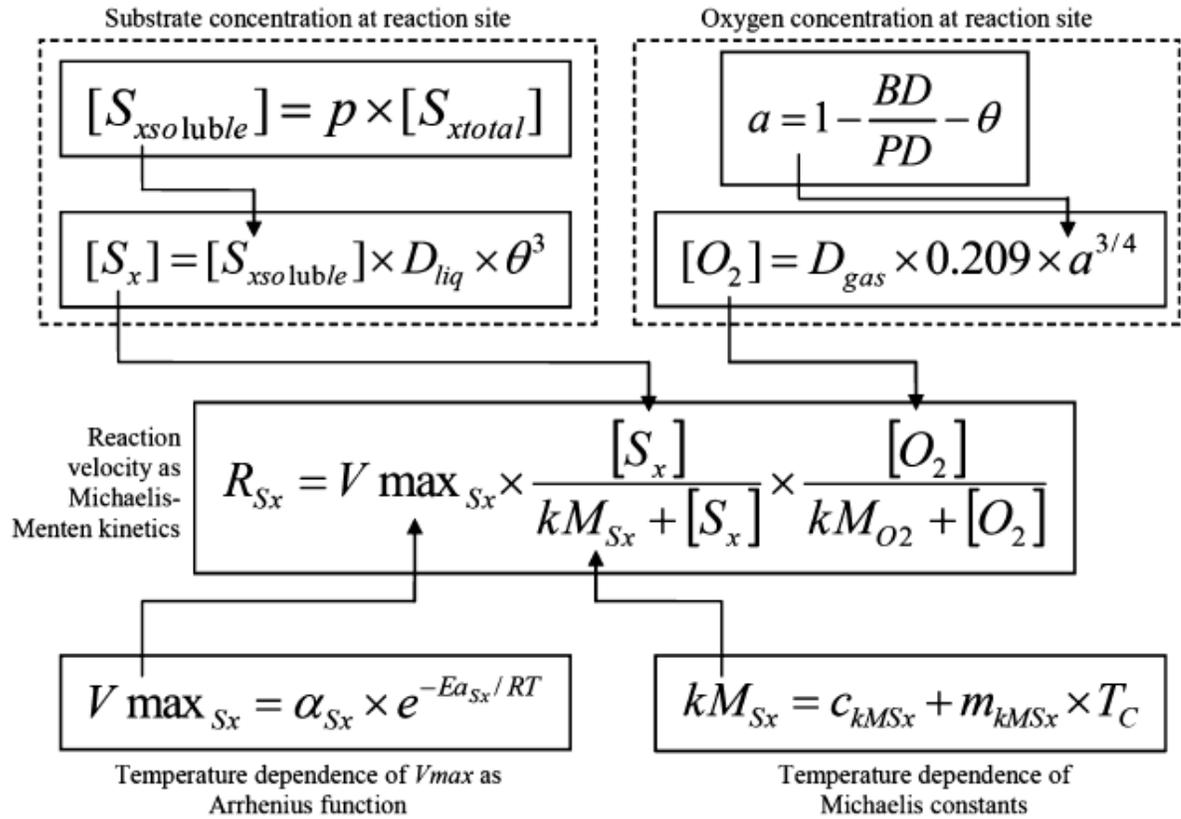
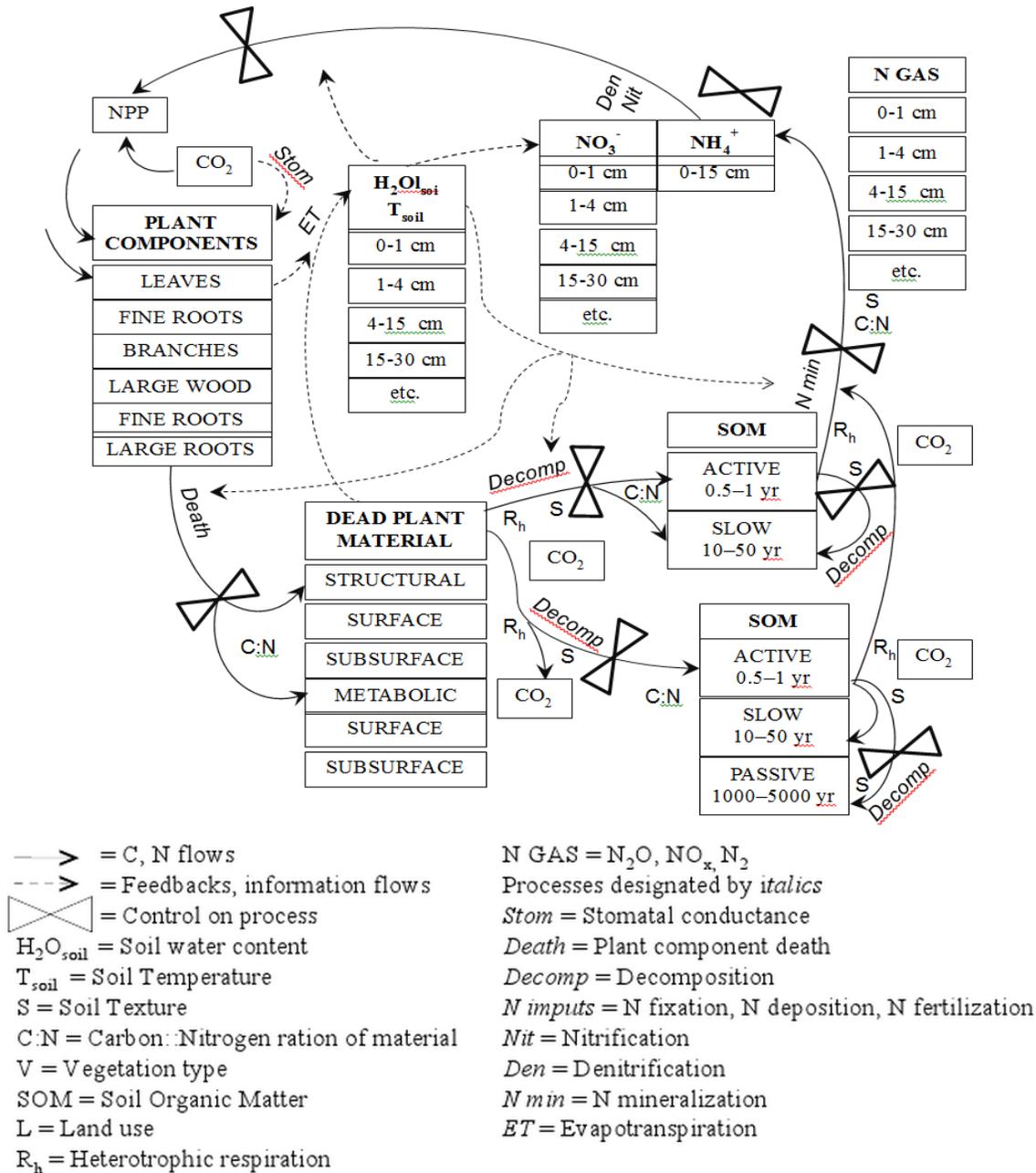


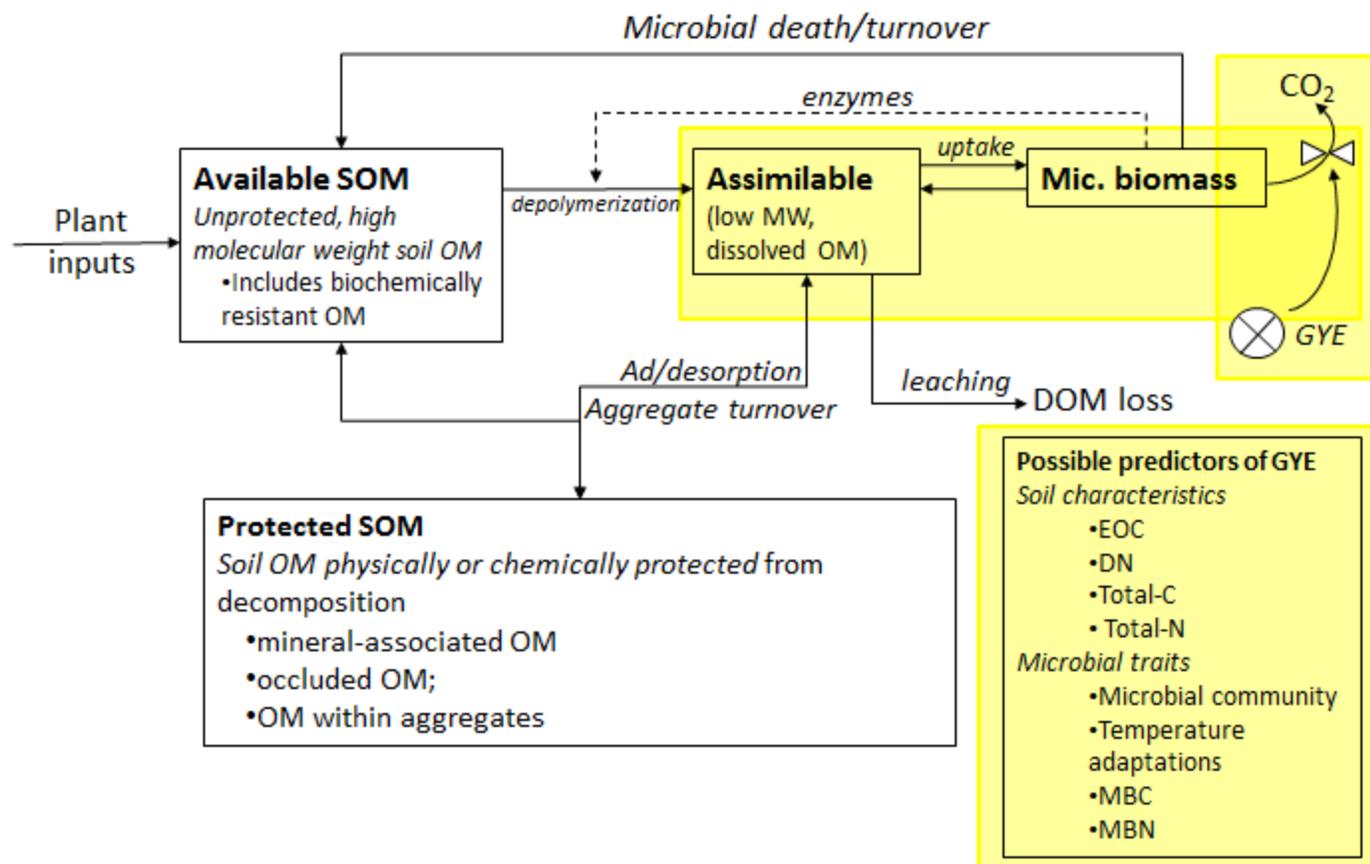
Fig. 1 A graphical representation of the compartmentalized structure of the DAMM model.

Contributed by:
Eric Davidson



Daycent Flow Diagram

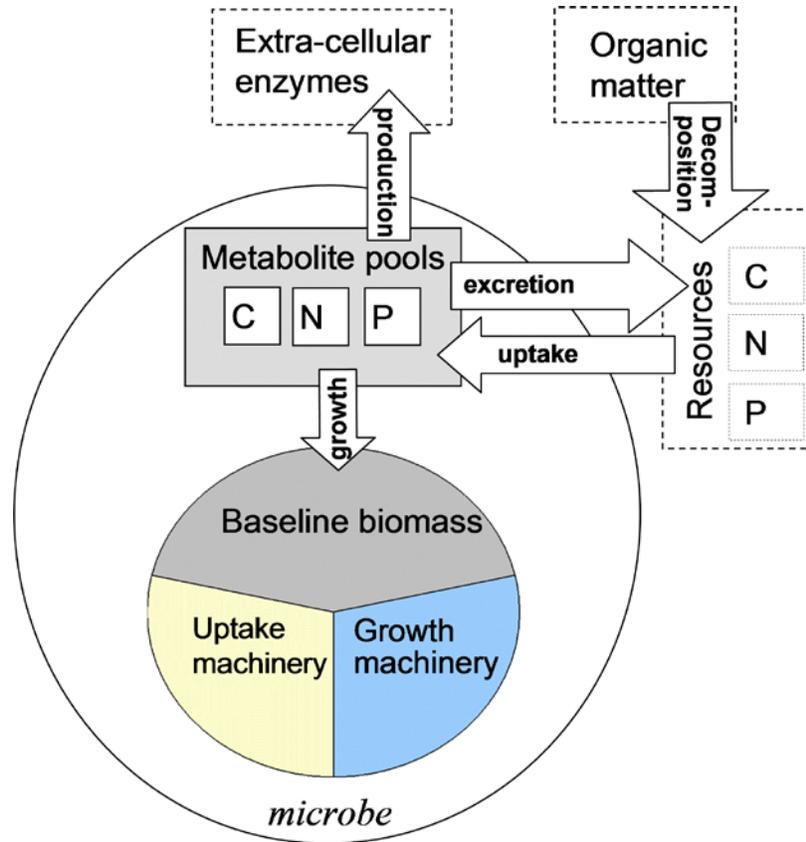
Contributed by:
 Steve Del Grosso



Adapted from Conant 2010.

GYE added as control on CO₂ release– the sections in yellow are those I have focused on with my experiment and MLR model

Contributed by:
 Jessica Ernakovich



Model components and fluxes (processes) of the Microbial biomass acclimation and enzyme production model. The model is based on the microbial biomass acclimation model described in: Franklin, O., E.K. Hall, C. Kaiser, T.J. Battin and A. Richter 2011. Optimization of biomass composition explains microbial growth-stoichiometry relationships. *American Naturalist*. 177:e29-e42.

Contributed by:
Oskar Franklin

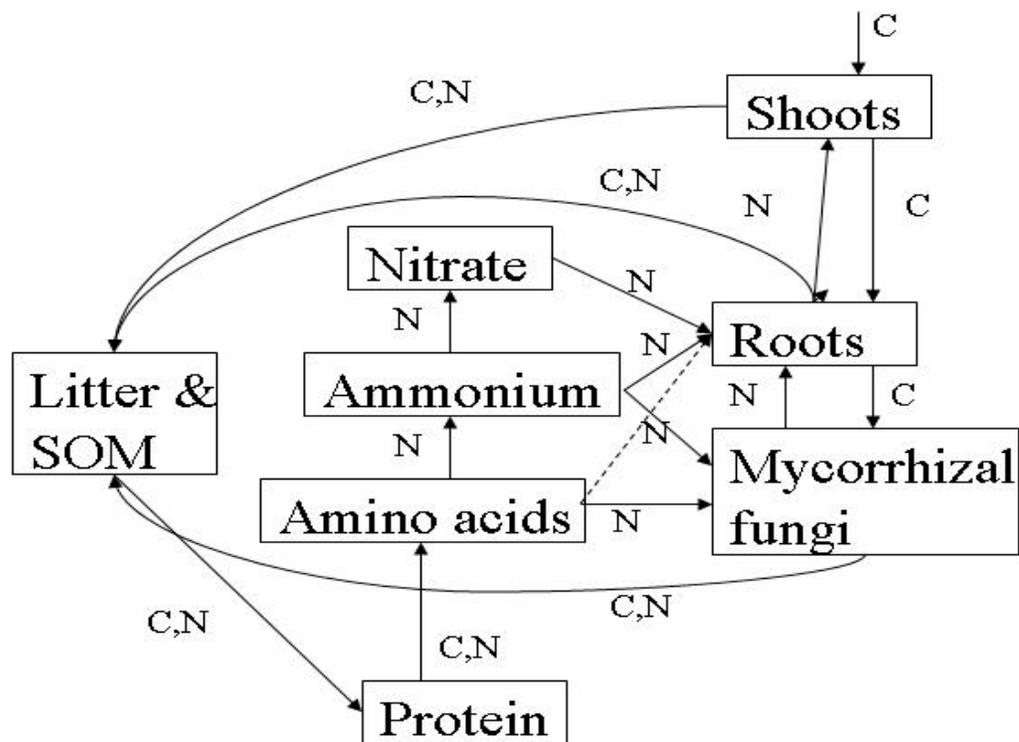
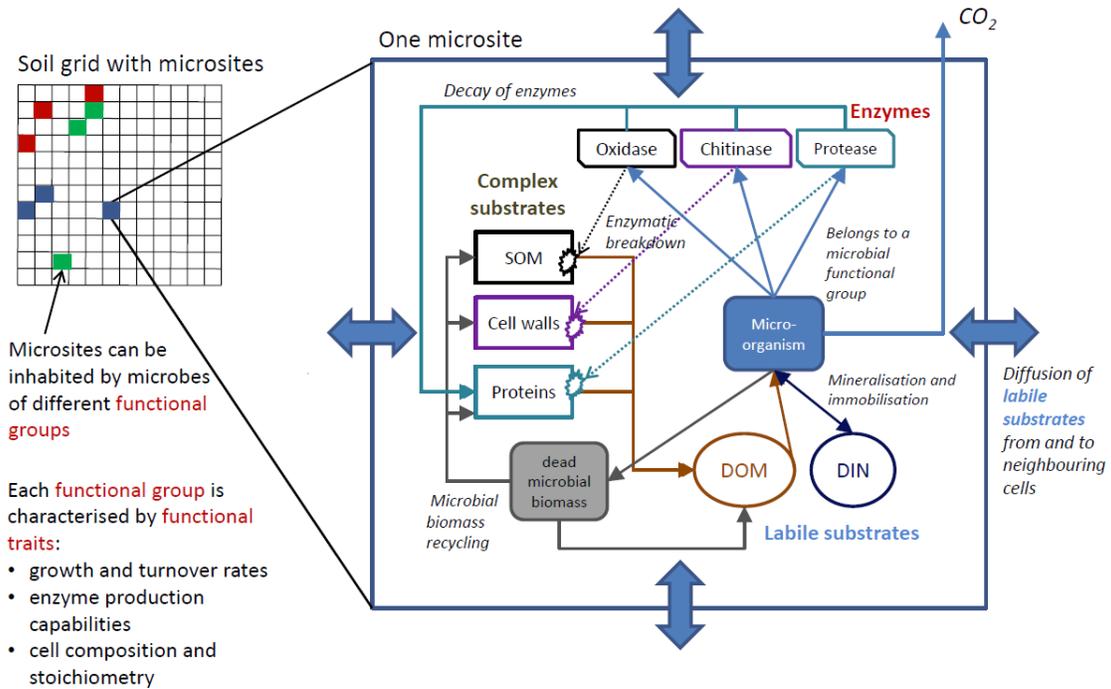


Figure 5. Diagram of C and N fluxes in forest ecosystems. For clarity, respiratory fluxes and leaching losses are not indicated. Stippled line indicates the unclear importance of root uptake of amino acids in forests. SOM, soil organic matter.

Contributed by:
Erik Hobbie and Andy Quimette



The microbial functional group model

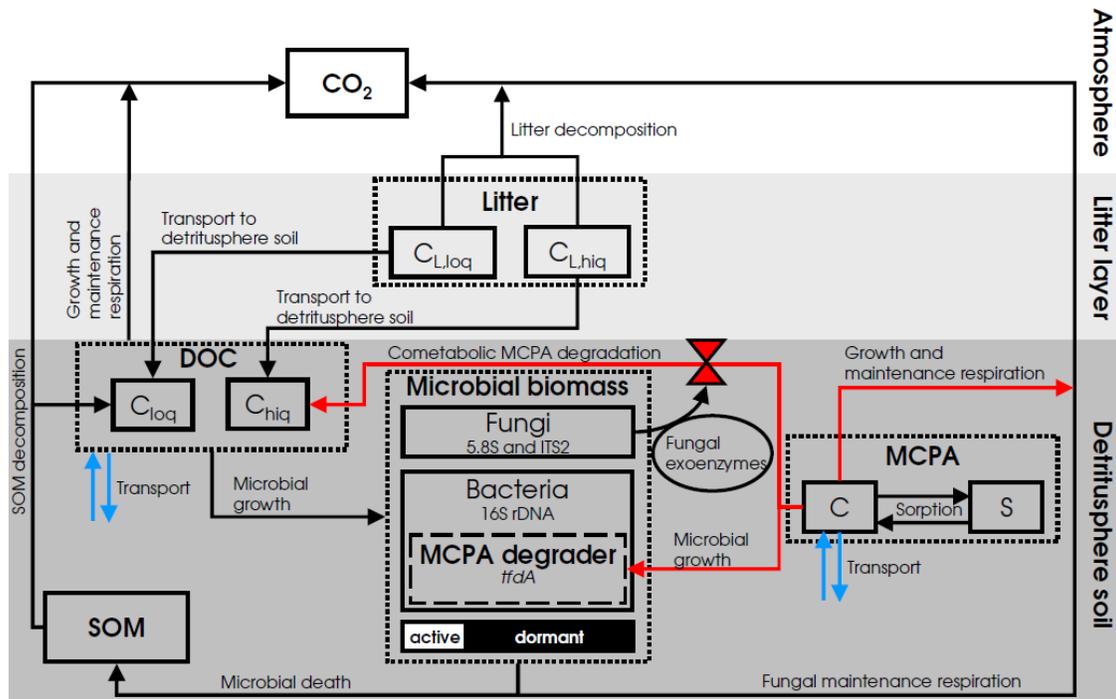


Christina Kaiser
 International Institute for Applied System Analysis (IIASA)

Contributed by:
 Christina Kaiser



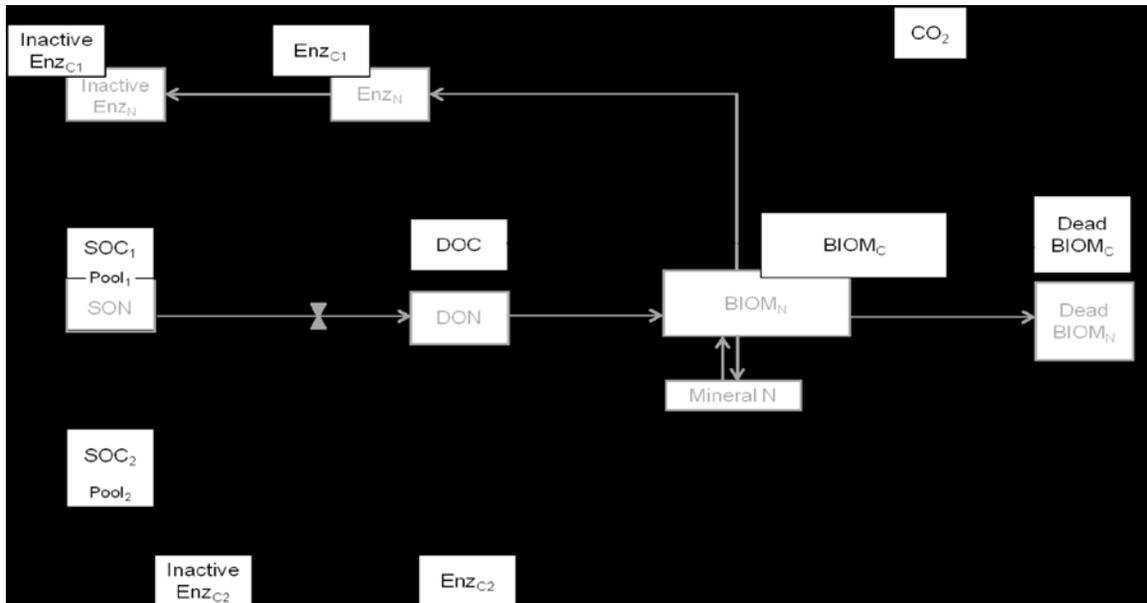
2nd International Enzymes in the Environment Workshop
Incorporating Enzymes and Microbial Physiology into Biogeochemical Models
Fort Collins, CO May 15-18 2012



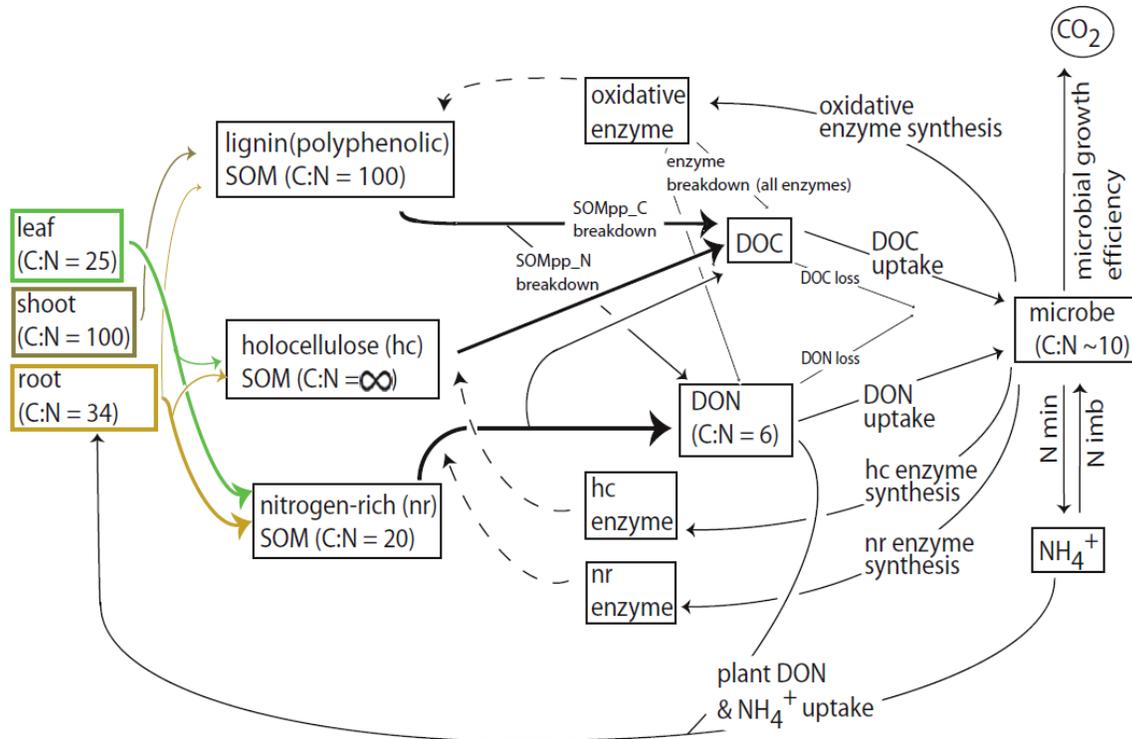
Contributed by:
Ellen Kandeler



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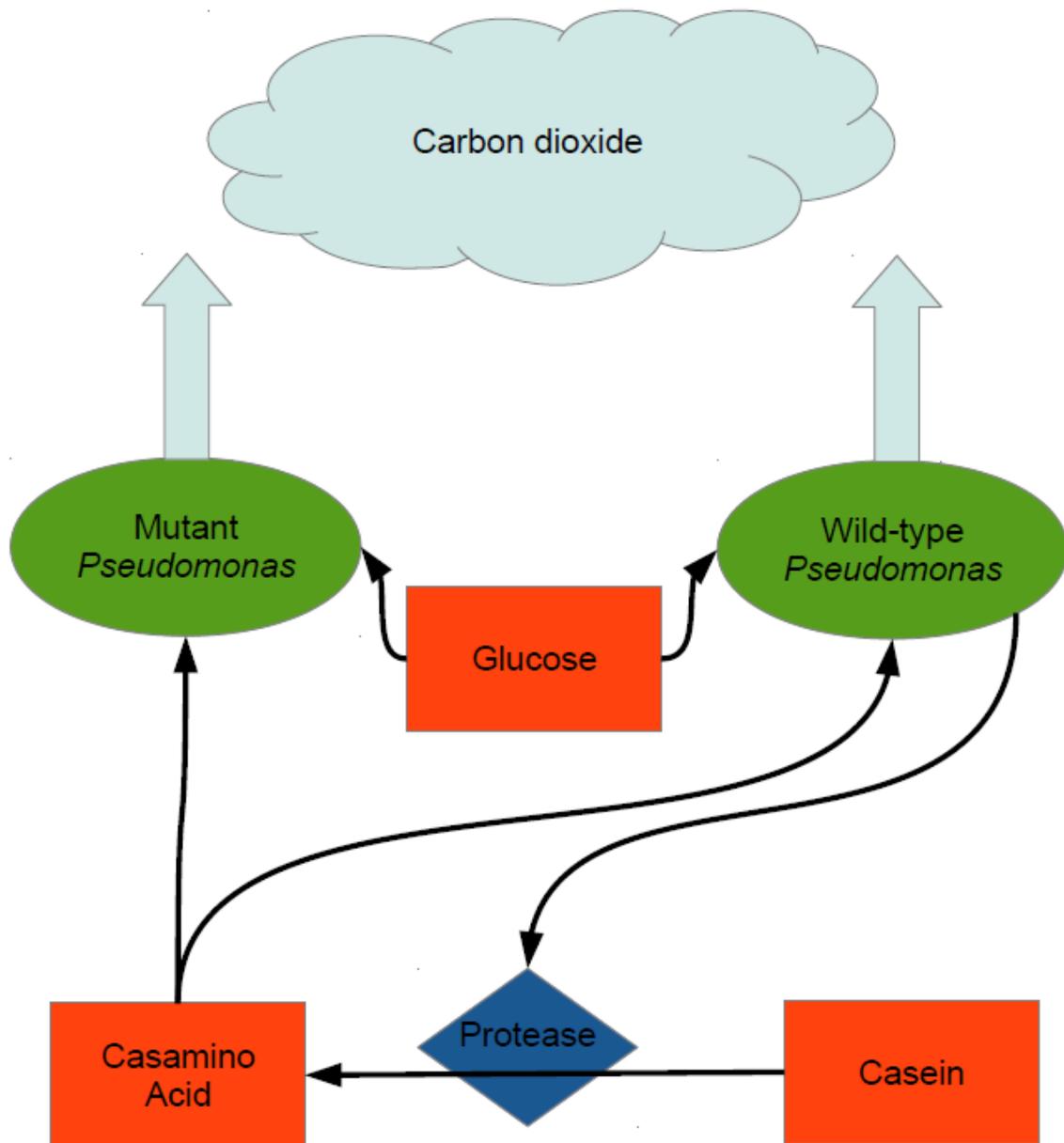


Contributed by:
Gwenaelle Lashermes,
Daryl Moorhead,
and Robert Sinsabaugh



A carbon (C) and nitrogen (N)-linked model of ecosystem biogeochemical cycling. Boxes represent pools of g C or N m⁻², while arrows represent processes, the flow of material between pools or out of the model system. The model includes three classes of soil organic matter (SOM), extracellular enzymes specific to these SOM pools, a microbial C:N ratio that varies from a bacterial-like to fungal-like community and dynamic plant allocation to wood, root and leaf growth. The microbes adapt between a more bacterial-like (lower C:N, faster turnover) and fungal-like community (higher C:N, slower turnover). Plants dynamically allocate to wood, root and leaf growth based on N-uptake. The plant pools provide inputs to the SOM pools at a higher C:N than their standing biomass (via N retranslocation). Not shown, but represented in the model, are inputs and losses of NH₄⁺, DON, or DOC, microbial death and extracellular enzyme turnover.

Contributed by:
 Seeta Sistla



Well-mixed biomass-enzyme driven decomposition model

Contributed by:
Kathy Todd-Brown



RFP - SUPPORT FOR FUTURE COLLABORATION

Purpose:

To enable further pursuit of important new ideas and collaborations stimulated by the workshop.

About the Application:

RCN funds are available on a competitive basis to provide **travel support** to workshop participants for developing ideas and solidifying new collaborations. Collaborative examples include (but are not limited to):

- Synthesis of existing cross-disciplinary ideas
- Development of a new biogeochemical model
- Development a new proposal or paper for publication.
- Research exchanges to acquire new skills in enzyme assays or modeling.
- Sharing of data and model testing

Applications should be formatted as single spaced, 12 point font, 1-inch margins, and should be one or two pages in length. The application should include the following information:

- Project title
- Names and contact information of workshop participants involved in the proposed work
- A description of the project, including its purpose(s), specific goal(s), envisioned impact and outcomes, and a brief timetable of activities.
- Estimated budget

Projects are limited to a one-year time frame and budgets should not exceed \$2,500. Because of NSF stipulations, the RCN cannot support international travel by scientists residing in the U.S., but can support travel of international scientists to the U.S.

Applications are due by 5 pm (Mountain Standard Time) **July 1, 2012**.

Contact the RCN for further details on the application process at enzymes@nrel.colostate.edu. Please put "Collaboration Support" in the subject area

Enzyme education

Dr Matthew Wallenstein, Professor Richard Dick and Dr Mary Stromberger discuss the impact enzymes have on the environment as well as the importance of forming a Research Coordination Network in order to advance findings through collaborative methodology and knowledge sharing



When did you first associate enzyme function with broader environmental issues? How did this project emerge and what are your specific goals?

MW: Enzymes play a critical role in the functioning of natural ecosystems, and their power is also harnessed for engineered processes such as sewage treatment, biofuel production, and bioremediation. My own interest in enzymes began as I was researching the vulnerability of the vast stores of carbon held in Arctic soils to climate warming.

As I began to incorporate enzymes into my own research, I realised that there are significant gaps in our understanding that make it difficult to interpret the mechanisms behind patterns in enzyme activities and their consequences for ecosystem functioning. I saw a great opportunity to revisit fundamental questions of how enzymes work, what controls enzyme production and degradation, and which microbes produce them, using recently developed genomic and proteomic technologies.

Along with co-investigators Richard Dick and Mary Stromberger, I organised the enzyme

research community to develop a proposal for a Research Coordination Network (RCM). We were successful in receiving funding from the U.S. National Science Foundation to help spur and coordinate fundamental research into environmental enzymology. While we cannot directly fund research, we bring together researchers to focus on advancing our knowledge of specific issues, and synthesising current knowledge.

Could you explain what enzymes are and their role in the decomposition process?

MW: Enzymes are proteins that catalyse chemical reactions, which would otherwise occur at rates too slow to sustain life. There are two primary types of enzymes found in the environment:

- Oxidative enzymes catalyse redox reactions, and are especially important in the degradation of lignin, the structural component of wood
- Hydrolytic enzymes are more specialised, targeting specific bonds in polymeric substrates. These enzymes are important in degrading proteins, cellulose and for obtaining phosphorus.

Decomposition accelerates with warming primarily because it is an enzymatic process, and enzymes work faster at warmer temperatures. However, not all enzymes are equally temperature sensitive. Even enzymes that perform the same function-degrading cellulose, for example, vary in their temperature sensitivity depending on the microorganism that produced them.

How important is it to standardise and advance enzyme methodology? What strategy are you employing to achieve this goal?

MW: There is considerable debate on the pros and cons of specific methods used in enzymology. My own view is that there are no perfect methods, and that they all have trade-offs. Thus, I encourage researchers to choose the most appropriate method for their particular objectives and questions. Our network activities have helped to clarify this debate and elucidate the advantages and disadvantages of alternative methods.

RD: The vast majority of bench scale enzyme activity assays – particularly those using

para-nitrophenyl as the substrate – are highly reproducible for skilled operators following the same rigorous set of conditions initially developed for a given assay. However, interpretation is another matter because a significant amount of activity for many soil enzymes stem from catalytic enzymes stabilised in the soil matrix that are no longer associated with a viable cell (abiotic enzymes). These lab methods do not measure in situ activity.

In this context, micro-plate methods present a challenge. They are attractive for high throughput, but because they use extremely small amounts of soil in the assay and substrates that produce fluorescing products; this creates problems of high variability and reproducibility across operators. Further research is needed to determine the critical components and to test a standardised micro-plate method across a range of labs.

What ultimate impact do you expect the project to have and what plans are in place to ensure the legacy of the funded work is successful?

MW: Ultimately, our goal is to increase fundamental knowledge of the ecology of enzymes in the environment so that we can better interpret enzyme data and predict how their activity will respond to environmental change.

I think we can take some credit for refocussing research on fundamental questions of the ecology of enzymes in the environment. There have been several recent papers that have really advanced our understanding of enzymes using creative approaches. Many of these studies are addressing questions brought to light by RCN activities.

MS: We have already made some important educational impacts, by providing opportunities for graduate students to come to workshops, conferences, and visit laboratories so that they can learn enzyme methodologies.

When is the next conference/workshop being held?

MW: This May, we are hosting a workshop on incorporating enzymes into ecosystem models. At the workshop, we will explore alternative approaches to modelling enzymes, the trade-offs involved in increasing model complexity, and identify the types of empirical data that will be needed to parameterize the next-generation of models.

Catalysing scientific discovery

The **Enzymes in the Environment** Research Coordination Network is currently halfway to completion, but has already gathered significant expertise and interest in advancing environmental enzymology

EXTRACELLULAR ENZYMES ARE produced by microorganisms and released into their environment in the hopeful act that the products of enzymatic activity will provide more resources than were invested in their production. They are involved in numerous invaluable biogeochemical processes and are central to important ecosystem services provided by both terrestrial and aquatic ecosystems. Their most critical function is the decomposition of organic matter – an essential process for reducing waste, recycling plant nutrients, and driving the carbon cycle in soils and other ecosystems. With further study, management of extracellular enzymes could make a significant impact on the reduction of polluted soils and waters within our ecosystem.

The study of extracellular enzymes is not only critical for natural and managed environments, but is also fundamental to the industrial, medical and food sectors. For example, the food industry must control post-harvest spoilage and polysaccharide wastes. In the U.S., industry has been unable to meet congressional mandates for cellulosic ethanol due to high production costs. The discovery or development of more efficient enzymes would make biofuel production more economically tractable.

However the ability to understand and manage extracellular enzyme production and stabilisation is constrained by a lack of methods for measuring in situ activity, limited communication between researchers from multiple disciplines and limited incorporation of current research into ecosystem models.

OVERCOMING GAPS IN ENZYMATIC RESEARCH

Although there has been staggered research into enzyme productively over past decades, Professor Matthew Wallenstein of Colorado State University noticed significant gaps in current knowledge: "In the early days of environmental enzymology there were many detailed studies of enzyme kinetics. Since that time, less detailed enzyme assays have largely been used as indices of soil quality or microbial activity," he observes.

With this in mind Wallenstein, together with his co-investigators Professors Richard Dick and

Mary Stromberger, applied for and received a grant from the U.S. National Science Foundation to form the Enzymes in the Environment: Research Coordination Network (RCN). The RCN has developed specific goals to enhance the understanding of extracellular enzymes. Foremost was to develop an international community of researchers spanning from students to senior scientists.

NEW DISCOVERIES

Prior to the first international conference on 'Enzymes in the Environment' held in 1999, terrestrial and aquatic microbiologists, biochemists and microbial ecologists conducted research on ecological and environmental enzymology independent of one another. Wallenstein believes that their approach to the RCN has initiated great discussion between groups who would not normally work together: "We are working with engineers focussed on optimising the production of biofuels from feedstock using a mixture of several manufactured enzymes," he elucidates. "We are connecting them to scientists studying the decomposition of those same plant materials in the natural environments, where hundreds or thousands of enzymes are involved. Both partners benefit – the engineers gain new ideas about how to develop a more efficient process, and the ecologists learn new techniques for studying enzymes."

The consortium feel that there is no better time to advance a field that has been using similar techniques for 25 years. Since forming the RCN, new developments in genomics, proteomics and new modelling approaches have started to yield significant advances in extracellular enzyme research. The RCN is working to increase coordination and communication among those developing and applying these new tools.

Genomic tools have provided new insights into the regulation of enzyme production through detection and quantification of mRNA. Until recently, it was only possible to detect the action of enzymes, but new mass spectrometry techniques allow scientists to detect the enzyme itself: "This is an exciting breakthrough that could revolutionise our understanding of enzymes in the environment," Wallenstein highlights.

INTELLIGENCE

ENZYMES IN THE ENVIRONMENT

OBJECTIVES

To advance understanding of the controls on the production of enzymes by different microbes, their stabilisation and turnover, and their in situ activity by enhancing collaboration and communication between the international community of scientists involved in various aspects of this research.

FUNDING

National Science Foundation (NSF) – award no. 0840869

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MATTHEW WALLENSTEIN is an Assistant Professor in the Department of Ecosystem Science and Sustainability, Colorado State University and a Research Scientist at the Natural Resource Ecology Laboratory. His research addresses the role of soil microbial communities in controlling ecosystem response to global change.

MARY STROMBERGER is an Associate Professor of Soil Microbiology. Her research objectives are to identify important ecosystem service providers within microbial communities, major abiotic and biotic factors that regulate their activities, and the spatial and temporal scales over which these microbes and their activities occur.

RICHARD DICK is an Ohio Eminent Scholar and Professor in the School of Environment and Natural Resources at The Ohio State University. His research focuses on soil biochemistry and ecology of microorganisms in relation to terrestrial management and manipulations for beneficial ecosystem services.

Stromberger adds that this is especially helpful in soil environments, where there are tens of thousands of different bacterial species in just one gram of soil: "With this incredible level of diversity, it has been challenging to demonstrate the importance of diversity to soil ecosystem functions. At least with enzymes and their associated genes, some researchers have been able to link microbial community structure with function".

Another discovery involves new models that have recently been developed to explicitly represent the activities of enzymes. These models have demonstrated that changes in enzyme production or in the temperature sensitivity of enzymes could have large impacts on climate-carbon feedbacks and other biogeochemical processes in the future. In 1992, the Kyoto Protocol called for increased research of carbon stabilisation in soils since any increase in soil decomposition rates could increase atmospheric CO₂ concentrations, accelerating global climate change. Wallenstein believes that enzyme activity should play a critical role in global change research: "Understanding how enzyme temperature sensitivity varies between seasons, or between different regions, is critical for making accurate predictions of ecosystem responses to climate change," he states.

OVERCOMING CHALLENGES

Half way through the five-year funding cycle, the RCN has helped to synthesise some key findings related to extracellular enzymes. However, many areas of uncertainty remain. One of their key objectives was to encourage the standardisation of enzyme methodology. The conditions under which enzyme assays are conducted have been debated for many years. Enzyme activities are affected by factors such as

levels of pH, water content and substrate concentration. Methodological differences can strongly affect the measured rates. Wallenstein points out how seemingly small details in protocols can have disproportionate effects on measured values: "One of our biggest challenges is the paucity of good standards because we lack standardised enzymes that can be used to calibrate between runs and between different labs. However, enzymes degrade during storage and vary in their activity even if produced under controlled conditions, and thus prevent their use as standards".

Another challenge is that labs differ in the types of equipment they use. However this is not to say that one method is more beneficial than another: "Rather than prescribe a specific protocol, the community has instead developed recommended best practices to ensure that the data collected is as accurate and meaningful as possible," Wallenstein explains. It should be stressed that the objective of the RCN is not to develop new methodologies, but rather help to bring together research from disparate fields using a range of technique into an integrated framework; by bringing experts from different fields together, their collective expertise can be applied to advancing the field.

THE FUTURE OF THE RCN

The RCN has already been recognised as having positively impacted not only research methodology but also for spurring new avenues for research: "We have generated interest in enzymes from early career researchers who have the training and skills to apply new technologies to enzyme research," Wallenstein adds.

Perhaps the interactive and informative Enzymes in the Environment RCN website could be pinpointed as influencing students. Features such as discussion forums, news and announcements are used to promote workshops, conferences and thought provoking topics: "The formal network we've developed solidifies a sense of community that will last well beyond the RCN," Wallenstein concludes.

