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LETTER

Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments

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Abstract

Extracellular enzymes allow microbes to acquire carbon and nutrients from complex molecules and catalyse the rate-limiting step in nutrient mineralization. Because the factors regulating enzyme production are poorly understood, I used a simulation model to examine how competition, nutrient availability and spatial structure affect microbial growth and enzyme synthesis. In simulations where enzyme-producing microbes competed with cheaters (who do not synthesize enzymes but take-up product), higher enzyme costs favoured cheaters, while lower rates of enzyme diffusion favoured producers. Cheaters and producers coexisted in highly organized spatial patterns at intermediate enzyme costs and diffusion rates. Simulations with varying nutrient inputs showed that nitrogen supply can limit carbon mineralization, microbial growth and enzyme production because of the nitrogen-demanding stoichiometry of enzymes (C : N = *c.* 3.5 : 1). These results suggest that competition from cheaters, slow diffusion and nitrogen limitation may constrain microbial foraging and the enzymatic decomposition of complex compounds in natural environments.

Keywords

Carbon, competition, extracellular enzyme, microbe, mineralization, model, nitrogen, phosphorus, stoichiometry.

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INTRODUCTION

Microorganisms drive critical processes in all ecosystems, including nutrient mineralization, decomposition and the removal of natural and anthropogenic toxins (Swift *et al.* 1979; McGill & Cole 1981; Soulas 1993; Schimel & Bennett 2004). Many of these processes depend on the conversion of complex substrates into smaller compounds that microbes then assimilate for growth and metabolism. Except in environments where simple nutrients are abundant, microbes rely on extracellular enzymes to degrade complex substrates into usable forms (Ratledge 1994).

The reliance of microbes on extracellular enzymes as a foraging strategy raises a number of important questions. When do microbes produce extracellular enzymes? What costs are involved? What is the fate of secreted enzymes and how effective are they? Answers to these questions are critical for understanding how microbial populations interact with chemical substrates to drive fundamental ecological processes.

Microbes that rely on extracellular enzymes to obtain essential resources face several important challenges. Because these enzymes function outside the cell, reaction products may diffuse away from the enzyme-secreting microbe. Other microbes in the system could intercept these products without secreting their own enzymes. This strategy of 'cheating' should arise whenever multiple organisms can benefit from the resource investment of a single organism (Velicer 2003), and could represent a competitive threat to enzyme-producing microbes. Nonetheless, extracellular enzymes are ubiquitous in aquatic, marine and terrestrial environments, and must represent a viable means of obtaining energy and nutrients under at least some conditions.

Economic theory can provide a useful framework for evaluating the conditions under which enzyme production is a viable foraging strategy. This framework is the basis

for several conceptual models of microbial function and enzyme production (Koch 1985; Sinsabaugh & Moorhead 1994; Schimel & Weintraub 2003) and predicts that the resource benefits of enzyme construction and secretion should outweigh the costs. Because enzymes are built from amino acids, the cost of enzyme production is largely in terms of carbon (C) and nitrogen (N) (Harder & Dijkhuizen 1983; Sterner & Elser 2002; Allison & Vitousek 2005), while the benefits may accrue from any nutrient in complex form. Therefore, enzyme production may be a particularly viable strategy for obtaining some nutrients, such as phosphorus (P), when C and N are abundant. In contrast, C and N are more challenging to acquire because enzyme synthesis is C- and N-demanding. The economic framework also predicts that if soluble products are already abundant in the environment, then microbes should allocate scarce resources to growth instead of enzyme production.

The viability of microbial enzyme foraging probably also depends on the spatial structure of the environment. Spatial heterogeneity can be an important determinant of microbial function and population dynamics (Chao & Levin 1981; Kerr *et al.* 2002), and populations often behave very differently in spatially structured vs. well-mixed environments. Mixing can favour cheaters because all organisms compete directly for a homogeneous resource pool (Codeço & Grover 2001; Amarasekare 2003). However, most natural systems contain a large degree of spatial heterogeneity. For example, organic substrates, microbes and mineral particles form a three-dimensional matrix of aggregates and pore spaces of different sizes in soils (Tisdall & Oades 1982). For enzyme-dependent microbes, these physical properties should influence the movement of substrates, enzymes and usable products (Vetter *et al.* 1998), and could mitigate the competitive threat from cheaters.

Here I use an individual-based simulation model (EnzModel) to explore whether resource acquisition through enzyme production is a viable strategy for microbes in the face of low nutrient supply and competition from cheaters. Because most natural environments are spatially structured, I modelled microbial competition and nutrient mineralization across a diffusional gradient ranging from low diffusion to well-mixed systems. I also used the model to examine how resource availability affects enzyme production, microbial growth and mineralization. My simulations explored three main hypotheses: (i) enzyme-producing microbes (producers) compete poorly against cheaters in well-mixed systems but coexist in spatially structured environments, (ii) rates of diffusion can limit the growth and activity of microbial populations and (iii) nutrients, particularly N, can limit microbes' ability to acquire resources with enzymes.

MATERIALS AND METHODS

Coding

I wrote the model code (available at http://www.stanford.edu/group/Vitousek/) using C++ and ran the model on computers running UNIX.

Grid initialization

The model begins by setting enzyme, substrate and product concentrations to default values of 0 fg μm^{-3} in each 1 μm^3 grid box of a 100×100 array (Fig. S1). To avoid edge effects in the model, I used periodic boundary conditions which force the grid edges to wrap around and meet each other to form a toroidal shape. Cheaters and producers were randomly assigned to each grid box at default initial frequencies of 0.01 with biomasses of 150 fg C $cell^{-1}$ (Button *et al.* 1998) (Table S1). Initial nutrient contents for microbes were assigned based on optimal C : N ratios of 6 and C : P ratios of 60.

Iteration

During each iteration, the model carried out one of 14 different microbial and physiochemical processes which could involve either C, N or P on the grid (Fig. S1). The grid box, the process and the nutrient were all chosen at random to avoid forcing a particular order on processes that occur simultaneously in nature. On average, the model must complete 420 000 iterations for each minute of real time because there are 14 processes, three nutrients and 10 000 grid boxes. However, not all processes involved nutrients (i.e. microbial death); rate parameters for these processes were adjusted to be temporally consistent with nutrient-dependent processes.

Inputs

As a default, I set C substrate inputs to 0.1 fg min^{-1} μm^{-2}, N product inputs to 0.1 fg min^{-1} μm^{-2}, P product inputs to 0.01 fg min^{-1} μm^{-2} and all other inputs to zero. Each substrate and product contains only one nutrient, either C, N or P. Products concentrations also increase via enzymatic degradation of substrates according to Michaelis–Menten kinetics. For each of three enzymes in the model (C-, N- and P-releasing), I used values of 10 fg fg^{-1} min^{-1} for V_{max} and 0.001 fg μm^{-3} for K_m, which fall within the range reported in the literature for hydrolytic enzymes (Schomburg & Schomburg 2001). Product formation via catalysis could not exceed the amount of substrate present in the grid box.

Uptake and enzyme production

The model assumes that microbes take up one nutrient product at a time, and only when they have a demand for the nutrient. Demand is defined as a deficit of the nutrient within a microbe's biomass relative to the initial biomass ratio; therefore microbes take up C product only when their biomass ratios are ≤ 6 and ≤ 60 for C : N and C : P respectively. Although microbes are actually more flexible in their nutrient uptake (i.e. luxury consumption), this constraint reflects a microbial tendency to maintain stoichiometric ratios within limits (Sterner & Elser 2002).

Microbes absorb products from the grid according to Michaelis–Menten kinetics using carrier proteins on their cell surface. The total number of carrier proteins on a cell is proportional to the surface area and the density of carriers per unit area, set at 0.1 fg μm^{-2}. This value and my uptake parameters of 10 fg fg^{-1} min^{-1} for V_{max} (maximum uptake rate *c.* 0.48 mg substrate mg^{-1} cells h^{-1}) and 0.001 fg μm^{-3} for K_m fall within the ranges reported by Button (1998).

I assumed that recent nutrient uptake would determine the quantity of resources available for enzyme synthesis, and whether or not resources should be invested in enzyme production. Therefore, enzyme production occurred immediately after the uptake step. If uptake was sufficient to eliminate nutrient demand, then enzyme production occurred at a low constitutive rate, $E_{constit}$ (a form of end-product inhibition). Otherwise, I assumed that 1% of product uptake would be allocated to enzyme production with the remainder stored in biomass. This value is within the range of 0.7–2.1% reported for α-glucosidase production by yeasts in continuous cultures (Giuseppin *et al.* 1993) and slightly higher than the 0.3–0.9% reported for protease production by *Bacillus clausii* (Christiansen & Nielsen 2002). I also assumed (conservatively) that a quantity of C equal to 10% of enzyme C mass would be respired to account for additional metabolic costs of enzyme production. A higher value would therefore decrease the growth and competitive ability of enzyme-producing microbes.

When microbes take up N or P, the model calculates enzyme production in N or P units and then uses stoichiometric ratios to calculate enzyme mass in C units. These ratios were based on the stoichiometry of proteins (C : N = 3.5), except that I assumed a small P cost to protein synthesis (C : P = 200). Small amounts of P could be lost during enzyme secretion, especially if protein phosphorylation is involved. Converting to C mass is not necessary for microbes that produce enzymes at constitutive levels because $E_{constit}$ is always expressed in C units.

Because enzyme construction requires C, N and small amounts of P, all three of these nutrients must be available to build enzymes. For example, a microbe absorbing 100 units of P and allocating one unit to enzyme production would require *c.* 200 units of C and *c.* 57 units of N because of the C : N : P stoichiometry of enzymes. Thus, calculating enzyme production based on only one nutrient can result in unrealistic losses of the other nutrients from the microbial biomass. To address this constraint, the model calculates the level of enzyme production that results in equal limitation by the nutrient in demand and the next most limiting nutrient within the microbial cell. This algorithm forces enzyme producers to maintain C : N : P near optimal levels and allows them to allocate abundant nutrients to obtain scarcer nutrients.

To account for constitutive enzyme production, the model ensured that enzyme production was never less than $E_{constit}$, regardless of nutrient demand or uptake. As a default, I set $E_{constit}$ to 10^{-7} fg fg^{-1} min^{-1} if enzyme production was turned on in the model. $E_{constit}$ scales positively but nonlinearly with total enzyme cost; at low values of $E_{constit}$, nutrient demand and product uptake largely determine enzyme production. At higher values, $E_{constit}$ determines total enzyme production (and cost) more directly.

Diffusion and loss

Products and enzymes diffuse at rates proportional to the concentration difference between two grid boxes. For well-mixed model scenarios (labelled 'mixed' in tables and figures), diffusion of all compounds occurred between any two randomly selected boxes on the grid. For all other scenarios, compounds diffused only between two adjacent grid boxes unless otherwise specified. Based on published diffusion coefficients for several organic compounds (Vetter *et al.* 1998), I assumed that products would mix rapidly on the scale of the grid and therefore assigned a product diffusion coefficient of 0.5 μm^2 min^{-1}. With a grid box of 1 μm^3 and time step of 1 min, this diffusion coefficient causes the product concentrations in two grid boxes to equilibrate immediately. This value was also applied to all products and enzymes in the well-mixed scenarios. Because substrates are insoluble, their diffusion coefficients were always set to zero. Products and enzymes were removed from the grid at a rate of 0.01 min^{-1} to represent loss pathways such as chemical breakdown (proteolysis), leaching or sorption. Substrates were also removed at this rate to represent abiotic degradation. These values fall within the range of 0.0006–60 min^{-1} used by Vetter *et al.* (1998) for all loss pathways.

Microbial processes

Microbes carry out basal metabolism by converting C mass to CO_2 at a rate of 0.00015 fg fg^{-1} min^{-1}. This metabolic rate is *c.* 10 times higher than the range reported by Price & Sowers (2004) because I assumed that actively growing

microbes would require more energy for maintenance metabolism. Thus my basal metabolic rate also includes growth metabolism. Microbes also lose N and P in proportion to their basal metabolic rate to represent uncontrollable nutrient losses from their biomass. These losses were calculated by multiplying the amount of C respired by a factor of 0.1 and by the optimal biomass C : nutrient ratios (in most cases, microbes maintained stoichiometric ratios very close to optimal values).

Microbial death occurred randomly at a rate of 3×10^{-5} min^{-1} and when cell mass dropped below 30 fg C, which is near the low end of bacterial cell sizes (Button *et al.* 1998). This death rate did not result in high turnover of the populations, but did allow grid boxes to become unoccupied. The C, N and P mass from a dead microbe immediately left the grid and was not re-metabolized by living microbes. Preliminary runs with a newer version of the model that includes remineralization produced similar results, but with faster overall microbial growth rates and higher variability within the system. Here I used the original model to ensure that microbial processes were only responding to nutrient inputs from outside the grid.

Microbial growth is defined as the change in biomass C over time. Positive growth rates occur when C uptake exceeds C loss because of metabolism and enzyme production. Low nutrient supplies decrease growth rates because microbes attempt to take up nutrients instead of C when their nutrient contents are suboptimal.

Microbes reproduce by splitting their mass into two halves, with the parent cell remaining in the original grid box and the daughter cell colonizing an adjacent, randomly chosen box. In well-mixed scenarios, any grid box can be colonized at random. Microbe C mass must exceed 300 fg before a cell may divide. If the box chosen for colonization is already occupied, one of the cells dies at random with probability 0.5 and leaves the grid. This value assumes that colonists and occupants are equal competitors. Lower values would favour colonists and allow an invading population to spread more rapidly, while higher values would favour occupants and slow down invasions.

Growth rates of producers alone in mixed and structured systems

I conducted model runs with an initial producer frequency of 0.001 and no cheaters to test whether producer populations could establish from low densities. The runs included a well-mixed scenario and spatially structured scenarios with diffusion coefficients (E_{diff}) of 10^{-4}–10^{-1} μm^2 min^{-1} for the C-releasing enzyme. In all of these runs, N- and P-releasing enzyme production was shut off, and N and P products were permitted to diffuse freely across the grid.

Gradients of enzyme diffusion and cost

To explore the response of enzyme producers to restricted diffusion, increasing enzyme costs, and competition from cheaters, I conducted model runs with parameters similar to those above, except that producer and cheater initial frequencies were always set to 0.01. I manipulated enzyme costs by varying E_{constit} of the C-releasing enzyme from 10^{-7} to 10^{-3} fg fg^{-1} min^{-1} for the well-mixed scenario and for each different value of E_{diff}.

Gradients of C, N and P supply

To isolate the effect of substrate availability on microbial processes, I set cheater initial frequency to zero and varied substrate input rates from 0.032–0.32, 0.0032–0.032 and 0.00032–0.0032 fg min^{-1} μm^{-2} for C, N and P, respectively, with no product inputs. I then held substrate inputs at 0.1, 0.01 and 0.001 fg min^{-1} μm^{-2} and added product at a rate of 0.032, 0.0032 and 0.00032 fg min^{-1} μm^{-2} for C, N and P, respectively, to test the effect of increased product availability on microbial processes. Production was turned on and E_{diff} was set to 10^{-2} μm^2 min^{-1} for all enzymes, with all other parameter values set to their defaults.

RESULTS

Growth rates of producers alone in mixed and structured systems

In systems with no cheaters, enzyme producers generally grew faster as diffusion increased, although populations of

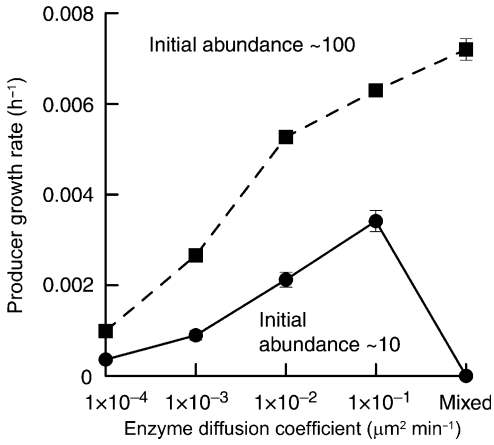

Figure 1 Mean (±SE) growth rates of enzyme producers alone across a gradient of enzyme diffusion at high (frequency = 0.01) and low (frequency = 0.001) initial abundance.

~10 individuals went extinct under well-mixed conditions (circles, Fig. 1). With small populations, enzyme and product concentrations were relatively uniform and low across the grid, and the microbes were never able to take up enough product to achieve positive growth. At larger population sizes (squares, Fig. 1), producers were able to drive the average product concentrations above a critical threshold in the system and achieve positive growth. This threshold is simply the product concentration that allows uptake to offset enzyme and metabolic costs. The more producer colonists there are, the faster this threshold is reached, as more microbes contribute enzyme to the grid.

Gradient of enzyme diffusion

At a given rate of constitutive enzyme production, increasing rates of enzyme diffusion generally favoured cheaters over producers. Cheaters grew poorly at an E_{diff} of 10^{-4} μm^2 min^{-1} (Figs 2 and S2A) but increased in dominance as E_{diff} increased (Figs 2 and S2B–E). Cheaters and producers coexisted at intermediate values of E_{diff} where resource 'microhabitats' formed (Fig. S3). In these runs, some regions of the grid were devoid of microbes and products but contained abundant substrate. Other regions were occupied by cheaters that exploited the enzymes and products emanating from adjacent producer colonies

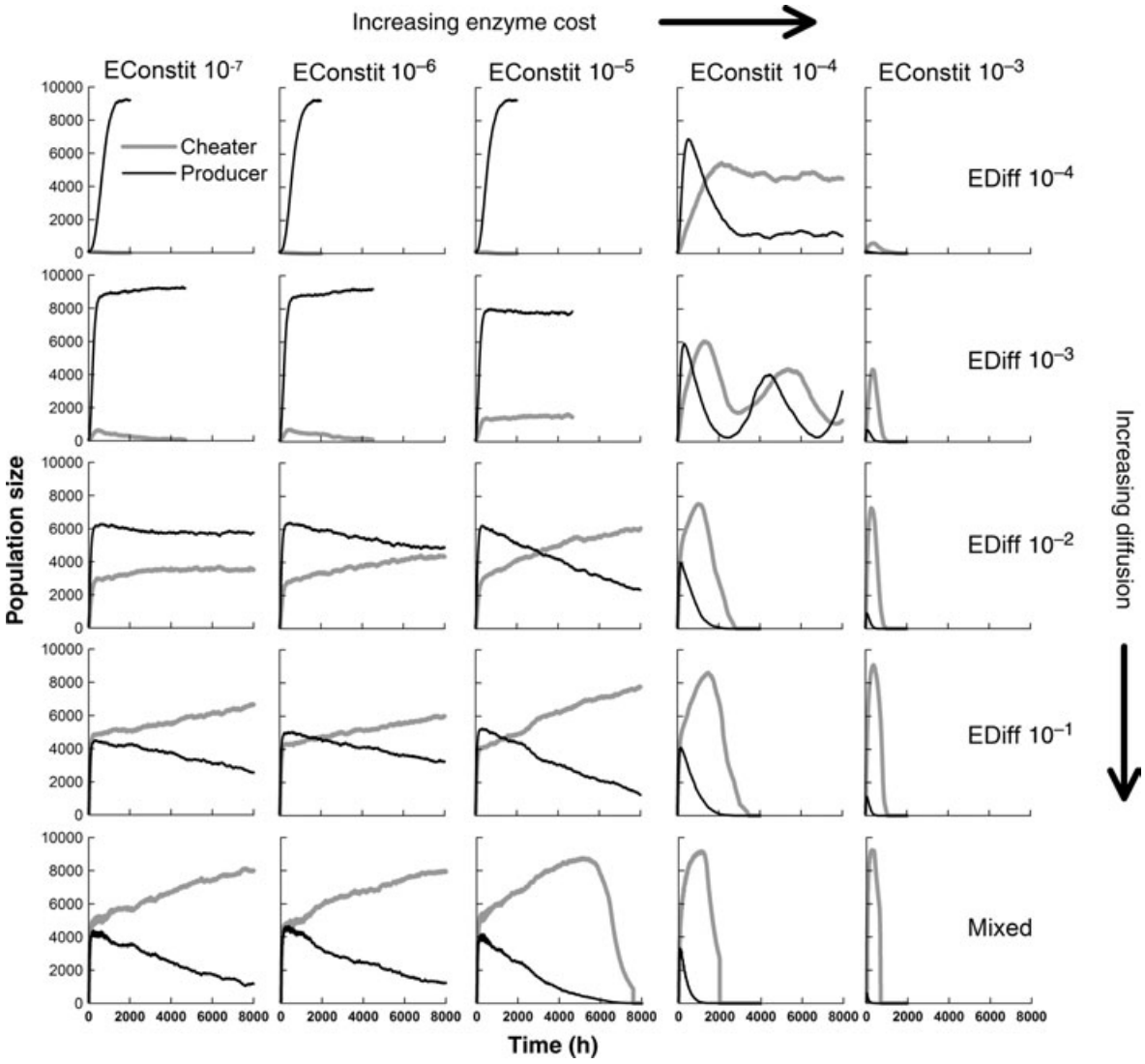

Figure 2 Population dynamics of cheaters and producers across gradients of constitutive enzyme costs and enzyme diffusion. Runs are representative examples of the 5–6 runs for each parameter combination (mean values and standard errors are shown in Fig. S2). Units of $E_{constit}$ are fg fg^{-1} min^{-1} and units of E_{diff} are μm^2 min^{-1}.

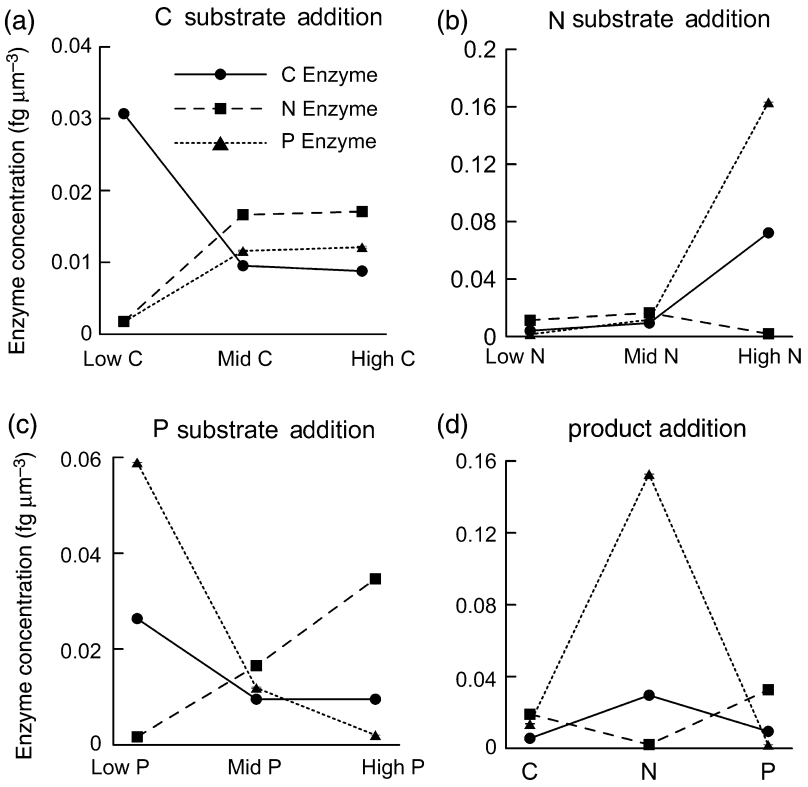

Figure 3 Mean (±SE) enzyme concentrations on the grid with producers alone under varying levels of C substrate (a), N substrate (b), P substrate (c) and product additions (d). Levels of resource addition are given in Tables 1–3 and Table S2.

(Fig. S3). Over time, microbial colonies migrated across the grid, with producers colonizing empty regions and cheaters following the producers.

Increasing enzyme costs

With constitutive enzyme production set to 10^{-7} fg fg^{-1} min^{-1}, producers dominated the system at low E_{diff}, and coexisted with cheaters as E_{diff} increased (Fig. 2). As $E_{constit}$ increased, costs to producers increased and cheaters became more dominant (Fig. 2). At the highest value of $E_{constit}$ (10^{-3} fg fg^{-1} min^{-1}), producers went extinct at all values of E_{diff} (Fig. 2 last column).

Interesting population dynamics developed in systems where $E_{constit}$ was 10^{-4} fg fg^{-1} min^{-1} (Fig. 2). With E_{diff} set to 10^{-4} μm^2 min^{-1}, a stable population of cheaters dominated a smaller, stable producer population. As E_{diff} increased to 10^{-3} μm^2 min^{-1}, the two populations oscillated, but then collapsed when diffusion increased further (Fig. 2). Similar oscillations occurred in additional simulations on a 200 × 200 grid, suggesting that these dynamics do not depend on grid size.

Gradients of C, N and P supply

Under low C supply (0.032 fg μm^{-2} min^{-1}), C-releasing enzymes dominated the system (Fig. 3a) and C mineralization was substrate limited (Table 1). When C substrate input was increased to 0.32 fg μm^{-2} min^{-1}, population size increased from 6190 to 9144 and growth rates increased from 0.0408 to 0.0706 day^{-1} (Table 1). The predominant enzymes were N-releasing because N became limiting (Fig. 3a) and equilibrium N product concentrations declined from 781 to 26 mg L^{-1} (Table 1). Despite a threefold increase in substrate supply, C mineralization changed little from the mid-C to the high-C scenario (5.25 vs. 5.26 fg μm^{-3} h^{-1}; Table 1) because enzyme production was constrained by N supply.

When N supply was reduced, total enzyme production declined, but N-releasing enzymes increased in concentration relative to other enzymes (Fig. 3b). Carbon mineralization only reached 2.5 fg μm^{-3} h^{-1} under the low N scenario compared with 5.9 fg μm^{-3} h^{-1} under high N supply and population size reached just 7431 instead of 9190 (Table 2). At the highest N supply rate, C- and P-releasing enzyme activities increased to 0.072 and 0.163 fg μm^{-3} (Fig. 3b), thereby mobilizing more C and P as these resources became limiting relative to N.

Under low P supply, microbial growth decreased and total population size declined to 7597 as with low N (Table 3), but total enzyme production actually increased. As expected, enzyme production was weighted toward P acquisition, but C-releasing enzyme activity also increased under P limitation (Fig. 3c). These changes occurred because P is only a minor constituent in enzyme mass and the excess C and N

Table 1 Effects of carbon-substrate manipulations (mean ± SE)

	Low C (0.032 fg μm^{-2} h^{-1})	Mid C (0.1 fg μm^{-2} h^{-1})	High C (0.32 fg μm^{-2} h^{-1})
Growth rate (day^{-1})	0.0408 ± 0.0011	0.0680 ± 0.0012	0.0706 ± 0.0033
Population size	6190 ± 6	9151 ± 11	9144 ± 11
C mineralization (fg μm^{-3} h^{-1})	1.880 ± 0.001	5.245 ± 0.006	5.262 ± 0.001
N mineralization (fg μm^{-3} $h^{-1} \times 10^{-1}$)	5.765 ± 0.004	5.941 ± 0.003	5.946 ± 0.003
P mineralization (fg μm^{-3} $h^{-1} \times 10^{-2}$)	5.934 ± 0.005	5.941 ± 0.004	5.934 ± 0.003
C product concentration (mg L^{-1})	70.2 ± 0.2	244.3 ± 0.2	259.9 ± 1.4
N product concentration (mg L^{-1})	780.85 ± 1.09	26.37 ± 0.08	26.18 ± 0.16
P product concentration (mg L^{-1})	81.755 ± 0.139	3.523 ± 0.023	3.446 ± 0.020

Table 2 Effects of nitrogen-substrate manipulations (mean ± SE)

	Low N (0.0032 fg μm^{-2} h^{-1})	Mid N (0.01 fg μm^{-2} h^{-1})	High N (0.032 fg μm^{-2} h^{-1})
Growth rate (day^{-1})	0.0276 ± 0.0006	0.0721 ± 0.0012	0.0838 ± 0.0017
Population size	7431 ± 12	9156 ± 12	9190 ± 10
C mineralization (fg μm^{-3} h^{-1})	2.499 ± 0.002	5.242 ± 0.006	5.935 ± 0.001
N mineralization (fg μm^{-3} $h^{-1} \times 10^{-1}$)	1.880 ± 0.001	5.945 ± 0.005	12.152 ± 0.016
P mineralization (fg μm^{-3} $h^{-1} \times 10^{-2}$)	5.943 ± 0.003	5.942 ± 0.006	5.943 ± 0.003
C product concentration (mg L^{-1})	125.9 ± 1.1	244.3 ± 1.3	1066.9 ± 4.9
N product concentration (mg L^{-1})	8.53 ± 0.07	26.29 ± 0.16	970.64 ± 3.42
P product concentration (mg L^{-1})	68.714 ± 0.099	3.504 ± 0.034	1.279 ± 0.009

Table 3 Effects of phosphorus-substrate manipulations (mean ± SE)

	Low P (0.00032 fg μm^{-2} h^{-1})	Mid P (0.001 fg μm^{-2} h^{-1})	High P (0.0032 fg μm^{-2} h^{-1})
Growth rate (day^{-1})	0.0315 ± 0.0005	0.0719 ± 0.0029	0.0793 ± 0.0014
Population size	7597 ± 9	9159 ± 13	9146 ± 16
C mineralization (fg μm^{-3} h^{-1})	5.913 ± 0.002	5.246 ± 0.003	5.251 ± 0.004
N mineralization (fg μm^{-3} $h^{-1} \times 10^{-1}$)	5.807 ± 0.005	5.941 ± 0.002	5.937 ± 0.002
P mineralization (fg μm^{-3} $h^{-1} \times 10^{-2}$)	1.881 ± 0.001	5.938 ± 0.005	18.792 ± 0.022
C product concentration (mg L^{-1})	5660.7 ± 2.0	245.8 ± 0.9	209.0 ± 0.3
N product concentration (mg L^{-1})	634.17 ± 0.82	26.57 ± 0.08	19.23 ± 0.14
P product concentration (mg L^{-1})	0.540 ± 0.002	3.480 ± 0.035	212.592 ± 4.418

products that accumulated in the system were used for enzyme synthesis. Under P limitation, a slight deficiency of C relative to N drove increases in C-releasing enzyme production and higher rates of C mineralization (5.91 vs. 5.25 fg μm^{-3} h^{-1}; Table 3).

Adding a product to the grid suppressed the enzyme associated with the product. Relative to control values, adding C product reduced the concentration of the C-releasing enzyme by 41%. Adding N product decreased the N-releasing enzyme by 87% but dramatically increased P-releasing enzyme to 0.152 fg μm^{-3}. The P-releasing enzyme declined by 83% when P product was added (Fig. 3d). Adding C and N products reduced C and N mineralization rates, respectively, but adding P product did not affect P mineralization (Table S2). N product addition also dramatically increased C-product concentration to 1080 mg L^{-1} (Table S2).

DISCUSSION

The outcome of microbial competition depends strongly on the amount of constitutive enzyme production and the spatial scale of microbial interactions as determined by E_{diff}. Many of the parameter combinations resulted in coexistence between producers and cheaters, as evidenced by population sizes that changed directionally but slowly, reached stable equilibria, or even oscillated (Fig. 2). Although existing theory can partially explain these results, my enzyme-dependent system also contains unique features that may contribute to coexistence. For example, the successional niche hypothesis of coexistence

states that inferior competitors persist by growing rapidly in resource-rich patches created by disturbance (Pacala & Rees 1998; Amarasekare 2003). In EnzModel, enzyme producers exploit a potential resource (the substrate) in temporary 'gaps' that form following local extinction of cheaters (Fig. S3). However, the disturbance is generated from within the system, rather than externally, and the unique life history trait that enables producers to persist is not rapid growth rates *per se*, but the ability to increase resource availability through enzyme production.

My results are also consistent with studies showing that spatial structure and non-hierarchical (or 'rock-paper-scissors') competitive interactions can promote coexistence (e.g. Kerr *et al.* 2002). However, my model is a special case of 'rock-paper-scissors' with only two players whose competitive abilities vary spatially. Cheaters dominate where products are abundant, but depend on producers to maintain sufficient product concentrations. Where producers grow and generate products, cheaters gain a competitive advantage and drive producers toward extinction. As producers near extinction (in structured systems), they regain their competitive advantage because substrates accumulate and product concentrations drop.

Although coexistence occurred in many simulations, competitive exclusion of enzyme producers occurred when E_{constit} and E_{diff} values were high. The negative effect of high E_{constit} is consistent with studies showing reduced competitive ability of microbes that secrete materials outside their cells (Chao & Levin 1981). In the model (and probably in most microbial environments), producing enzymes beyond a certain point is not beneficial to the microbe–product formation saturates at relatively low levels of enzyme production. A model by Schimel & Weintraub (2003) also predicts this type of asymptotic relationship between decomposition and enzyme concentration because of increasing competition for enzyme-binding sites on substrates.

As the rate of enzyme diffusion increased, producer competitive ability declined because the benefits of enzyme production became more accessible to cheaters. By contrast, at low values of E_{diff}, few enzymes diffused away to generate products that cheaters could exploit (Fig. 2). These E_{diff} values were much lower than published diffusion coefficients for proteins in liquid media (Vetter *et al.* 1998; He & Niemeyer 2003), suggesting that enzyme diffusion must be extremely slow for producers to compete well in natural environments. Such slow diffusion could occur if enzymes encounter more tortuous diffusion pathways in soils, sediments or particles because of complex pore structures (Moldrup *et al.* 2001). Alternatively, extracellular enzymes may be physically bound to the surfaces of microbial cells, organic matter or minerals (Burns 1982; Lynd *et al.* 2002).

In the absence of cheaters, higher rates of mixing actually stimulated the growth of enzyme producers because enzymes converted substrates to products across the entire grid, instead of just near the producer. However, the positive effect of mixing on growth is contingent upon a high initial population size and occurs after a (sometimes substantial) lag phase. If population size is too low and mixing too rapid, producers may be unable to raise product concentrations in the grid enough to achieve positive growth. Such a scenario could occur in a well-mixed flask that contains only a few enzyme-producing microbes and a complex substrate. Although surrounded by a potentially useful resource, dilution would prevent these producers from accessing enough product to survive (Fig. 1).

In natural environments, enzyme producers probably contend with both ends of the diffusional spectrum. I hypothesize that diffusion may act as a selective agent and affect the distribution of enzyme-producing microbes in ecosystems. In systems with high rates of diffusion, such as aquatic environments or microsites within the soil matrix, mineralization and decomposition could decline dramatically if enzyme producers lose in competition with cheaters. Microbes in these environments may be under selection to evolve mechanisms to overcome dilution, such as cell wall polysaccharides that adhere to sinking particles (Biddanda 1986). However, in most of the soil volume and even within the suspended particulate organic matter of aquatic systems, decomposition may be constrained by the slow diffusion of enzymes towards their substrates. Here, selection might favour the evolution of enzymes with smaller sizes or other chemical properties that enhance movement through the soil matrix.

Enzyme producers and cheaters are also likely to exert selective pressures on one another, resulting in coevolution. Cheating may drive the evolution of countermeasures in enzyme producers, such as quorum sensing and antibiotic production (Travisano & Velicer 2004). Tight regulation of enzyme production could also enhance producer competitive ability by lowering the costs of enzyme foraging. For example, producers could secrete enzymes at extremely low constitutive rates, increase production when substrates become abundant and then down-regulate production when products increase. Numerous studies provide evidence for significant regulation; for example, cellulase production is induced by cellulose and cellobiose substrates but repressed by many simple C metabolites (Beguin 1990; Hanif *et al.* 2004). However, additional studies are required to determine whether regulation is strong enough in most systems to overcome competition from cheaters.

In my simulations, cheaters were completely dependent on producers and went extinct after producer populations collapsed, but in natural ecosystems selection has probably increased cheater adaptability. Some cheaters may employ a

'hybrid' strategy and produce enzymes facultatively when product concentrations are low. Other cheaters may specialize on simple compounds derived from root exudation, litter leaching or microbial turnover rather than enzymatic degradation and only 'cheat' when they happen to be close to enzyme producers. In systems where cheaters are absent, selective pressures probably drive *in situ* evolution of cheater strategies (Velicer 2003).

The results from EnzModel are consistent with previous studies and conceptual models of resource allocation to microbial enzymes (Koch 1985; Sinsabaugh & Moorhead 1994). My model assumes that microbes synthesize enzymes to acquire limiting resources and highlights the potential for resources to limit enzyme production, especially N and C. At the lowest input rates of C, N or P, the enzymes that acquired these nutrients dominated the system (Fig. 3). As the supply of the limiting element increased, both the distribution and total quantity of enzyme production changed. For example, increased N supply caused P limitation and increases in P-releasing and total enzyme concentrations, even as N-releasing enzymes declined.

Nitrogen supply represents a particularly important constraint on the growth and function of enzyme-dependent microbes. Low N supply reduced total enzyme production far more severely than low P supply (Fig. 3) because of high N requirements for enzyme production. Consequently, C mineralization declined >50%, while reduced supplies of C or P did not strongly affect the mineralization of other elements. In turn, growth rates declined most dramatically under reduced N supply (Table 2) and N additions increased growth more than C and P additions (Tables 3 and S2). Reduced P supply also decreased growth rates but through a different mechanism – microbes were allocating C and N to P-acquiring enzymes rather than to growth (Table 3).

My enzyme allocation results are also consistent with empirical studies of enzyme production and mineralization under varying nutrient supplies. Many studies have shown that adding P to aquatic and terrestrial systems reduces phosphatase activity (Chróst 1991; Clarholm 1993; Olander & Vitousek 2000). Allison & Vitousek (2005) found that enzyme production in N-limited Hawaiian soils depends on the availability of C and N and that excess C and N stimulates production of phosphatase enzymes. Numerous studies show that mineralization of organic matter can be limited by N availability (e.g. Melillo *et al.* 1982) and EnzModel confirms that N could limit decomposition through constraints on degradative enzyme synthesis [but see Berg & Matzner (1997) and Neff *et al.* (2002)].

EnzModel provides a useful framework for examining microbes and enzymes that control the breakdown of organic matter into low molecular weight compounds. For the sake of interpretation, the model leaves out many important features of real microbial systems, such as evolution, facultative and cooperative enzyme production, substrates containing multiple nutrients, competition with plants and variable turnover rates for enzymes. Use of the model for quantitative prediction of experimental results will also require greater certainty in parameter values and additional validation of the simulation modelling framework (Wilson 1998). While these issues warrant further attention, the current version of the model clearly demonstrates that slow diffusion, competition from cheaters and nutrient limitation may constrain microbially catalysed decomposition. In some cases, low enzyme costs and tight regulation of enzyme production may help enzyme producers overcome these challenges. However, constraints on enzyme production and function probably do limit the turnover of large quantities of organic matter (e.g. Freeman *et al.* 2001). These constraints should be evaluated experimentally to determine which mechanisms most strongly regulate the degradation of complex organic compounds.

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SUPPLEMENTARY MATERIAL

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/ELE/ELE756/ELE756sm.htm

Figure S1 Schematic of EnzModel structure.
Figure S2 Mean population sizes of cheaters and producers with changes in E_{constit} and E_{diff}.
Figure S3 Spatial patterns of microbe populations, enzymes and products on the grid.
Table S1 Model parameter descriptions and values.
Table S2 Effects of C, N and P product additions.

REFERENCES

Allison, S.D. & Vitousek, P.M. (2005). Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol. Biochem.*, 37, 937–944.

Amarasekare, P. (2003). Competitive coexistence in spatially structured environments: a synthesis. *Ecol. Lett.*, 6, 1109–1122.

Beguin, P. (1990). Molecular-biology of cellulose degradation. *Annu. Rev. Microbiol.*, 44, 219–248.

Berg, B. & Matzner, E. (1997). Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environ. Rev.*, 5, 1–25.

Biddanda, B.A. (1986). Structure and function of marine microbial aggregates. *Oceanologica. Acta*, 9, 209–211.

Burns, R.G. (1982). Enzyme activity in soil: location and a possible role in microbial ecology. *Soil Biol. Biochem.*, 14, 423–427.

Button, D.K. (1998). Nutrient uptake by microorganisms according to kinetic parameters from theory as related to cytoarchitecture. *Microbiol. Mol. Biol. Rev.*, 62, 636–645.

Button, D.K., Robertson, B.R., Lepp, P.W. & Schmidt, T.M. (1998). A small, dilute-cytoplasm, high-affinity, novel bacterium isolated by extinction culture and having kinetic constants compatible with growth at ambient concentrations of dissolved nutrients in seawater. *Appl. Environ. Microbiol.*, 64, 4467–4476.

Chao, L. & Levin, B.R. (1981). Structured habitats and the evolution of anticompetitor toxins in bacteria. *Proc. Natl. Acad. Sci. USA*, 78, 6324–6328.

Christiansen, T. & Nielsen, J. (2002). Production of extracellular protease and glucose uptake in *Bacillus clausii* in steady-state and transient continuous cultures. *J. Biotechnol.*, 97, 265–273.

Chróst, R.J. (1991). Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: *Microbial Enzymes in Aquatic Environments* (ed. Chróst, R.J.). Springer-Verlag, New York, NY, pp. 29–59.

Clarholm, M. (1993). Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. *Biol. Fertil. Soils*, 16, 287–292.

Codeço, C.T. & Grover, J.P. (2001). Competition along a spatial gradient of resource supply: a microbial experimental model. *Am. Nat.*, 157, 300–315.

Freeman, C., Ostle, N. & Kang, H. (2001). An enzymic 'latch' on a global carbon store. *Nature*, 409, 149.

Giuseppin, M.L.F., Almkerk, J.W., Heistek, J.C. & Verrips, C.T. (1993). Comparative study on the production of guar alpha-galactosidase by *Saccharomyces cerevisiae* SU50B and *Hansenula polymorpha* 8 2 in continuous cultures. *Appl. Environ. Microbiol.*, 59, 52–59.

Hanif, A., Yasmeen, A. & Rajoka, M.I. (2004). Induction, production, repression, and de-repression of exoglucanase synthesis in *Aspergillus niger*. *Bioresour. Technol.*, 94, 311–319.

Harder, W. & Dijkhuizen, L. (1983). Physiological responses to nutrient limitation. *Annu. Rev. Microbiol.*, 37, 1–23.

He, L. & Niemeyer, B. (2003). A novel correlation for protein diffusion coefficients based on molecular weight and radius of gyration. *Biotechnol. Prog.*, 19, 544–548.

Kerr, B., Riley, M.A., Feldman, M.W. & Bohannan, B.J.M. (2002). Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature*, 418, 171–174.

Koch, A.L. (1985). The macroeconomics of bacterial growth. In: *Bacteria in their Natural Environments* (eds Fletcher, M. & Floodgate, G.D.). Academic Press, London, UK, pp. 1–42.

Lynd, L.R., Weimer, P.J., van Zyl, W.H. & Pretorius, I.S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.*, 66, 506–577.

McGill, W.B. & Cole, C.V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma*, 26, 267–286.

Melillo, J.M., Aber, J.D. & Muratore, J.F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, 63, 621–626.

Moldrup, P., Olesen, T., Komatsu, T., Schjonning, P. & Rolston, D.E. (2001). Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. *Soil Sci. Soc. Am. J.*, 65, 613–623.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J. & Bowman, W.D. (2002). Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature*, 419, 915–917.

Olander, L.P. & Vitousek, P.M. (2000). Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, 49, 175–190.

Pacala, S.W. & Rees, M. (1998). Models suggesting field experiments to test two hypotheses explaining successional diversity. *Am. Nat.*, 152, 729–737.

Price, P.B. & Sowers, T. (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc. Natl. Acad. Sci. USA*, 101, 4631–4636.

Ratledge, C. (1994). *Biochemistry of Microbial Degradation*. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Schimel, J.P. & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, 85, 591–602.

Schimel, J.P. & Weintraub, M.N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.*, 35, 549–563.

Schomburg, D. & Schomburg, I. (2001). *Springer Handbook of Enzymes*. Springer, New York, NY.

Sinsabaugh, R.L. & Moorhead, D.L. (1994). Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. *Soil Biol. Biochem.*, 26, 1305–1311.

Soulas, G. (1993). Evidence for the existence of different physiological groups in the microbial community responsible for 2,4-D mineralization in soil. *Soil Biol. Biochem.*, 25, 443–449.

Sterner, R.W. & Elser, J.J. (2002). *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.

Swift, M.J., Heal, O.W. & Anderson, J.M. (1979). *Decomposition in Terrestrial Ecosystems*. Blackwell Scientific Publications, Oxford, UK.

Tisdall, J.M. & Oades, J.M. (1982). Organic matter and water-stable aggregates in soils. *J. Soil Sci.*, 33, 141–164.

Travisano, M. & Velicer, G.J. (2004). Strategies of microbial cheater control. *Trends Microbiol.*, 12, 72–78.

Velicer, G.J. (2003). Social strife in the microbial world. *Trends Microbiol.*, 11, 330–336.

Vetter, Y.A., Denning, J.W., Jumars, P.A. & Krieger-Brockett, B.B. (1998). A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microb. Ecol.*, 36, 75–92.

Wilson, W.G. (1998). Resolving discrepancies between deterministic population models and individual-based simulations. *Am. Nat.*, 151, 116–134.

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