

Bacterivorous grazers facilitate organic matter decomposition: a stoichiometric modeling approach

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Abstract

There is widespread empirical evidence that protist grazing on bacteria reduces bacterial abundances but increases bacteria-mediated decomposition of organic matter. This paradox has been noted repeatedly in the microbiology literature but lacks a generally accepted mechanistic explanation. To explain this paradox quantitatively, we develop a bacteria-grazer model of organic matter decomposition that incorporates protozoa-driven nutrient recycling and stoichiometry. Unlike previous efforts, the current model includes explicit limitation, via Liebig's law of minimum, by two possible factors, nutrient and carbon densities, as well as their relative ratios in bacteria and grazers. Our model shows two principal results: (1) when the environment is carbon limiting, organic matter can always be decomposed completely, regardless of the presence/absence of grazers; (2) when the environment is nutrient (such as nitrogen) limiting, it is possible for organic matter to be completely decomposed in the presence, but not absence, of grazers. Grazers facilitate decomposition by releasing nutrients back into the environment, which would otherwise be limiting, while preying upon bacteria. Model analysis reveals that facilitation of organic matter decomposition by grazers is positively related to the stoichiometric difference between bacteria and grazers. In addition, we predict the existence of an optimal density range of introduced grazers, which maximally facilitate the decomposition of organic matter in a fixed time period. This optimal range reflects a trade-off between grazer-induced nutrient recycling and grazer-induced mortality of bacteria.

Introduction

Heterotrophic bacteria are primarily responsible for the decomposition of organic matter in many environments. Empirical studies of decomposition and its relationship to food web structure have revealed a seemingly incongruous set of results, which, when viewed together, we term the 'decomposition-facilitation paradox.' The paradox is as follows: the rate or the extent of organic matter decomposition often increases in the presence of bacterivorous protists that substantially reduce bacterial abundances (Fenchel & Harrison, 1976; Coleman *et al.*, 1978; Nisbet, 1984; Sherr *et al.*, 1988; Ratsak *et al.*, 1996; Jiang, 2007). Multiple hypotheses have been proposed to explain this paradox. Perhaps the earliest and prominent of these – the 'nutrient recycling' hypothesis – suggests that excretion of mineral

nutrients by protists results in stimulated physiological status of bacteria and thus enhanced usage of carbon by bacteria (Johannes, 1965; Coleman *et al.*, 1978).

Nutrient recycling is a common feature of consumers across a range of sizes. Consumer-driven nutrient recycling occurs when consumers return a portion of their ingested nutrients back to the environment. Nitrogen and phosphorus are two elements that are often limiting in natural environments, and their densities differ among different organisms. For example, bacteria are comparatively nutrient rich, relative to most living organisms including protozoa (Sturner & Elser, 2002; Cross *et al.*, 2005). As a result, bacterivorous consumers, particularly protists, can recycle nutrients when grazing on bacteria for reasons of uptake inefficiency and compositional mismatch (Caron *et al.*, 1985, 1988; Goldman *et al.*, 1985; Andersen *et al.*, 1986;

Caron & Goldman, 1988; Jürgens & Güde, 1990; Sterner & Elser, 2002). The mismatch in elemental composition among prey and predators is but one example of how trophic interactions in ecosystems can be strongly affected by stoichiometric imbalances (Elser *et al.*, 1988; Andersen & Hessen, 1991; Elser & Urabe, 1999; Daufresne & Loreau, 2001; Grover, 2002; Wang *et al.*, 2008).

There have been a few attempts at reconciling empirical observations with mechanistic explanations linked to nutrient recycling. First, Barsdate *et al.* (1974) performed a microcosm experiment on bacteria-mediated plant material decomposition (with or without grazers) by tracing the phosphorus cycle, and compared it with a phenomenological model. Their experiment together with the model suggested that grazed bacterial systems had considerably faster phosphorus cycling than nongrazed systems and that detritus mineralization was stimulated by protozoan grazing. Next, Hunt *et al.* (1977) developed a chemostat-type cell component model, which incorporates the stoichiometry of bacteria. In so doing, Hunt and colleagues highlighted the importance of grazing and protozoa-driven nutrient recycling without explicitly incorporating them into their model. Although nutrient recycling is commonplace, the stimulation of bacterial physiological status, as proposed by Johannes (1965) and Coleman *et al.* (1978), remains unproven as the primary cause of the decomposition–facilitation paradox.

One stumbling block in resolving the decomposition–facilitation paradox quantitatively has been the lack of suitable mathematical models of ecological interactions with explicit treatment of stoichiometry. Recently, a number of mathematical models have been developed that incorporate the stoichiometry of bacteria and nutrient cycling (Codeço & Grover, 2001; Wang *et al.*, 2007), and bacteria-grazer trophic interactions (Grover, 2003). Here we present and analyze a dynamic, ecological model of organic matter decomposition that considers the effects of protist grazing, consumer-driven nutrient recycling, and stoichiometry. We use the mathematical model to systematically explore how protozoa-driven nutrient recycling affects bacteria-mediated decomposition of organic matter. Specifically, model results indicate that nutrient recycling by grazers releases bacteria from nutrient-limitation and therefore facilitates organic matter decomposition. Next, we show that stoichiometric mismatches between bacteria and grazers can enhance decomposition when bacteria are more nutrient rich than grazers. Finally, we predict that there will be an optimal density range of grazers that maximally facilitates the decomposition of organic matter in a fixed time period.

Model, analysis, and simulations

Our model depicts a situation where bacteria decompose organic matter and bacterivorous grazers graze upon bacteria,

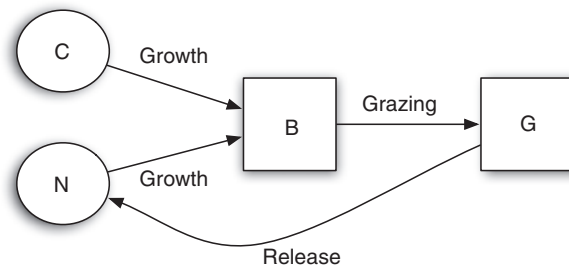


Fig. 1. A depiction of the relationship among variables in the model, where C is organic carbon, B is bacteria, G is grazers, and N is available nitrogen. Arrows denote flow of nutrients and carbon.

in a potentially nitrogen- or carbon-limiting environment. The results of our model, however, can be readily generalized to situations where other elements, such as phosphorus, are limiting. Organic matter is organic carbon linked with organic nitrogen. We use organic carbon decomposition as a proxy for organic matter decomposition. In addition, for simplicity we combine organic nitrogen in organic matter with inorganic nitrogen together as the available nitrogen pool. Our study system consists of four compartments: bacteria (B), grazers (G), organic carbon in organic matter (C), and the available (inorganic and organic) form of nitrogen (N), as shown in Fig. 1.

Model presentation

The stoichiometrically explicit model of B (density of bacterial carbon), G (density of grazer carbon), C (density of organic carbon), N (density of available nitrogen) is described by the following set of equations:

$$\left. \begin{aligned} \frac{dB}{dt} &= \mu_B B \min\{f(N), g(C)\} - \mu_G h(B) G \\ \frac{dG}{dt} &= \alpha \mu_G h(B) G - \omega G \\ \frac{dC}{dt} &= -\frac{1}{r} \mu_B B \min\{f(N), g(C)\} \\ \frac{dN}{dt} &= \theta_C \omega G + \underbrace{(\theta_B - \theta_C \alpha) \mu_G h(B) G}_{\text{Nitrogen exudation of grazers}} - \theta_B \mu_B B \min\{f(N), g(C)\} \end{aligned} \right\} \quad (1)$$

The bacterial growth follows the Liebig's law of the minimum. The N-dependent dimensionless uptake rate $f(N)$, the C-dependent dimensionless uptake rate $g(C)$, and per capita grazing efficiency $h(B)$ satisfy the properties that they be monotonically increasing, saturating functions, which are equal to 0 when their argument is 0. The parameter α (< 1) is the conversion efficiency of bacteria to grazers. The decomposition rate of organic carbon is proportional to the bacterial growth rate, where the constant of proportionality r (< 1) is the yield constant. Other parameters include the maximal growth rate of bacteria (μ_B), the maximal grazing rate (μ_G), the biomass loss rate of

grazers (ω) (reflecting combined processes of death and maintenance), and the nitrogen : carbon ratio of grazers and bacteria (θ_G and θ_B , respectively). Table 1 contains a complete list of parameters, definitions, and values.

According to this model, available nitrogen can be increased by the nitrogen exudation of grazers and the nitrogen recycling from biomass loss of grazers, and can be decreased by bacterial uptake. The nitrogen exudation of grazers is the difference between nitrogen uptake from bacteria, $\theta_B\mu_G h(B)G$, and nitrogen used in the growth of grazers, $\theta_G\alpha\mu_G h(B)G$. Empirical evidence indicates that bacteria are more nutrient rich than protozoa, i.e. $\theta_B > \theta_G$ (Sturner & Elser, 2002; Cross *et al.*, 2005). Grazers release extra nitrogen obtained from bacteria, because there is a mismatch of their cellular nitrogen : carbon ratios ($\theta_B > \theta_G$). Inefficient grazer growth ($\alpha < 1$) strengthens this nitrogen recycling.

The model that we analyze in this paper describes a closed system in nitrogen, though the model generalizes to open systems (see Discussion for more details on the difference). As such, the total nitrogen remains fixed throughout the course of the dynamics, i.e. $T_N = \theta_B B(0) + \theta_G G(0) + N(0)$ is a constant for all times. To derive the model in the absence of grazers, we choose $G(t) = 0$ for all t . In the absence of grazers, the total organic carbon is also fixed, i.e. $T_C = B(0) + rC(0)$ is a constant for all times (given our closed system dynamics, we defer treatment of bacterial respiration and mortality until later in the manuscript). Hence, we can reduce our model from four variables to a model in terms of one variable only, $B(t)$ (for more details on this derivation see Appendix A):

$$\frac{dB}{dt} = \mu_B B \min\{f(T_N - \theta_B B), g((T_C - B)/r)\} \quad (2)$$

This model describes the population dynamics of bacteria, $B(t)$, given initial pools of carbon, T_C , nitrogen T_N , and

additional information on growth and stoichiometric parameters.

Analysis of model without grazers

For model (2), the system in the absence of grazers, we assume that the functions f and g take the Monod form; i.e. $f(T_N - \theta_B B) = \frac{T_N - \theta_B B}{K_f + T_N - \theta_B B}$ and $g((T_C - B)/r) = \frac{(T_C - B)/r}{K_g + (T_C - B)/r}$, where K_f and K_g are half-saturation constants. We can show that $B(t) \rightarrow \min\{T_N/\theta_B, T_C\}$ as $t \rightarrow \infty$ (see Appendix B for more details). The fraction T_N/θ_B is the nitrogen-dependent steady state density of bacteria while the parameter T_C is the carbon-dependent steady state density of bacteria. Environmental conditions determine the fate of organic matter: (1) If $T_N/T_C > \theta_B$, i.e. the environment is *initially carbon limiting* [there is a left-over of available nitrogen to bacteria, called 'nitrogen immobilization'; see Postgate (1998) for details], then $B(t) \rightarrow T_C, C(t) \rightarrow 0$; i.e. organic matter is completely decomposed in a carbon-limiting environment. (2) If $T_N/T_C < \theta_B$, i.e. the environment is *initially nitrogen limiting*, then $B(t) \rightarrow T_N/\theta_B, C(t) \rightarrow (T_C - \frac{T_N}{\theta_B})/r > 0$ [($T_C - \frac{T_N}{\theta_B})/r$ is positive due to the condition $T_N/T_C < \theta_B$], i.e. organic matter cannot be completely decomposed in a nitrogen-limiting environment.

Analysis of model with grazers

Here, we analyze the full model (1), which incorporates grazers. The grazing term takes the Michaelis–Menten form in our analyses and simulations. The dynamics of bacteria, grazers, and carbon converge to a steady state. For any steady state of this model, (B^*, G^*, C^*) , G^* has to be zero (we prove it briefly in Appendix B). There are infinitely many equilibria of the form (B^*, G^*, C^*) with the following three types. First, bacteria and grazers can die out locally, leading to a steady state: $(0, 0, C_1)$. Next, grazers may die, but bacteria may become nutrient limited, leading to a steady

Table 1. Definitions and values of parameters in Eqn. (1)

Parameter	Meaning	Value	References
μ_B	Maximum bacterial growth rate	1–4 day ⁻¹	Connolly <i>et al.</i> (1992); Codeço & Grover (2001)
K_h	H.s.c. for grazing	$0.2 \times 10^{-3} - 0.25 \times 10^{-3}$ mgC mL ⁻¹	Mohapatra & Fukami (2004)
K_f	Nitrogen-dependent h.s.c. for bacterial growth	$0.96 \times 10^{-6} - 6.4 \times 10^{-6}$ mgN mL ⁻¹	Codeço & Grover (2001) with Redfield ratio N : P = 16 : 1
K_g	Carbon-dependent h.s.c. for bacterial growth	$5 \times 10^{-6} - 50 \times 10^{-6}$ mgC mL ⁻¹	Connolly <i>et al.</i> (1992)
μ_G	Maximum grazing rate	0.06 – 0.36 day ⁻¹	Nishimura <i>et al.</i> (2005)
α	Conversion efficiency of bacteria to grazers	0–1	
ω	Biomass loss rate of grazers	$\ll \alpha \times \mu_G$	
R	Yield constant	0.31–0.75	del Giorgio & Cole (1998); Wang <i>et al.</i> (2007)
θ_G	Nitrogen : carbon ratio of grazers	$< \theta_B$	Cross <i>et al.</i> (2005)
θ_B	Nitrogen : carbon ratio of bacteria	1/9–1/4	Sturner & Elser (2002)

h.s.c., half-saturation constant.

state: $(T_N/\theta_B, 0, C_2)$. Finally, grazers may die, and bacteria may be able to use up available carbon and nutrients, leading to a steady state: $(B_1, 0, 0)$. The precise steady state values depend on initial conditions and parameters. Note that one eigenvalue of the type $(0, 0, C_1)$ is positive; thus, the equilibria of the type $(0, 0, C_1)$ are unstable. The stability of the equilibria of the other two types depends on initial nutrient availability. Also note that the current model assumes that density-independent mortality of bacteria is negligible (otherwise the only equilibria of a closed system would be local extinction of both bacteria and grazers).

Case 0. When the environment is *initially carbon limiting*, then each solution $[B(t), G(t), C(t)] \rightarrow (B_1, 0, 0)$ for some $B_1 \geq 0$, i.e. organic matter can always be decomposed.

When the environment is *initially nitrogen limiting*, there are two possible outcomes for the stability of the equilibria of the other two types $(T_N/\theta_B, 0, C_2)$ and $(B_1, 0, 0)$.

Case 1. If organic matter is completely decomposed before the grazers go extinct, then each solution $[B(t), G(t), C(t)] \rightarrow (B_1, 0, 0)$ for some $B_1 \geq 0$ (Fig. 2a).

Case 2. If the grazers go extinct before the organic matter is completely decomposed, then each solution $[B(t), G(t), C(t)] \rightarrow (T_N/\theta_B, 0, C_2)$ for some $C_2 > 0$, i.e. organic matter cannot be decomposed completely (Fig. 2b).

Grazing effect on decomposition

We have the following observations based on the previous analysis subsections: (1) In an initially carbon-limiting environment, $C(t) \rightarrow 0$ as $t \rightarrow \infty$ for both systems in the presence/absence of grazers, i.e. organic matter can always be decomposed completely by bacteria. Hence, grazers have no effect on organic matter decomposition when the environment is (initially) carbon limiting. (2) In an initially nitrogen-limiting environment for the system with grazers, $C(t) \rightarrow 0$ as $t \rightarrow \infty$ if grazers can survive until the complete decomposition. Otherwise, $C(t) \rightarrow C_2 > 0$ as $t \rightarrow \infty$ if grazers go extinct before the complete decomposition. (3) In an initially nitrogen-limiting environment for the system without grazers, $C(t) \rightarrow (T_C - \frac{T_N}{\theta_B})/r > 0$ as $t \rightarrow \infty$, i.e. organic matter cannot be decomposed completely in the absence of grazers. Therefore, when the environment is (initially) nitrogen limiting, it is possible for the organic matter to be completely decomposed in the presence of grazers, but impossible in the absence of grazers. As a result, we find that the grazers have no effect on carbon decomposition in a carbon-limiting environment while they have strong positive effects on carbon decomposition in a nitrogen-limiting environment.

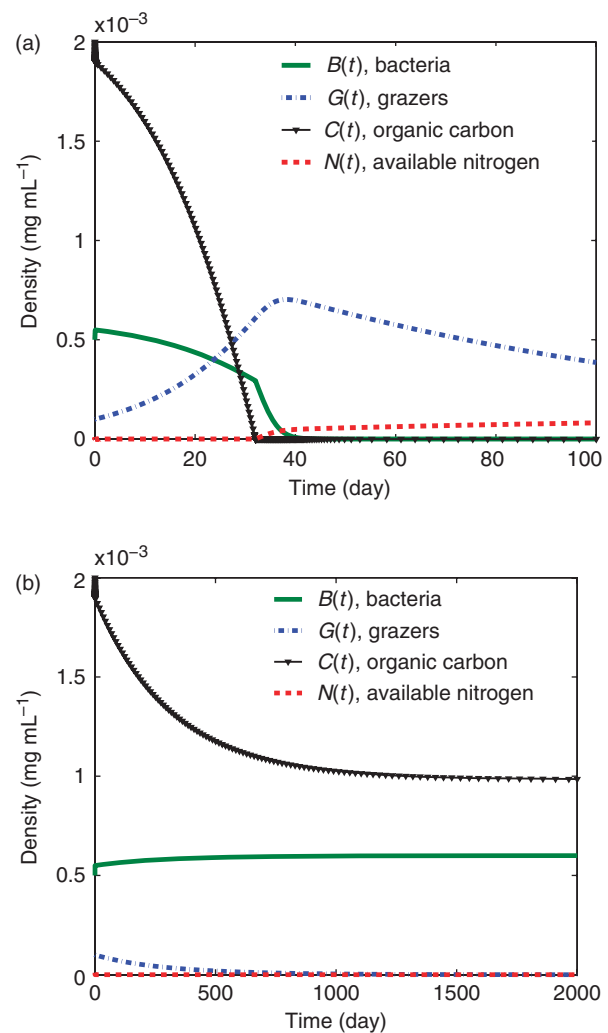


Fig. 2. Two possible outcomes of model (1) for an initially nitrogen-limiting environment. The grazer in (a) has higher grazing efficiency than the grazer in (b). Parameter values: $\mu_B=2$, $K_f=3 \times 10^{-6}$, $K_g=15.9 \times 10^{-6}$, $\mu_G=0.2$, $K_h=0.225 \times 10^{-3}$ (a), and $K_h=8 \times 10^{-3}$ (b), $\alpha=0.5$, $\omega=0.1 \times \alpha \times \mu_G$, $r=0.5$, $\theta_G=1/10$, $\theta_B=1/5$. The initial conditions are $B(0)=0.5 \times 10^{-3}$, $G(0)=0.1 \times 10^{-3}$, $C(0)=2 \times 10^{-3}$, and $N(0)=10 \times 10^{-6}$.

Moreover, our model provides quantitative descriptions of the grazing effect. For example, numerical simulations in Fig. 3 demonstrate that organic matter is decomposed to a greater extent with grazers than without grazers in a nutrient-limiting environment. Figure 3a shows the short-term transient solution behaviors while Fig. 3b shows the long-term stabilized solution behaviors. Both of them exhibit positive effects of grazers on organic matter decomposition. In a carbon-limiting environment, we speculate that grazing can modestly facilitate the speed of decomposition although organic matter is always completely decomposed.

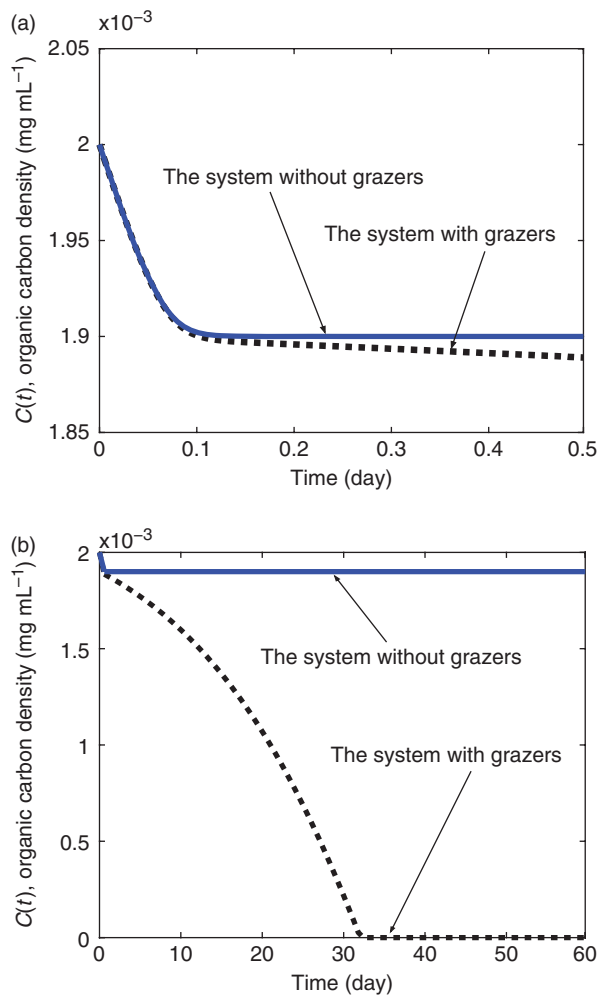


Fig. 3. Comparison of model dynamics with or without grazers, using parameter values and initial conditions in Fig. 2a.

Thus far, we have not presented analysis of the dynamics of decomposition with density-independent bacterial mortality. The qualitative predictions of our model are robust when we incorporate non-zero bacterial mortality. In fact, bacterial mortality has far less effect on nutrient regeneration than protist grazing. In the analysis, we include a bacterial mortality term of the form $-mB$ to the dB/dt equation and add a nitrogen recycling term from bacterial death into the dN/dt equation in system (1). In addition, one could add a carbon recycling term from bacterial death into the dC/dt equation. However, we assume it is negligible. We choose bacterial mortality rates $m = 0, 0.01, 0.1, 1 \text{ day}^{-1}$ and assume that 20% of its nitrogen content is recycled to the nitrogen pool in Fig. 4 (model results are robust to choices of finite fractions of recycling). Incorporating density-independent bacterial mortality does not change the qualitative dependence of organic matter decomposition on grazers. As is evident in Fig. 4, increased

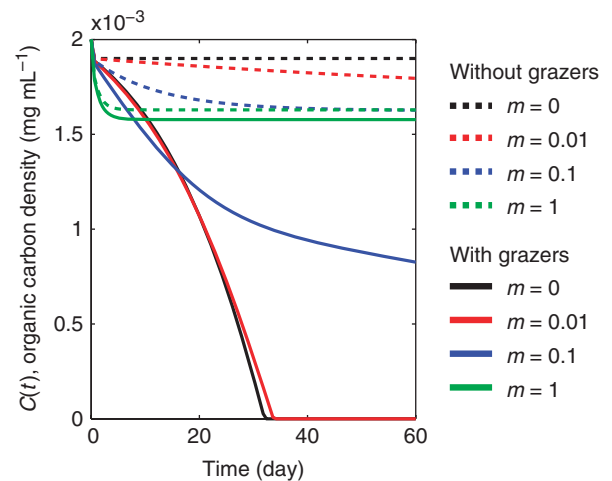


Fig. 4. A comparison of organic matter decomposition with various rates of bacterial mortality. $m = 0, 0.01, 0.1, \text{ and } 1 \text{ day}^{-1}$; all other parameters are the same as in Fig. 2a.

mortality rate weakens the facilitation of grazers on decomposition. The facilitation effect approaches zero as the mortality rate increases, because bacteria are dying largely independently of grazers, eliminating the facilitation effect. If we consider 100% recycling, these results are robust so long as the bacterial mortality rate m is less than or comparable to the grazer mortality/waste rate. Results can be different for unrealistically large m .

Influences of stoichiometry on decomposition

The stoichiometric imbalance between bacteria and grazers is another factor governing the dynamics of organic matter decomposition. There are two cases to consider: $\theta_G > \theta_B$ and $\theta_G < \theta_B$, in which grazers are either richer or poorer, respectively, in nutrients than bacteria. We find that facilitation of organic matter decomposition by bacterivorous grazers is positively related to the stoichiometric difference between bacteria and grazers when $\theta_G < \theta_B$. Figure 5 plots the decomposition time vs. $\theta_B - \theta_G$ for three different θ_G values. This simulation illustrates that the increased stoichiometric difference between bacteria and grazers substantially reduces the decomposition time and thus substantially enhances organic matter decomposition.

That bacteria should be more nutrient rich than grazers ($\theta_G < \theta_B$) is an important but not a necessary condition for the facilitation of grazers on organic matter decomposition. In the case of $\theta_G > \theta_B$, grazers release less nutrients back to the environment because bacterial nutrient contents are insufficient for grazers to grow. When their food source is nutrient poor, grazer growth is limited by nutrients (in this case nitrogen) instead of carbon. Thus, nitrogen will not be exuded by grazers as waste and grazer growth will be limited

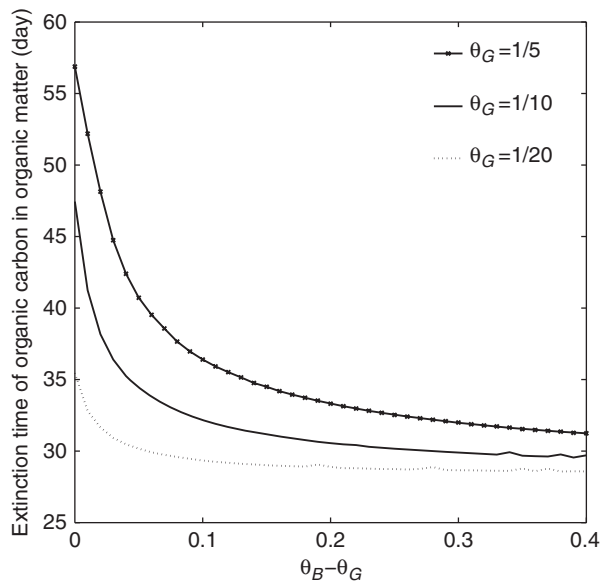


Fig. 5. The decomposition time of organic matter vs. the stoichiometric difference $\theta_B - \theta_G$. The parameters θ_G and θ_B are the nitrogen : carbon ratios of grazers and bacteria, respectively. The parameters are the same as in Fig. 2a except that the conversion efficiency for $\theta_G < \theta_B$ is $\alpha = 0.5$. For a deterministic model, organic carbon can only asymptotically tend to zero. Numerically, we define that organic carbon goes extinct whenever its density is $< 10^{-10} \text{ mg mL}^{-1}$.

not by inefficiencies but rather by stoichiometric mismatch. We can rewrite the original model Eqn. (1) as follows:

$$\begin{aligned} \frac{dB}{dt} &= \mu_B B \min\{f(N), g(C)\} - \mu_G h(B)G \\ \frac{dG}{dt} &= \frac{\theta_B}{\theta_G} \mu_G h(B)G - \omega G \\ \frac{dC}{dt} &= -\frac{1}{r} \mu_B B \min\{f(N), g(C)\} \\ \frac{dN}{dt} &= \theta_G \omega G - \theta_B \mu_B B \min\{f(N), g(C)\} \end{aligned} \quad (3)$$

For this model, organic matter can also be decomposed completely with grazers due to inefficiency of grazing, instead of compositional mismatch. Simulation results depicting this scenario are presented in Appendix C.

Optimal density of introduced grazers

As we have shown, grazers facilitate the decomposition of organic matter in a nitrogen-limiting environment. This finding leads to the prediction that increasing the density of introduced grazers should speed-up decomposition by bacteria. To test this prediction, we numerically simulated Eqn. (1) using a range of grazer densities as initial conditions. Contrary to our expectation, when we introduce a large density of grazers into the system, they

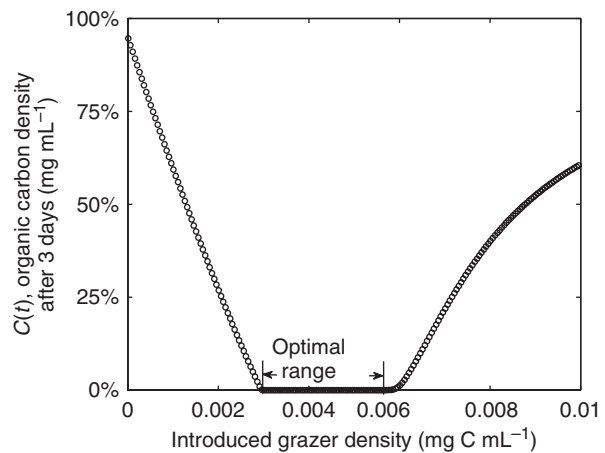


Fig. 6. The effects of varying grazer density on decomposition. We plot organic carbon density after 3 days. All parameters are the same as in Fig. 2a except for $G(0)$ which varies from 0 to $0.01 \text{ (mg C mL}^{-1}\text{)}$.

quickly consume most of the bacteria, which dominates the nutrient regeneration effect. In this situation, Fig. 6 demonstrates that there is an optimal range of the grazers, which maximally facilitates the decomposition of organic matter in 3 days. For a fixed time period, this optimal window will vary with the physiological properties of bacteria and grazers as well as with the initial carbon and nutrient availabilities.

Discussion

We have developed a mathematical model that explains how consumer-driven nutrient recycling can explain the decomposition-facilitation paradox. Our model supports the nutrient recycling hypothesis (Johannes, 1965; Coleman *et al.*, 1978) while specifying a more precise mechanism for how grazers can modify the physiological status of bacteria. They do so by releasing bacteria that are not grazed from nutrient limitation. Grazers have strong positive effects on organic matter decomposition in a nutrient-limiting environment, while they have no effect on organic matter decomposition in a carbon-limiting environment. We also predict that when bacteria have higher nutrient content than grazers, i.e. $\theta_G < \theta_B$, this will greatly strengthen the facilitation of organic matter decomposition by grazers. Moreover, the facilitation of organic matter decomposition by protozoa is positively related to the stoichiometric difference between bacteria and protozoa. Finally, we predict that there will be an optimal density range of introduced grazers that best facilitates the decomposition of organic matter in a fixed time period. These final two predictions are amenable to experimental tests, the former by linking decomposition dynamics to data of nutrient : carbon ratios and the latter by evaluating the efficiency of decomposition in habitats with

Table 2. Experimental studies of decomposition facilitation

Microcosm experiment source	Organic matter	Time duration (days)	% Loss of organic matter		Bacterivorous grazers	References
			Without grazers	With grazers		
Sea water	Barley hay	40	22	82	Flagellates, ciliates	Fenchel & Harrison (1976)
Lake water	Lyophilized <i>Peridinium</i> cells	14	0	70	Microflagellates	Sherr <i>et al.</i> (1982)
Stream water	Leaf litter	120	1	11	Flagellate (<i>Spumella</i> sp.), ciliate (<i>Dexiostoma campyla</i>)	Ribblett <i>et al.</i> (2005)
Carolina Biological Supply Company	Wheat seeds	28	33	67	Ciliate (<i>Colpidium striatum</i>)	Jiang & Krumins (2006)
Pond water	Wheat seeds	42	15	23	Ciliate (<i>Tetrahymena</i>)	Jiang (2007)
Saltmarsh sediment	Cordgrass litter	75	65	63	Nematodes (<i>D. meylli</i> , <i>D. dievengatensis</i> , <i>D. oschei</i> , <i>P. paetzoldi</i>)	Mesel <i>et al.</i> (2003)
Eutrophic lake water	Macrophyte leachate	27	41	43	Heterotrophic nanoflagellates (<i>Bodosaltans</i> , <i>Spumella</i> sp.)	Sala & Jürgens (2004)

The first five experiments exhibit strong facilitation of organic matter decomposition by grazers. The last two experiments have nutrient-rich conditions and thus show that grazers have no effect on the decomposition of organic matter. All these experimental observations are consistent with our principal findings. The percentages of organic matter loss are computed from the weights in the beginning and in the end of experiments. If one experiment has more than one data set, then we take the average of all data sets.

varying grazer densities. In most cases analyzed in this paper, we consider the strength of decomposition in terms of the decrease of available carbon over long times. However, as we found (see Fig. 4), the initial speed of decomposition and the final density of carbon in our models need not concur. Further, the speed of decomposition can itself be variable, i.e. the speed changes with time.

In this paper, we model a nitrogen-closed system because many microcosm experiments are closed in nutrient elements. As but one real-world example, an ecosystem may be considered to be nitrogen closed if we examine the decomposition of a cohort of leaves over short time scales (weeks–months) in a seasonal deciduous forest. We can derive an open system by incorporating standard chemostat in- and outflow processes into the model. When the dilution rate is small, the results are robust. When the dilution rate is large, the chemostat setting will strongly weaken the facilitation effect because the nitrogen recycled from grazers will be washed out eventually. To simplify our model, we assume that organic carbon is a proxy for organic matter and we combine organic nitrogen in organic matter with inorganic nitrogen together as the available nitrogen pool. In addition, the current model assumes that organic carbon is fully accessible to bacteria, but some sources of organic carbon (such as lignin) are difficult for bacteria to access. Such sources require other forms of readily available carbon (such as sugars) to be available for subsequent decomposition. This is the well-known ‘priming effect’ (Fontaine *et al.*, 2003). These processes of organic matter decomposition are not

linked with the stoichiometric balance between carbon and nitrogen in our model. However, it is possible to incorporate such additional complexities into the current framework.

The environment-dependent effects of grazers on decomposition are qualitatively consistent with previous experiments (Sherr *et al.*, 1982; Caron *et al.*, 1985, 1988; Bloem *et al.*, 1989; Ratsak *et al.*, 1996; Jiang, 2007). The facilitation of bacteria-mediated decomposition by grazers, although common, is not universal. Most of the previous experiments suggest that decomposition is associated with substrate carbon : nutrient ratio. Grazing enhances carbon decomposition in situations where substrates have high carbon : nutrient ratios (i.e. nutrient poor) (Fenchel & Harrison, 1976; Sherr *et al.*, 1982), but not in situations where substrates have low carbon : nutrient ratios (i.e. nutrient rich) (Bloem *et al.*, 1988, 1989; Tezuka, 1990).

Multiple experiments have been performed to illustrate the facilitation of organic matter decomposition by grazers when the environment is nutrient limiting (Fenchel & Harrison, 1976; Sherr *et al.*, 1982; Ribblett *et al.*, 2005; Jiang & Krumins, 2006; Jiang, 2007). We summarize these experiments in Table 2. In Fenchel & Harrison’s (1976) experiment, the decomposition rate was measured as the liberation rate of ^{14}C CO_2 , and the system with a natural assemblage of protozoa showed a rapid, nearly constant decomposition rate. Sherr *et al.* (1982) examined the decomposition of lyophilized *Peridinium* cells suspended in lake water and found that the decomposition rate was greatly facilitated by adding laboratory microflagellate

culture or a natural protozoan population. Ribblett *et al.* (2005) isolated live protists from decomposing leaves collected in a stream in Northern Virginia and established laboratory cultures of common bacterivorous protists. They found that the rate of leaf decomposition was significantly higher in treatments with protists than without. One author of this paper performed microcosm experiments on the decomposition of wheat seeds. In Jiang & Krumins (2006), bacterivory of a ciliated protist *Colpidium striatum* resulted in an increase in wheat seed decomposition. The authors hypothesized that this increased decomposition was largely due to an increase in the abundance of one single bacterial species that appeared resistant to predation. Later, Jiang performed another microcosm experiment on the decomposition of wheat seeds using different combinations of four bacterial species in a nutrient-limiting environment (Jiang, 2007). He introduced a small number of the bacterivorous ciliate, *Tetrahymena pyriformis*, into microcosms, when bacterial densities had reached a stationary phase. By comparing all treatments with grazers and without grazers, 40 out of 45 (c. 89%) treatments with grazers had larger wheat seed weight loss than those without grazers.

The last two experiments in Table 2 show a different result. Mesel *et al.* (2003) observed no stimulation of the decomposition process nor of the microbial community in the presence of nematodes. They hypothesized that nitrogen recycling is not important for the activity of bacteria in eutrophic systems such as salt marshes. In Sala & Jürgens's (2004) batch enrichment experiment with macrophyte leachate as natural DOC source, they show that bacterivorous grazers have a strong impact on bacterial biomass and phenotypic community structure but relatively little impact on rates of DOC degradation. This may be due to eutrophic conditions in lake where they took water samples. Both of these experiments found that protist grazing has almost no effect on the decomposition of organic matter in nutrient-rich environments. This conclusion is consistent with our model findings.

To clearly answer whether the 'nutrient recycling' hypothesis is the central, or only, mechanism by which protist grazing facilitates organic matter decomposition, we need to examine other possible hypotheses. Fenchel (1977) proposed several mechanisms: protozoan excretion of bacterial growth-promoting substances, mechanistic activity of protozoa that might increase availability of nutrients or oxygen to bacteria, and selection by protozoan grazing of more quickly growing forms among the mixed assemblage of bacteria. The first two mechanisms are similar to the 'nutrient recycling' hypothesis tested in this paper. The third mechanism was also proposed in Sherr *et al.* (1988) and in Jiang & Krumins (2006). Moving forward, it would be useful to develop and test quantitative models that evaluate the relative importance of both nutrient recycling and selection hypotheses.

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Appendix A

Derivation of model without grazers

When total nitrogen is fixed, model (1) can be reduced to:

$$\begin{aligned}\frac{dB}{dt} &= \mu_B B \min\{f(T_N - \theta_B B - \theta_G G), g(C)\} - \mu_G h(B)G \\ \frac{dG}{dt} &= \alpha \mu_G h(B)G - \omega G \\ \frac{dC}{dt} &= -\frac{1}{r} \mu_B B \min\{f(T_N - \theta_B B - \theta_G G), g(C)\}\end{aligned}\quad (A1)$$

To derive the model in the absence of grazers, we choose $G(t) = 0$ for all t . Therefore, we obtain the model in

the absence of grazers:

$$\begin{aligned}\frac{dB}{dt} &= \mu_B B \min\{f(T_N - \theta_B B), g(C)\} \\ \frac{dC}{dt} &= -\frac{1}{r} \mu_B B \min\{f(T_N - \theta_B B), g(C)\}\end{aligned}\quad (\text{A2})$$

In this case, the total carbon remains fixed, i.e. $d(B + rC)/dt = 0$. Thus $T_C = B(t) + rC(t)$ is a constant for all t . As a result, the model in the absence of grazers becomes a single equation:

$$\frac{dB}{dt} = \mu_B B \min\{f(T_N - \theta_B B), g(C)\} \quad (\text{A3})$$

combined with an algebraic equation $C(t) = [T_C - B(t)]/r$. In summary, we obtain the differential equation

$$\frac{dB}{dt} = \mu_B B \min\{f(T_N - \theta_B B), g((T_C - B)/r)\} \quad (\text{A4})$$

for the model in the absence of grazers.

Note: While many modelers have used multiplicative Monod functions for the limitation of bacterial growth, our model applies a minimum function of Monod functions. We think this approach is more biologically realistic as has been adopted in other contexts, for example in the analysis of phytoplankton dynamics (Klausmeier *et al.*, 2004; Ballantyne *et al.*, 2008).

Appendix B

Proof of nonexistence of internal equilibria

Any equilibrium (B^*, G^*, C^*) should satisfy the following:

$$\begin{aligned}0 &= \mu_B B^* \min\{f(T_N - \theta_B B^* - \theta_G G^*), g(C^*)\} - \mu_C h(B^*) G^* \\ 0 &= \alpha \mu_G h(B^*) G^* - \omega G^* \\ 0 &= -\frac{1}{r} \mu_B B^* \min\{f(T_N - \theta_B B^* - \theta_G G^*), g(C^*)\}\end{aligned}\quad (\text{B1})$$

Suppose that $G^* \neq 0$, then the second equation of Eqn. (B1) gives a positive $B^* = h^{-1}\left(\frac{\omega}{\alpha \mu_G}\right)$. Thus, we have $G^*, B^* \neq 0$. On the contrary, from the first and third equations of Eqn. (B1), $\mu_C h(B^*) G^* = 0$, then either $B^* = 0$ or $G^* = 0$, contradicting to $G^*, B^* \neq 0$. This completes the proof.

Appendix C

Necessity of the stoichiometric order

We compare the case $\theta_G < \theta_B$ and the case $\theta_G > \theta_B$ in Fig. C1. $\theta_G < \theta_B$ strengthens the facilitation of grazers on

decomposition by comparing the decomposition time cost with the case $\theta_G > \theta_B$. However, $\theta_G < \theta_B$ is not a necessary condition for the facilitation, because organic matter can also be decomposed completely in the presence of grazers in the case $\theta_G > \theta_B$.

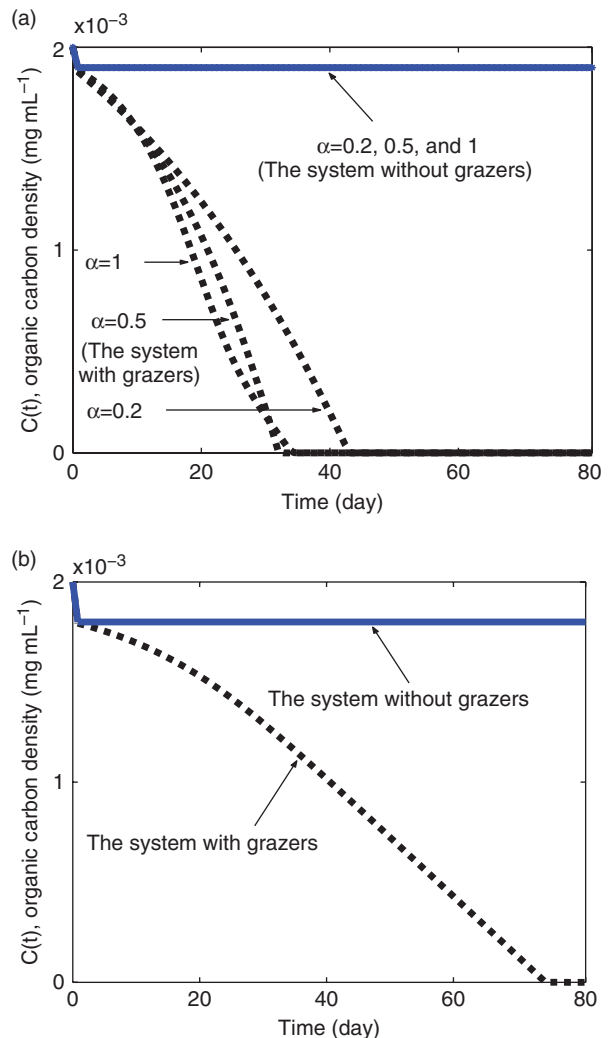


Fig. C1. A comparison of the case $\theta_G < \theta_B$ with various α values and the case $\theta_G > \theta_B$. The parameter α is the conversion efficiency of bacteria to grazers. The parameters θ_G and θ_B are the nitrogen : carbon ratios of grazers and bacteria, respectively. All other parameters are the same as in Fig. 2a.