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Chapter 6

Microbial growth, biomass production and controls

In the previous chapter we learned about the degradation and the mineralization of organic material back to its original inorganic constituents, most notably carbon dioxide. The chapter pointed out the importance of heterotrophic microbes, such as fungi in soils and bacteria in all environments, in carrying out this degradation. In many aquatic ecosystems and soils, heterotrophic microbes are responsible for a large fraction (50% or more) of this degradation and thus they consume an equally large fraction of primary production. Microbes degrade organic material not as a community service to ecosystems, but rather to support their survival and growth with the evolutionary goal of passing on their genes to future generations. So, to understand organic material degradation, we need to understand microbial growth and what controls it. In addition to biogeochemical questions, growth and production along with standing stock are fundamental properties of populations in nature. This chapter will discuss these properties.

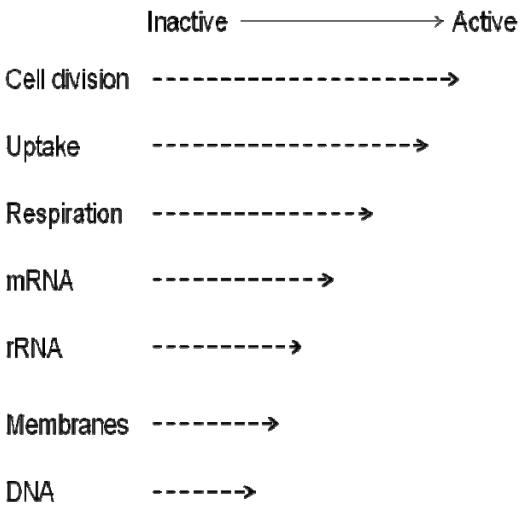
Here we focus on heterotrophic bacteria and fungi in oxic environments. But many of the topics discussed here are also relevant to other microbes and anoxic environments. Some of the factors controlling growth and biomass levels of heterotrophic bacteria undoubtedly have impacts on heterotrophic archaea. Likewise, these factors also have an impact on the growth of microbes in anoxic waters and sediments, but in these systems, the lack of oxygen and of other electron acceptors has to be considered first, as it is often the limiting factor (Chapter 11).

Are bacteria live or dead?

The high abundance of bacteria was an important discovery back in the 1970's when epifluorescence microscopy was first applied to natural samples. The question then became, are these cells really active and alive? It was possible that the observed degradation of organic material was mediated by a small number of live bacteria and that most of the cells visible by epifluorescence microscopy were dead. Questions about the metabolic state of bacteria were raised in part because it was known that the number of bacteria that grew up on agar plates (the plate count method) was much smaller (by 1000-fold or more) than the "direct count" estimate from epifluorescence microscopy (Chapter 1). Although the usual cultivation methods may miss bacteria (which we know is true), it seemed also possible that the difference could be due to a large fraction of dead bacteria, 99% of the total or more.

We now know that the extreme estimate—99% dead—is not correct, but the actual number of alive and dead cells for a given environmental sample is rather hard to pin down. Part of the difficulty is that microbial cells can be in different states of "activity" (Fig. 1), ranging

Figure 1. Possible activity states of microbes in nature. The diagram also illustrates how the definition of "activity" depends on what is being measured. Cell properties at a particular level depend on those below it. All actively dividing heterotrophic cells take up organic compounds, respire and so on. All cells taking up compounds may not be dividing, but are respiring and synthesizing mRNA and so on down the ladder.



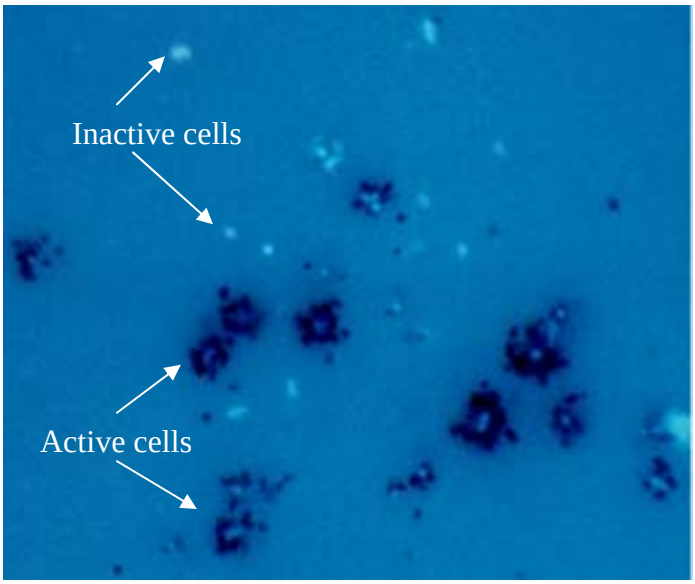
from truly dead cells which never could be resuscitated to microbes that are actively metabolizing and dividing. These metabolic states can be explored by a variety of single cell methods. In the end, the estimate of the number of active or inactive cells depends on the method and what aspect of microbial activity is being examined.

Microautoradiography is one method used to examine the number of active cells in natural environments. The method was first used in microbial ecology to examine the uptake of ^{3}H-thymidine by cells growing in coastal marine waters (Brock 1967), and it is still easier to use to examine single-cell activity in aquatic environments than in soils. Here is how it works. A radiolabeled organic compound, ^{3}H-amino acids, for example, is added to a sample, incubated for a few hours, and then filtered or the cells harvested by other means. The microbes are placed into photographic film emulsion. After an exposure time ranging from hours for highly active samples to days for relatively inactive samples, the film emulsion is developed, the microbes are stained for DNA, and the sample is viewed with epifluorescence microscopy. Cells that have

Photography before the digital age. A photograph once was a product of chemistry, rather than of electronics. In the pre-digital age, a photograph resulted from chemical reactions driven by light hitting compounds in an emulsion that coated photographic paper. Microautoradiography uses a similar emulsion to detect radioactivity. While regular photography uses visible light energy, microautoradiography uses energy in the particles given off by the decay of unstable (radioactive) elements. Marie Curie (1867-1934) inadvertently discovered the radioactive properties of uranium when she noticed that photographic plates turned black when stored next to uranium-rich ores ("pitchblende"). While radioactive uranium is dangerous (Curie's death was caused in part by exposure to uranium radiation), nearly all of the radioisotopes used in microautoradiography and other approaches in microbial ecology are quite safe. The isotopes most commonly used by microbial ecologists include ^{3}H, ^{14}C, ^{35}S, and ^{33}P, all of which emit low energy beta particles.

taken up ^{3}H-amino acids have silver grains associated with them (Fig. 2). These silver grains arise from the decay of ^{3}H which produces beta particles that strike compounds in the photographic emulsion.

Figure 2. Example of microautoradiography showing cells with silver grains that have taken up ^{3}H-amino acids.



The relative number of active bacteria detected by this method varies from <10% to 50% or even higher, depending on the environment and radiolabeled compound. This is a large fraction, given that microautoradiography probably detects only cells actively synthesizing new biomass because the radiolabeled compound has to be incorporated into biomass if a cell is to be scored as being active. So, a large fraction of cells are active to some extent in natural environments, although a sizable fraction also can be inactive. Microautoradiography also has been used to examine which microbes—bacteria or phytoplankton—assimilate organic material. Studies using microautoradiography, along with other methods, demonstrated that uptake of dissolved organic material (DOM) is dominated by heterotrophic bacteria (Chapter 5).

Other methods have been used to examine the metabolic state of bacteria (Del Giorgio and Gasol 2008). These include the CTC method (5-cyano,2,3-tolyl-tetrazolium chloride is reduced by the electron transport chain of respiration), propidium iodine (membrane integrity), and commercial kits for counting live and dead cells. These methods tend to give lower estimates of active cells than those from microautoradiography even though they should theoretically detect more cells, those that are respiring, for example, not necessarily synthesizing new biomass. Several approaches take advantage of the counting and sorting capabilities of flow cytometry to examine the number of active cells in a sample. This approach can be used with CTC and other fluorescence-based assays of activity.

Another general index of the activity state of bacteria in all environments is whether or not ribosomes can be detected. Ribosome presence is usually examined by a microscopic technique, fluorescence in situ hybridization (FISH), which is used to identify bacteria in natural environments. In FISH, microbes are identified by binding of an oligonucleotide probe. One such probe (Eub338) binds to all bacteria—if the bacterium has a sufficient number of ribosomes. A large fraction (50% or more) of bacteria in all natural environments can be detected by FISH and thus have ribosomes. The mere presence of ribosomes does not say anything about growth rates or other metabolic activity, but bacteria with detectable ribosomes are not likely to be dead or even just dormant.

Another argument against the hypothesis that natural environments have many dead or dormant bacteria—cells that are not growing. It is that in most natural environments, ranging from the water column of aquatic habitats to soils and sediments, grazing by detritivores and bactivores (predators of bacteria) would remove cells that were not growing. As discussed in

Chapter 7, these grazers often eat anything within a particular size range. A counter argument is that nongrowing cells may be produced as fast as they are being consumed by grazers.

Activity state of bacteria in soils and sediments The same questions about whether bacteria are active or not are relevant to thinking about soils and sediments. However, the physical complexity of these environments creates problems. There are many practical problems in trying to assay single cells within the complex matrix of detritus and inorganic particles, and there are conceptual problems in dealing with the range of possible activity states of bacteria inhabiting the many microhabitats in a single sample. A bacterium on one side of a particle may be quite active while another on the other side may not. Other microscopic techniques, such as confocal microscopy, can be useful for sorting out this complexity. Nevertheless, a few generalizations can be made.

Bacteria in soils and sediments seem to be as active, if not more so, than those in the water column of aquatic habitats. For example, using a method similar to the CTC approach, one study found that about 50% of bacteria were actively respiring in the rhizosphere of pine seedlings (Norton and Firestone 1991). Likewise, over 50% of cells in many soils are recognized by the Eub338 FISH probe, indicating that these bacteria at least have ribosomes (Eickhorst and Tippkotter 2008), although that may not be the case in all soils. In contrast, one of the few studies to use microautoradiography in sediments found few bacteria (<10%) took up acetate (Carman 1990), a key organic compound in anaerobic systems (Chapter 11). Likewise, <5% of the bacteria used naphthalene in an aquifer (Rogers et al. 2007). Naphthalene is often examined by studies trying to understand the degradation of polyaromatic hydrocarbons, common pollutants (Chapter 5).

Activity state of soil fungi It may not make much sense to ask what fraction of soil fungi is active because of the hyphal growth form of many soil fungi. For this reason and because of technical difficulties with working in soils, few microautoradiographic studies have examined activity of fungi in soils (Baath 1988). However, microbial ecologists can gain insights into the activity state of fungi by examining the portion of the hyphae that is filled with cytoplasm (Fig. 3). Fungi are capable of moving cytoplasm out of regions without adequate resources to support cytoplasmic metabolism in more favorable microhabitats, leaving behind empty or evacuated hyphae. The length of cytoplasm-filled hyphae to total hyphae is then one index of the activity state of the fungal community, analogous to the fraction of active bacterial cells.

Figure 3. A network of filled and empty fungal hyphae. Cytoplasm has moved into the tips, leaving behind a empty sheath of the hyphae. Panel B from Figure 1 of (HKlein and Paschke 2004H).

The activity state of soil fungi is similar to what we have seen for bacteria. Cytoplasm-filled hyphae make up 10-50% of total hyphal length, with the exact percentage varying with many environmental properties (Klein and Paschke 2004). For example, cytoplasm-filled hyphae are more prevalent near plant roots and other sources of organic material, and the active fraction of fungal hyphae vary with plant species (Klein et al. 2006). As with bacteria, these percentages also vary with treatments that stimulate growth, such as the addition of organic

carbon (e.g. sugars), nitrogen (ammonium), or water. Physical disturbances also can change the distribution and fraction of cytoplasm-filled hyphae.

Microbial growth and biomass production

As microbial ecologists were answering the question about the number of active cells, it became clear that we did not know how fast bacteria were growing in natural environments. Even if all bacteria were alive, their growth still could be very slow. The same question applies to fungi in soils. Microbial ecologists need information about growth rates and biomass production of the entire bacterial or fungal assemblage for understanding the role of these microbes in material and energy fluxes. These rates are basic properties of organisms in nature.

Before we examine growth in natural environments, let's review some basic parameters and definitions of bacterial growth. These parameters are summarized in Table 1.

Table 1. Terms for basic parameters of microbial biomass and growth.

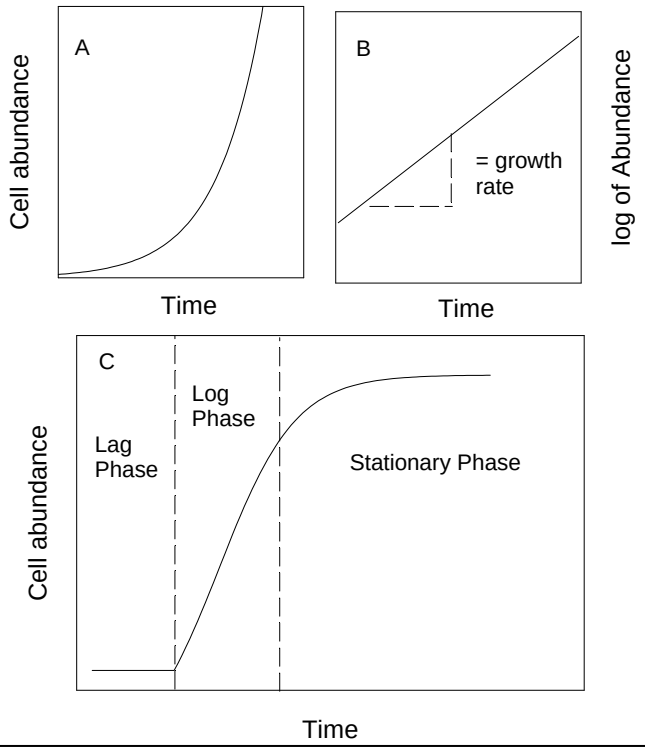
Parameter	Symbol	Units[a]	Method
Cell numbers	N	cells liter $^{-1}$	Microscopy, flow cytometry
Biomass	B	mgC liter^{-1}	Cell numbers, biomarkers
Growth rate	μ	d^{-1}	From production and biomass
Biomass production	BP	mgC liter $^{-1}$ d^{-1}	Leucine incorporation, others
Generation time	g	days	From the growth rate
Growth yield	Y	cells liter $^{-1}$	Cell numbers or biomass
Growth efficiency	BGE	Dimensionless	Various

[a]Volumetric units are given, but for soils the analogous units would be per gram of dry weight. Also, the appropriate parameters can be expressed per unit area, such as m^{-2}.

Growth of pure cultures in the lab: batch cultures Microbes growing as a single species in the laboratory provide a couple of models for growth in nature. The simplest model consists of growth in fresh media in a closed environment, such as a laboratory flask, that is, a batch culture.

When inoculated into new, fresh media, growth usually does not begin immediately but only after a delay of a few hours or longer depending on the bacterial strain and how different the media is from the media the strain was growing in previously (Fig. 4). This delay is

Figure 4. Bacterial growth in batch culture. (A) Exponential growth with no lag period or stationary phase; (B) Also exponential growth, but cell numbers are plotted on a log scale; (C) Growth phases of bacteria growing after a lag phase before the log or exponential phase. After growth-limiting substrate (usually organic carbon) is used up, the culture reaches the stationary phase when cell numbers do not change.



called the lag phase. Once microbes start to grow, they enter into the log or exponential phase during which abundance increases exponentially. The change in bacterial numbers (N) as a function of time (t) is:

$$dN/dt = \mu N \qquad (1)$$

where μ is the specific growth rate (sometimes called the instantaneous growth rate) of the bacterial population. Growth rates in pure cultures are calculated from the slope of ln(N) vs time. ("ln(N)" is the natural log of N or 2.30*log(N)). For this reason, the exponential phase is sometimes called the log phase. The change in numbers or biomass (dN/dt) is equal to bacterial production. The solution to Equation 1 is:

$$N_t = N_0 e^{\mu t} \qquad (2)$$

where N_t is the number of cells at time=t and N_0 is the initial abundance (t=0). Note that the units for μ are per time; for example, for rapidly growing lab cultures, convenient units are per hour whereas they would be per day for bacterial assemblages growing more slowly in nature.

Parameters related to the growth rate (μ) include the turnover time of the population (1/μ) and the generation time (g), both of which having units of time (e.g. hours or days). The generation time is defined as the amount of time required for a population to double. That is,

$$2N_t = N_t e^{\mu g} \qquad (3)$$

which yields after some algebra:

$$g = \ln(2)/\mu = 0.692/\mu \qquad (4).$$

Even in pure cultures, some resource becomes limiting, and growth slows down and eventually stops completely. At this point, the culture enters the stationary phase (Fig. 4). In some cultures, growth may continue, but other cells may die and lyse. The end result is the same; abundance is constant over time in the stationary phase. The equation sometimes used to define this sigmoid growth is the logistic equation:

$$dN/dt = rN/(K-N). \qquad (5)$$

where r is the specific growth rate and K the maximum population size or carrying capacity of the environment. Note that when N is small relative to K, Equation 5 becomes similar to

Equation 1. The symbols r and K reflect the historical roots of Equation 5. Both are part of terms used classically to define two types of selection pressures faced by organisms: r-selection and K-selection.

The terms, r-selection and K-selection, were originally derived for large eukaryotes (insects and invertebrates) colonizing a new habitat. The initial colonizers are r-selected and grow rapidly to take advantage of free space and new habitats. As the carrying capacity of the new habitat is reached, rapid growth is no longer favored, but rather K-selected organisms with traits for surviving crowded conditions win out. Traits of r-selected organisms allow them to flourish in unstable environments where growth conditions change rapidly, preventing the buildup of dense populations. In contrast, K-selected organisms dominate stable environments with invariant growth conditions that promote dense populations. While the concepts are from large organism ecology, they can be applicable to thinking about microbes in some environments.

Growth of pure cultures in the lab: continuous cultures The key feature of a batch culture is that it is a closed system with no inputs or outputs; the inoculum is exposed to one dose of growth substrates at the beginning, and any waste byproducts excreted during growth are not removed, except for gases. In contrast to this model of microbial growth, microbes in a continuous culture are provided fresh media continuously and the old media--along with waste products and cells--are removed at the same rate. A chemostat is a continuous culture in which the concentrations of all chemicals are constant. All chemostats are continuous cultures, but continuous cultures are not necessarily chemostats.

Continuous cultures can be quite elaborate and sophisticated, but the basic design is simple (Fig. 5). A reaction chamber is inoculated with microbes and is allowed to operate in batch mode initially; at first there are no inputs or outputs as the microbes multiply. Then new, sterile medium is pumped into the reaction chamber at a fixed rate, and the media in the reaction chamber is pumped out at the same rate in order to maintain a constant volume within the reaction chamber. Initially, abundance decreases when the pump is turned on, but then microbes increase as they take advantage of the new media. These oscillations continue until a steady-state is reached when abundance is constant. At this point, it can be shown that

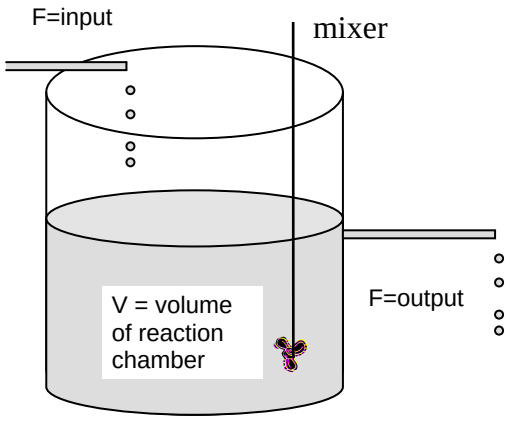
$$\mu = D \qquad (6)$$

where D is the dilution rate, defined by:

$$D = f/V \qquad (7)$$

where f is the flow rate (e.g. liters per h) and V the volume of the reaction chamber (e.g. liters). The dilution rate has the same units (e.g. per h) as the growth rate.

Figure 5. A simple continuous culture. The rate (F) at which new medium is added (input) must equal the rate at which medium from the reaction chamber flows out (output). The flow rate can be controlled by simple gravity or by pumps. In addition to a mixer, gases can be introduced to help with circulation and to provide oxygen or other gases.



Equation 6 is a very simple but powerful statement about growth; it says that growth is set by the dilution rate (if we ignore problems like growth on the walls of the reaction chamber), which is under experimental control. It also says that growth rates are independent of the supply and concentration of organic material in the continuous culture. However, the concentration of organic material, along with the growth efficiency, sets biomass levels.

Continuous cultures provide a different model of growth in nature than batch cultures. Like continuous cultures, microbial abundance is mostly constant over time in nature because growth is balanced by removal: the outflow in the case of continuous cultures, mortality caused by grazing and viral lysis in nature. The implication is that over some time and space scales in some environments, microbial communities are in a quasi-steady-state. Growth conditions may change, but perhaps not on times scales relevant to microbes. On the other hand, growth conditions do change, and a batch culture may be a more accurate description of microbial growth. One example is the initial stages of a spring bloom in temperate aquatic habitats where initial microbial abundance is low and concentrations of many nutrients are high, similar to a batch culture.

Neither batch nor continuous cultures are perfect models for growth in nature. But both provide useful terms and concepts for examining the processes controlling microbial standing stocks and production in natural environments.

Measure growth and biomass production in nature

Measuring growth rates in the lab is usually very easy because the rate is calculated simply from the change in abundance or biomass over time. In nature, however, microbes occur in complex communities, and growth is usually balanced by mortality caused by grazing and

viral lysis. Microbial abundance and biomass are usually quite constant over time and space, even though other data, like numbers of active cells, indicate that microbes must be growing. So, changes in cell numbers or biomass over time in nature, in the absence of any manipulations to minimize mortality, tell us nothing about growth rates.

Table 2 summarizes the methods that have been proposed over the last 30 years for measuring bacterial growth and production in aquatic ecosystems. A few of these same methods have been used to measure bacterial growth in soils. The two most commonly used methods, both in soils and in aquatic environments, are based on thymidine and leucine incorporation (Fuhrman and Azam 1980; Kirchman et al. 1985). The two methods are quite similar.

Table 2. Some of the methods used to estimate biomass production by microbes.

Method	Principle	Comments
$^{14}CO_2$ fixation	Light-dependent fixation of CO_2 into biomass	Targets autotrophs
Dark $^{14}CO_2$ fixation	Light-independent CO_2 fixation due to anaploretic processes	Variable relationship between CO_2 fixation and total biomass production
Frequency of dividing cells (FDC)	Frequency of paired cells about to divide increases with growth rate	Variable relationship between FDC and growth rate
^{3}H-adenine incorporation	Adenine is used in RNA synthesis. rRNA synthesis scales with growth rate	tRNA and mRNA synthesis may not scale with growth
^{35}S-sulfate incorporation	Sulfate is used in protein synthesis which scales with growth	Phytoplankton use sulfate; hard to measure in seawater
^{14}C-acetate-in-ergosterol	Acetate is used for ergosterol synthesis which is coupled to growth	Targets fungi
Dilution or filtration	After minimizing grazing and viral lysis, increase in biomass is followed	Labor-intensive and intrusive
^{3}H-thymidine (TdR) incorporation	TdR is used in DNA synthesis which scales with growth.	See text
^{3}H-leucine (Leu) incorporation	Leu is used in protein synthesis which scales with growth.	See text

Thymidine, which is one of the four nucleotides in DNA, is used to trace DNA synthesis whereas leucine, an amino acid, is used to trace protein synthesis. Dividing cells must make more DNA and thus incorporate more thymidine as they grow. Similarly, fast growing cells make more protein and thus incorporate more leucine than slow growing cells. The same basic idea is used for estimating fungal growth, except that the starting radiolabeled compound is ^{14}C-acetate. After incubation, the common fungal sterol, ergosterol, is isolated and radioassayed for the incorporated ^{14}C (Rousk and Baath 2007b); this approach is called the "acetate-in-ergosterol" technique. The text box gives simple directions for using the thymidine method with a water sample.

How to estimate bacterial production from thymidine (TdR) incorporation. The procedure given here is for aquatic habitats, but a similar approach is used for soil and sediment samples.

1. Add 1.5 ml of sample to a 2 ml microcentrifuge tube, along with enough ^{3}H TdR for a final concentration of 20 nM. (This concentration minimizes de novo synthesis of TdR.)
2. Incubate at the in situ temperature for 15 min to 24 h, depending on the environment.
3. Put the tube on ice until water reaches 4 ^{o}C. (The sample should be cold to prevent hydrolysis of the DNA during the next step.)
4. Add 80 μl of 100% trichloroacetic acid (TCA) (5%, final concentration) and centrifuge. Discard supernatant. (All macromolecules precipitate in cold TCA whereas only protein remains after hot TCA.)
5. Add 1.0 ml cold 5% TCA and repeat centrifugation. (This removes the unincorporated ^{3}H TdR.)
6. Add 1.0 ml cold ethanol and repeat centrifugation. (This step removes any remaining unincorporated ^{3}H TdR and the TCA. The macromolecules will remain precipitated in ethanol, but unlike TCA ethanol can be removed by drying.)
7. After allowing the sample to dry to remove the ethanol, add 1.0 ml scintillation cocktail to the microcentrifuge tube and radioassay the incorporated ^{3}H.

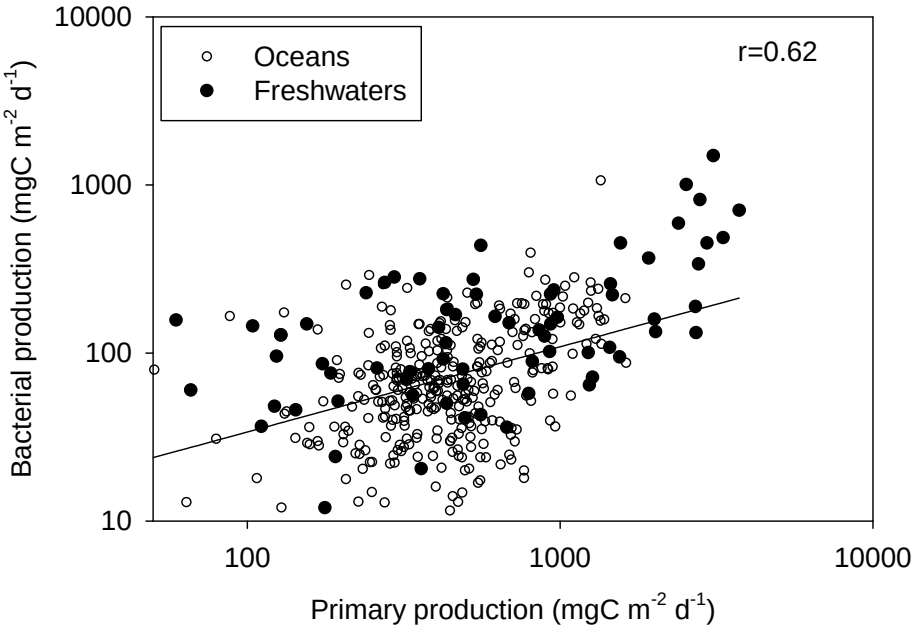
Bacterial biomass production in aquatic environments

Production rates are useful for evaluating the general importance of heterotrophic bacteria in ecosystems and for exploring what controls production and biomass levels. The most important observation is that bacterial production usually correlates with primary production, but with great variation. Higher primary production leads to higher bacterial production. In Figure 6, the overall correlation coefficient was significant ($r=0.62$; $n=441$; $p<0.001$), but the linear regression line on this log-log plot captures only about 40% of the variation in the data. So, while the two production rates covary, there is much variation in the relationship. Sometimes the relationship is much closer, indicating a tighter "coupling" between the two microbial processes, while in other habitats and times, there is no significant relationship. Microbial ecologists often say that bacterial production and primary production are coupled over large spatial and temporal scales but not over small ones. Part of the explanation is methodological. It is easier to detect the correlation between bacterial and primary production when differences are large (large scales), especially compared to errors in the measurements.

Another important observation is about the magnitude of bacterial production compared with primary production and the ratio of the two rates (BP:PP). (Some ecologists multiply this ratio by 100 and report percentages, but here let's reserve percentages for another purpose.) This ratio is a measure of the importance of heterotrophic bacteria and the rest of the microbial loop in consuming primary production. The BP:PP ratio varies greatly over time and space, but usually it is low, about 0.1, in the open oceans whereas sometimes it is as high as 0.3 to 0.5 in lakes. The ratio is higher in lakes in part because of the input of terrestrial organic carbon. Consequently, small lakes which are impacted more by terrestrial organic carbon should have higher BP:PP ratios than large lakes. Because of terrestrial organic carbon, the BP:PP also can be high in

estuaries. At the other extreme, the BP:PP ratio for the Arctic Ocean and Antarctic seas is very low (<0.05).

Figure 6. Bacterial production versus primary production in a variety of aquatic environments. The correlation coefficient and least-squares line are from the entire data set of oceans and freshwaters, but here only environments with primary production greater than 100 mgC m^{-2} d^{-1} are shown for clarity. Data provided by Eric Fouilland, taken from (HFouilland and Mostajir 2010H).



A BP:PP ratio of 0.1 or less may not seem impressive, but its significance becomes clear when it is coupled with bacterial growth efficiency (BGE). Remember that BGE is

$$BGE = BP/(BP+R) \quad (8)$$

where R is respiration. If we define total bacterial carbon demand (BCD) as the sum of both production and respiration, then

$$BCD = BP/BGE \quad (9).$$

We can now relate bacterial growth and the total use of organic carbon (BCD) by heterotrophic bacteria to primary production with data on production rates and bacterial growth efficiency. That is, the percentage of primary production processed by heterotrophic bacteria (% BCD/PP) is

$$\% \text{ BCD/PP} = \text{BP/BGE/PP} * 100 \quad (10).$$

We can now use the data reported in Table 3 and calculate the total impact of heterotrophic bacteria on carbon flows through aquatic environments.

Table 3. Average production rates for phytoplankton (primary) and heterotrophic bacteria (bacterial), the ratio of primary production to bacterial production (BP/PP), bacterial growth efficiency (BGE) and the % of primary production consumed by heterotrophic bacteria, calculated from BP/PP divided by BGE. The production data are from Figure 6 and the BGE values are from Figure 13 in Chapter 5.

Environment	Production rates Primary (mgC $m^{-2}d^{-1}$)	Bacterial (mgC $m^{-2}d^{-1}$)	BP/PP	BGE	% of Primary Production
Open ocean	1000	98	0.10	0.15	65
Arctic and Antarctica	1063	17	0.02	0.15	11
Other Marine	780	179	0.23	0.35	66
Lakes	1385	224	0.16	0.25	65

These data once again indicate the importance of heterotrophic bacteria in processing primary production. Although the open oceans tend to have lower BP:PP ratios, these are offset by low BGE, leading to the observation that about 65% of primary production is routed somehow through DOM and heterotrophic bacteria. Other marine habitats and lakes arrive at the same percentage but with higher BP:PP ratios and BGE values. A thorough analyses of these data suggested higher BP:PP ratios and fluxes through DOM and heterotrophic bacteria (Fouilland and Mostajir 2010). The exception is the Arctic Ocean and Antarctic seas. In these perennially-cold environments, the extremely low BP:PP ratios are not offset by equally low

BGE values, leading to a low fraction of primary production routed through DOM and heterotrophic bacteria.

Bacterial growth rates in aquatic environments In contrast to the ease of estimating growth rates for bacteria in a laboratory pure culture, it is difficult to do this in nature for the same reasons why it is difficult to measure biomass production; microbes live in complex communities with growth usually being balanced by mortality. A few approaches, such as the frequency of dividing cells and the amount of ribosomal RNA per cell, yield estimates of growth rates directly. Other approaches use estimates of bacterial production and standing stocks (cell abundance or biomass). That is, the growth rate (μ) is bacterial production divided by cell abundance or biomass. Care must be taken to make sure the units of production and standing stocks are compatible. For example, if production is expressed as cells per liter per day, then the standing stock estimate has to be in cells per liter. Likewise, if production is mmol C m^{-3} d^{-1}, then biomass must be given as mmol C m^{-3}.

A problem with this approach is that the calculated growth rate is a composite of all microbes in the sampled community potentially growing at quite different rates, ranging from zero (dead or dormant cells) to potentially high values. While the approach of using bulk production and standing stocks to estimate this composite growth rates has its flaws, the estimates give a good general picture of the time scale on which microbes grow in natural environments.

Data from aquatic habitats, which is where this approach has been used the most, illustrate a general point about microbes in most natural environments. Figure 7 plots generation times of heterotrophic bacteria versus generation times of the phytoplankton for various aquatic

habitats. (Phytoplankton growth rates can be calculated from primary production and chlorophyll data, similar to the approach used for bacteria.) These data indicate that heterotrophic bacteria grow relatively slowly in aquatic habitats, usually on the order of days, much longer than the generation time of bacteria growing in the lab where bacteria can double every 30 minutes. The record for a lab culture is less than 10 minutes, held by the marine bacterium *Vibrio natriegens*, orders of magnitude faster than the month or more generation time of bacteria growing in polar waters in the winter.

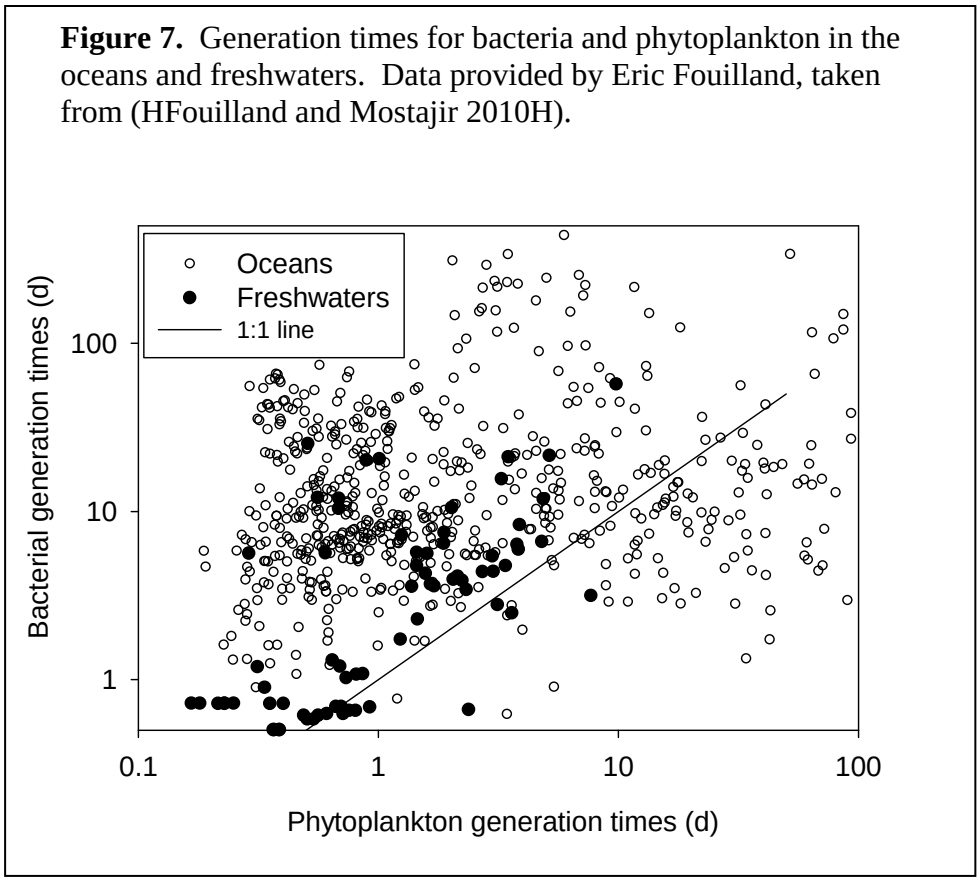


Figure 7. Generation times for bacteria and phytoplankton in the oceans and freshwaters. Data provided by Eric Fouilland, taken from (HFouilland and Mostajir 2010H).

Another point to be learned from Figure 7 is that heterotrophic bacteria often grow more slowly than phytoplankton. The exception may be some estuaries where bacterial generation times are quite short and bacteria seem to be growing much more quickly than phytoplankton. One explanation is that estuaries are often light limited, preventing rapid phytoplankton growth.

Similarly, growth of heterotrophic bacteria in freshwater lakes is more similar to rates for phytoplankton than seen in other environments. Bacteria may grow quickly at the expense of organic carbon from terrestrial sources. Even so, bacterial growth is slower than phytoplankton in these lakes, some of which are represented in Figure 7. In most freshwaters and marine environments, bacterial generation times are longer than those of phytoplankton.

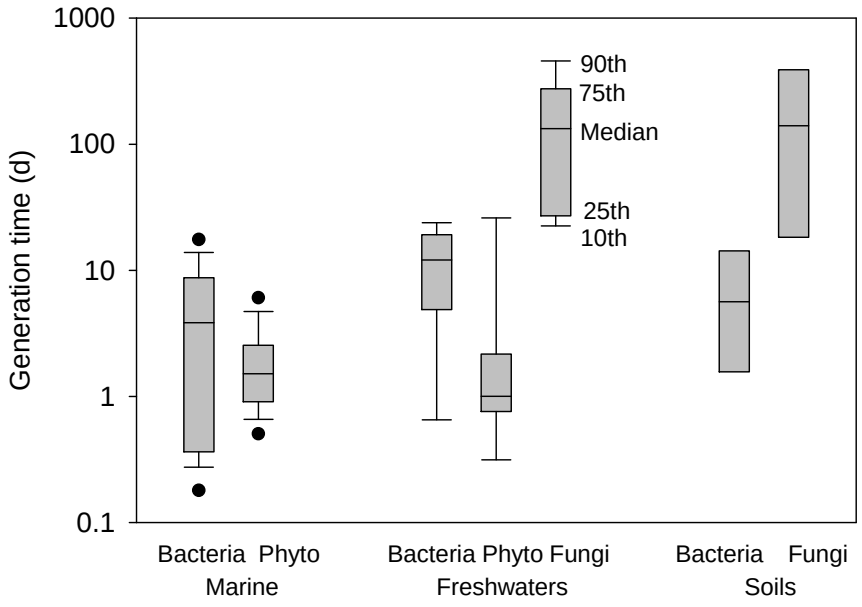
Growth rates of bacteria and fungi in soils

Similar to work on bacteria in aquatic ecosystems, microbial ecologists can address questions about growth of bacteria and fungi in soils using the same methods as introduced above: leucine or thymidine incorporation (or both) for bacteria, and the acetate-in-ergosterol technique for fungi.

Bacteria appear to grow faster than fungi in soils, a difference that is also true in aquatic systems (Fig. 8). Early studies emphasize the very fast potential growth rates of bacteria with generation times on the order of an hour, 10-fold faster than for fungi (Coleman 1994). Studies using the modern methods mentioned above found much slower growth rates for both microbial groups, but still faster rates for bacteria than for fungi. In sandy loam, for example, fungi have generation times over 100 days (Rousk and Baath 2007b), about 10-fold slower than the typical growth of bacteria in soils (Baath 1998). The few direct comparisons also indicate that bacteria grow faster than fungi (Buesing and Gessner 2006). Fungal growth is also slow in aquatic environments, similar to rates in soils and aquatic habitats (Gulis et al. 2008; Newell and Fallon 1991; Pascoal and Cassio 2004). Some soil microbial ecologists have concluded that bacteria grow more slowly in soils than in aquatic habitats (Baath 1998), but this hypothesis needs more data especially for soils because there is much variability in these rates for both environments

(Fig. 8). Even so, the growth rate data fit the general hypothesis about slow and fast carbon pathways mediated by slow-growing fungi and fast-growing bacteria, respectively, as discussed in Chapter 5.

Figure 8. Generation times for bacteria, phytoplankton ("phyto") and fungi in soils and aquatic habitats. The number "10th", 25th, 75th and 90th refer to the percentiles containing the data. The points for the marine data are the 5th and 95th percentiles. Data from (HCarter and Suberkropp 2004H; HCole et al. 1988H; HDemoling et al. 2007H; HGulis et al. 2008H; HKirchman et al. 2009H; HRousk and Baath 2007aH; HRousk and Nadkarni 2009H).



Remember that these growth rates are for the entire community of bacteria and fungi being sampled. Studies of growth rates in soils have not attempted to distinguish saprophic from mycorrhizal fungi. Growth by these two fungal groups probably differs greatly because of the large differences in their environments. More generally, the physical-chemical environments of soils differ greatly for microbes over very small scales (Chapter 3), leading to huge heterogeneity in growth of both bacteria and fungi. It is very difficult to estimate rates for individual microbes

in nature. Still, estimates for the entire community are still useful for examining controls of growth.

What sets biomass production and growth by microbes in nature?

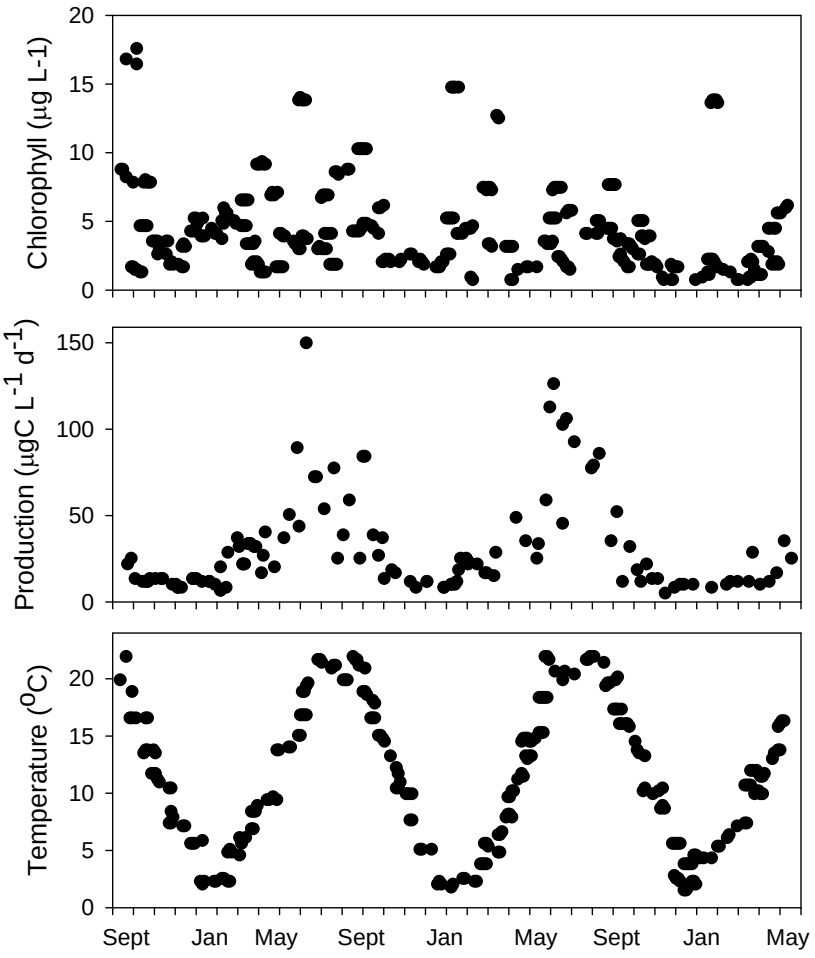
The growth rates of bacteria and fungi are much lower than what can be achieved in laboratory cultures. What then prevents these microbes from growing faster in nature? For phototrophic microbes, we saw in Chapter 4 that the answer is fairly simple: light and the supply of inorganic nutrients, such as compounds containing nitrogen, phosphorus and sometimes iron. For heterotrophic microbes, the answer is more complex. Here we will focus on bottom-up factors, leaving top-down factors for future chapters.

Temperature effects on growth and carbon cycling Of all bottom-up factors, temperature is arguably the most important. Chapter 3 discussed how temperature affects all chemical reactions and rates of processes in nature, and microbial growth rates are no exception. As a general rule of thumb, the Q_{10} of growth rates is about 2, but it varies, of course. The precise value for this temperature effect is important perhaps especially for soil ecosystems. Many studies have examined how soil respiration and organic material decomposition may respond to predicted changes in temperature due to global warming (Davidson and Janssens 2006). The problem is very important in the Arctic where warming by only a few degrees may melt permafrost and release not only organic carbon that can be mineralized to carbon dioxide, but may lead to higher fluxes of methane, a potent greenhouse gas, to the atmosphere (Dorrepaal et al. 2009). At the ecosystem level, respiration of the soil community has a Q_{10} of 1.4 even though controlled

experiments typically lead to much higher estimates of Q_{10} (>2) (Mahecha et al. 2010). It has been argued that the experiments exclude too many important variables.

Temperature also affects the growth of bacteria in temperate aquatic environments. Often, bacterial biomass production correlates the best with temperature rather than other properties, such as dissolved organic carbon (DOC), chlorophyll and primary production. One example is Narragansett Bay, Rhode Island (Fig. 9). In this environment, temperature ranges

Figure 9. Example of how bacterial production varies with temperature but not with chlorophyll in a temperate environment, Narragansett Bay, Rhode Island. Data from (HStaroscik and Smith 2004H).



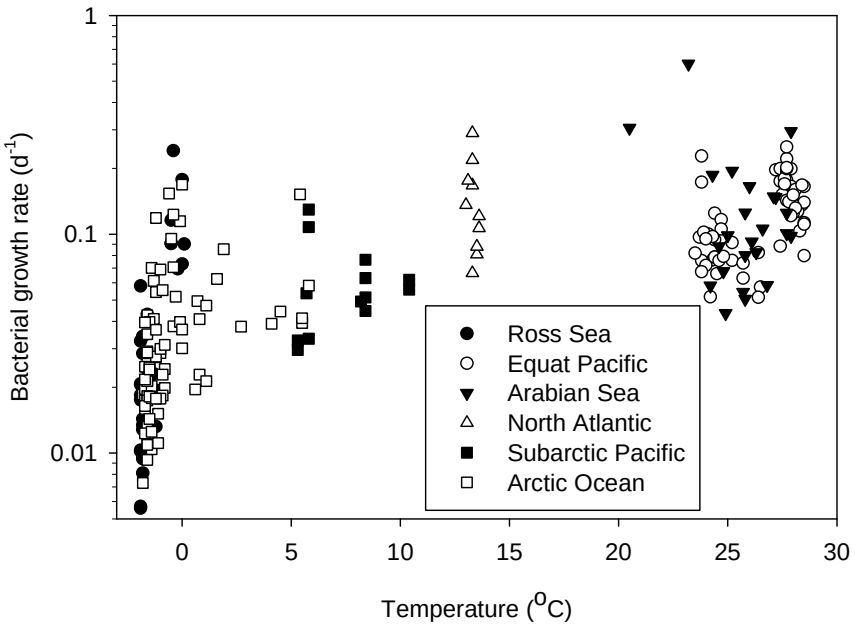
from -1 to nearly 23 ^{o}C while biomass production varies by over 100-fold (Staroscik and Smith 2004). The correlation between the two parameters was high during this study (r=0.70) whereas in contrast, there was no significant correlation with chlorophyll, which is often used as a proxy for the supply of organic carbon. The investigators examining this system concluded that temperature was the most important factor controlling bacterial biomass production. However, the relationship between temperature and production varied during the year, and the Q_{10} implied by the field data was much higher than 2, suggesting that other factors also affected bacterial production and growth. Soil microbial ecologists have also concluded that high Q_{10} values indicate that factors other than temperature are at work (Davidson et al. 2006).

The Narragansett Bay study is one example of a problem often faced by microbial ecologists who need to use correlations to examine functional relationships between, in this case, microbial growth and temperature. The problem is that correlations do not necessarily imply causation. In temperate environments, temperature varies greatly along with other ecosystem properties potentially affecting growth. So, temperature may correlate significantly with bacterial production in part because temperature covaries with another, hidden property of the ecosystem that also affects bacterial growth.

In addition to within an ecosystem, we can examine how much growth can be explained by differences in temperature among ecosystems, the biggest difference being between polar systems and low latitude waters. Growth rates of bacteria are low in the perennially-cold waters of the Arctic Ocean and in Antarctica's Ross Sea and are higher in the slightly warmer subarctic Pacific Ocean and the North Atlantic Ocean (Fig. 10). But this increase is substantially more (about 10-fold more) than what is predicted from the known response of bacteria to temperature, that is a Q_{10} of about 2. What's more, rates do not get much higher in the warm waters of the

equatorial Pacific and Arabian Sea; growth rates remain, albeit with much variation, at about 0.1 d^{-1} (generation time of about 7 days) for temperatures ranging from 5 to 28 ^{o}C. This leveling off of rates with temperature has also been seen in freshwaters and estuaries.

Figure 10. Growth rates of bacteria in various oceanic ecosystems. Taken from (HKirchman et al. 2009H)



So, temperature is important, but not necessarily in all ecosystems, and its effect can be overestimated. Other ecosystem properties affecting growth, which are more difficult to measure, covary with temperature and may be the real reason for the high temperature-growth correlation. Even in polar systems, there is evidence that bacteria are in fact adapted to cold temperatures and that growth rates are low for other reasons, most likely low concentrations and supply rates of DOM.

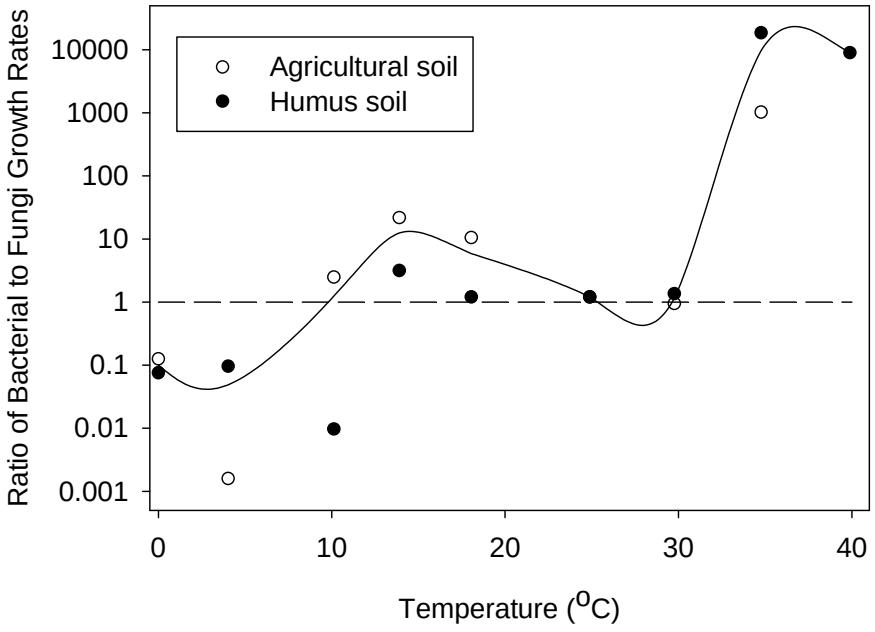
Temperature effects on fungi versus bacteria in soils Since temperature explains much of the variation in soil respiration, it is likely that it is equally powerful in examining the variation in bacterial and fungal growth rates in soil ecosystems, just as is the case for aquatic habitats. The same general rule of thumb, that is, $Q_{10} = 2$, applies to soils as it does for freshwater, and marine environments. Although soil microbes never experience the extremely hot waters of a hydrothermal vent, temperatures can drop to low levels in soils; there is evidence of bacterial activity even in frozen permafrost colder than -39 ^{o}C (Panikov et al. 2006). What may be especially important is the difference in how fungi and bacteria respond to temperature.

We saw in Chapter 3 that bacteria and cyanobacteria can grow in much hotter water than algae and other eukaryotes. The difference between prokaryotes and eukaryotes in temperature tolerance holds true for bacteria and fungi in soils. The optimal temperature for bacterial growth in agricultural and forest soils is about 5 ^{o}C warmer than that of fungi (Pietikainen et al. 2005). But fungi do better at the other end of the temperature scale and can grow in soils 4-5 ^{o}C colder than can bacteria; one study calculated temperature minima of -12 and -17 ^{o}C for bacteria and fungi, respectively (Pietikainen et al. 2005). Consequently, the ratio of bacterial biomass production to fungal biomass production increases with temperature in soils (Fig. 11). These results are consistent with the observation that fungi dominate soils in winter but less so in summer when bacterial biomass is higher; likewise, the ratio of bacterial biomass to fungal biomass is lower in snow-covered soils than in uncovered soils (Schadt et al. 2003).

While correlations between temperature and microbial activity in soils are often very high, like seen in aquatic habitats, it remains unclear how much temperature determines rates among various soil ecosystems. There is little relationship between temperatures between 5 and 28 ^{o}C and organic material decomposition in soils (Giardina and Ryan 2000). Likewise,

decomposition rates often return back to the original rates after temperatures have been experimentally increased (Knorr et al. 2005).

Figure 11. Ratio of bacterial production to fungal production as determined by leucine incorporation (bacteria) and acetate-into-ergosterol (fungi). The dashed horizontal line indicates when the ratio of growth rates is one. The solid line is the average of growth rates in the two soils. Data from (HPietikainen et al. 2005H).



Limitation by organic carbon The concentration and supply of organic material are often the most important factors determining the growth of heterotrophic bacteria and fungi in both soils and aquatic systems. As mentioned in Chapter 5, concentrations of organic material and of especially labile components are very low in nature, which explains why growth rates of heterotrophic microbes are usually far lower in nature than seen in the laboratory. One line of evidence for carbon limitation in aquatic systems comes from studies comparing rates of

bacterial biomass production with rates of primary production. As indicated in Figure 6, there is an overall correlation between bacterial and primary production in lakes and the oceans. The easiest way to explain this correlation is that primary production determines directly or indirectly the supply of DOM and detritus which in turn drives heterotrophic bacterial activity. Any change in primary production leads to a change in the DOM supply with consequences for heterotrophic bacteria. Few analogous data from soils and for fungal growth are available. There is a correlation between organic matter content and fungal growth (Rousk and Nadkarni 2009) and between soil respiration and primary production (Sampson et al. 2007), all evidence for organic carbon limitation of soil bacteria and fungi.

Another line of evidence indicating carbon limitation is based on addition experiments. In brief, organic compounds are added to incubations of water or soil, and microbial production is followed over time. Often bacterial and fungal growth is higher in incubations with the organic compounds than in the no-addition control in experiments with samples from soils and aquatic habitats (Baath 2001; Demoling et al. 2007). The addition of organic carbon usually stimulates growth more so than the addition of inorganic nutrients, such as ammonium or phosphate, but there are important exceptions as discussed below.

Both the concentration and the supply rate are important in thinking about limitation by organic carbon and other elements. The relationship between concentrations and growth rates is described by the Monod equation

$$\mu = \mu_{max}\, S/(K_s+S) \qquad (11)$$

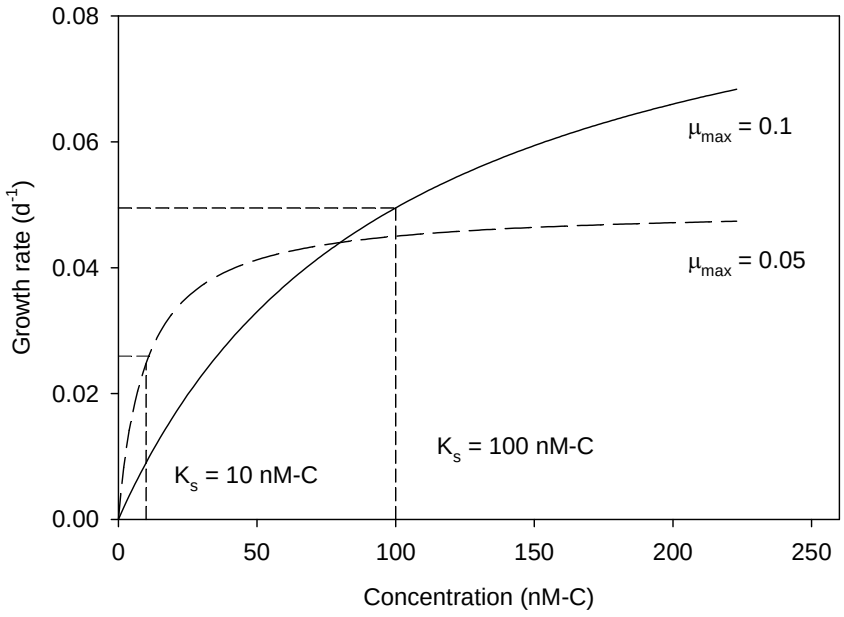
where μ is the growth rate, μ_{max} the maximum growth rate, S the substrate concentrations, and $K_s$the substrate concentration at which the growth rate is half of the maximum (Fig. 13). Notice the similarities between the Monod equation and the Michaelis-Menten equation given in

Chapter 4. But it is difficult to use the Monod equation to examine growth rates of microbes in nature because as mentioned in Chapter 5, the concentration of labile organic carbon is not only very low in natural ecosystems but also hard to measure. Concentrations are only high when the growth of heterotrophic microbes is in fact low, such as during early spring in temperate aquatic habitats,

Freedom fighter and microbiologist *par excellence* The Monod equation is named after Jacob Monod (1910-1976) who won the Nobel Prize (along with his compatriots, François Jacob and André Lwoff) for work on the *lac* operon in *E. coli*. This operon was the one of the first models of gene regulation at the transcription level. But before his work in microbiology, Monod was a member of the French Resistance that fought against the German occupation of France during World War II (1939-1945).

implying a concentration-growth relationship opposite from that of the Monod equation. This paradox also applies to limiting substrates other than organic carbon.

Figure 12. The Monod equation describing growth rates by two competing species as a function of limiting substrate concentrations.



In addition to amounts—concentrations and supply rates—of organic material, intuitively, one would think that the "quality" of the organic components would have an impact on growth rates of heterotrophic bacteria and fungi. In fact, there is little direct evidence from field studies for this reasonable hypothesis. We do know that degradation rates vary with substrate quality (Chapter 5), implying that heterotrophic microbes grow faster on organic components such as protein and simple polysaccharides rather than on lignin, for example. In any case, differences in the quality of organic material are likely to lead to variation in growth rates even if concentrations or supply rates of organic material are similar.

Limitation by inorganic nutrients The concentration of many inorganic nutrients potentially used by microbes is low in soils, lakes, and the oceans, raising the possibility of these compounds limiting growth of heterotrophic microbes. However, many of the same studies that demonstrated organic carbon limitation using additions experiments also examined the impact of adding ammonium or phosphate on bacterial growth; analogous experiments with fungi have not been done. Only a few studies found any evidence of heterotrophic bacteria being limited by phosphate; even fewer studies have reported that addition of ammonium alone stimulates bacterial growth (Church 2008). This work raises two questions: why is heterotrophic growth generally limited by organic carbon and not by inorganic nutrients? And why is phosphate limitation more common than nitrogen limitation?

One answer is that organic carbon is used by aerobic microbes for both biomass synthesis and respiration, but N and P is used only for biomass synthesis. In Chapter 12, we will see that given typical C:N ratios for the organic material used by microbes and for microbial biomass, both bacteria and fungi should excrete ammonium, not assimilate it, implying that these

microbes are not limited by this compound. A similar argument can be built for C:P ratios and phosphate use versus excretion. Another answer involves competition for these inorganic nutrients between the heterotrophic microbes and autotrophic microbes in aquatic systems and higher plants in terrestrial systems. In Chapter 4, we learned that small cells such as heterotrophic bacteria with their high surface area to volume ratio should out-compete large phytoplankton and higher plants for ammonium, phosphate and other dissolved compounds. However, uptake of inorganic nutrients by heterotrophic microbes eventually would lead to lower growth of autotrophic organisms and lower production of organic material, resulting in organic carbon limitation of the heterotrophs.

There are some interesting exceptions to the general rule of organic carbon limitation. Addition experiments demonstrate that phosphate limits growth of heterotrophic bacteria in the Sargasso Sea and the Mediterranean Sea (Church 2008). In fact, primary production in both systems is thought to be limited by phosphate, unlike the general rule of marine waters being limited by nitrogen. The N_2 fixing cyanobacterium *Trichodesmium* is abundant in the Sargasso Sea and may alleviate nitrogen limitation in that system. Parts of the Gulf of Mexico also can be phosphate-limited due to the nitrogen inputs from the Mississippi River.

The other question is about why phosphate limitation of heterotrophic bacteria is more common than nitrogen limitation. The answer may be related to heterotrophic bacteria being exceptionally phosphorus-rich and thus having very low C:P ratios in waters like the Sargasso Sea; few data are available to test this idea. Another part of the answer may be the biochemicals containing nitrogen and phosphorus in microbes. As mentioned in Chapter 2, nitrogen is mainly in protein, which generally is not degraded and synthesized (“turn over”) independent of growth

in bacteria, whereas phosphorus is in nucleic acids, lipids, and nucleotides, some of which (e.g. mRNA and ATP) turn over greatly.

Co-limitation and interactions between controlling factors Microbes have adapted to live on very low concentrations of many compounds in natural ecosystems, so it can be overly simplistic to focus on a single limiting factor. We see the consequences of these low concentrations in addition experiments where often the addition of both an organic compound and inorganic nutrient stimulates bacterial biomass production more so than the addition of either compound alone. For example, in high nutrient-low chlorophyll oceans (see Chapter 4), addition of iron along with an organic carbon source stimulates bacterial production more so than either alone (Church et al. 2000; Kirchman et al. 2000). Some authors call this co-limitation by organic carbon and iron, but it is seems likely that in these experiments, iron just became the next limiting factor, once the addition alleviated organic carbon limitation.

There are several clearer examples of co-limitation for microbes where the limiting factors are physiologically linked (Table 4). For example, microbes may be prevented from using nitrate, and thus are limited by nitrogen, because low iron levels interfere with nitrate reductase, an iron-containing enzyme essential for reducing nitrate to ammonium and in so doing making nitrate available for biomass synthesis. Nitrogenase, the critical enzyme for N_2 fixation, is another enzyme that requires iron as a co-factor. Several enzymes require other trace metals, such as cobalt and zinc (Table 4), which occur in very low concentrations, especially in the open oceans. These cases are clear-cut examples of co-limitation because one compound or element is required for acquisition of the other.

Table 4. Some cases of co-limitation of microbial growth by at least two bottom-up factors. Based on (Saito et al. 2008).

Microbe	Primary factor	Secondary factor	Comments
Algae	Light	Nitrate	Nitrate use requires energy.
All microbes	Nitrate	Iron	Nitrate use requires iron-containing nitrate reductase.
All microbes	Phosphate	Zinc	Alkaline phosphatase requires zinc.
All microbes	Nitrogen (urea)	Nickel	Urease requires nickel.
Diazotroph	Nitrogen	Iron	Nitrogenase requires iron.
Bacteria	Organic carbon	Temperature	
Soil microbes	Organic carbon	Water	

Two important examples of co-limitation involving temperature should be mentioned. Growth of microbes in polar environments may be co-limited by organic carbon and temperature. One physiological link between the two factors is that low temperature causes stiffer membranes and impedes transport of dissolved compounds. According to this hypothesis, higher DOM concentrations are needed for a heterotrophic microbe to grow in cold water at the same rate as in warmer waters. In soil microbial ecology, there has been much discussion about whether the sensitivity of organic matter degradation to temperature, as measured by Q_{10}, varies with organic material quality (Fang et al. 2005; Knorr et al. 2005). The other example of co-limitation involving temperature is the interaction between it and water content in controlling microbial activity in soils. We know that addition of water can increase bacterial growth rates in soils (Iovieno and Baath 2008), while a glucose addition may not, implying water limitation of growth. There has been more work examining how respiration and decomposition in soils may be affected by both water and temperature (Howard and Howard 1993). Rising temperatures alone would stimulate decomposition and presumably microbial growth in soils, but it also leads to more evaporation and less moisture, which potentially limits microbial activity. The

confounding effects of moisture complicate efforts to estimate Q_{10} for soils and to its use in global models to predict the response of terrestrial ecosystems to global warming (Davidson and Janssens 2006).

Competition and chemical communication between organisms

So far, we have discussed the abiotic factors controlling microbial growth without reference to the abundance of these microbes or of other organisms. In some cases, these factors are referred to as being density-independent, because their effect does not vary with microbial abundance. Temperature is a good example. Predation, on the other hand, is a density-dependent factor because it does vary with population and prey abundance (Chapter 7). Many abiotic factors are density-independent, but not all. Physical space or room, for example, may limit microbial growth in a soil microenvironment or in a biofilm. Soil moisture is a product of both density-independent factors, such as the frequency and intensity of rain events, and density-dependent factors, such as the role of microbes and their biopolymers in retaining water within the soil matrix.

Competition is another important density-dependent factor. We have already discussed competition between small and large microbes, e.g. heterotrophic bacteria competing with eukaryotic phytoplankton for inorganic nutrients. In Chapter 4, competition was examined with the Michaelis-Menten equation describing transport of dissolved nutrients and other compounds, but it can also be viewed in terms of the Monod equation (Fig. 13). Depending on the substrate concentration, a microbe with a low Ks and high μ_{max} will win over another microbe with high Ks and low μ_{max}. We have also seen that the abundance and growth of bacteria and fungi vary with some physical factors, such as water content and temperature (Table 5), but these

differences may not be the result of true competition. Bacteria and fungi may each respond differently to a particular factor independent of the other microbial group, giving the appearance of competition without any actual interactions between the two. However, experiments have been done to directly test competition between bacteria and fungi.

Table 5. Summary of factors affecting bacteria and fungi in soils. The positive effects are indicated by the various number of "+" while "-" and "--" indicated negative and strongly negative impacts.

	Impact on		
Factor	**Bacteria**	**Fungi**	**Reference**
Moisture	+++	++	See text
Temperature	+++	++	(Pietikainen et al. 2005)
Acidity	--	++	(Rousk et al. 2009)
Disturbance	++	+	(Six et al. 2006)
Metals	--	+	(Rajapaksha et al. 2004)
C: N *	-	+	(Six et al. 2006)

These experiments show that bacteria affect fungi in a density-dependent fashion, a strong sign of direct competition between the two microbial groups for the same growth-limiting organic substrates. The experiments consist of following bacterial and fungal growth after adding or removing fungi, or by adding inhibitors of bacterial activity (Rousk et al. 2008). In the latter case, stimulation of fungal growth was inversely correlated with inhibition of bacterial growth by the inhibitors (oxytetracycline, tylosin and bronopol). Bacteria and fungi compete with each other in spite of evidence that the two microbial groups differ in their capacity to degrade various organic compounds (Chapter 5) and to grow on these compounds (Steinbeiss et al. 2009).

In addition to competition, microbes can interact via chemical cues, which affect growth as well as many other aspects of microbial behavior and metabolism. For example, some types of bacteria can negatively affect fungi by excreting organic compounds, one example being the

polyene nystatin. The genus *Streptomyces*, a bacterium found in soils, is famous for producing these anti-fungal compounds as well as those (antibiotics) that work against other bacteria. However, we know little about how these antimicrobial compounds actually work in natural environments, and what happens in the lab or in the human body may not be representative of what happens in nature (Davies 2009). For example, while nystatin is an effective drug against fungal infections, it also signals some bacteria to form biofilms (López et al. 2009). In addition to chemical warfare, microbes release various organic and inorganic compounds to communicate with themselves and each other.

References

Baath, E. 1988. Autoradiographic determination of metabolically-active fungal hyphae in forest soil. Soil Biol. Biochem. 20**:** 123-125.

Baath, E. 1998. Growth rates of bacterial communities in soils at varying pH: A comparison of the thymidine and leucine incorporation techniques. Microbial. Ecol. 36**:** 316-327.

Baath, E. 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biol. Biochem. 33**:** 2011-2018.

Brock, T. D. 1967. Bacterial growth rate in the sea: direct analysis by thymidine autoradiography. Science 155**:** 81-83.

Buesing, N., and M. O. Gessner. 2006. Benthic bacterial and fungal productivity and carbon turnover in a freshwater marsh. Appl. Environ. Microbiol. 72**:** 596-605.

Carman, K. R. 1990. Radioactive labeling of a natural assemblage of marine sedimentary bacteria and microalgae for trophic studies: an autoradiographic study. Microbial. Ecol. 19**:** 279-290.

Carter, M. D., and K. Suberkropp. 2004. Respiration and annual fungal production associated with decomposing leaf litter in two streams. Freshwater Biology 49**:** 1112-1122.

Church, M. J. 2008. Resource control of bacterial dynamics in the sea, p. 335-382. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. John Wiley & Son, Inc.

Church, M. J., D. A. Hutchins, and H. W. Ducklow. 2000. Limitation of bacterial growth by dissolved organic matter and iron in the Southern Ocean. Appl. Environ. Microbiol. 66**:** 455-466.

Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. 43**:** 1-10.

Coleman, D. C. 1994. The microbial loop concept as used in terrestrial soil ecology studies Microbial. Ecol. 28**:** 245-250.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440**:** 165-173.

Davidson, E. A., I. A. Janssens, and Y. Q. Luo. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q(10). Glob Change Biol 12**:** 154-164.

Davies, J. 2009. Everything depends on everything else. Clinical Microbiology and Infection 15**:** 1-4.

Del Giorgio, P. A., and J. M. Gasol. 2008. Physiological structure and single-cell activity in marine bacterioplankton, p. 243-298. *In* D. L. Kirchman [ed.], Microbial Ecology of the Ocean. Wiley.

Demoling, F., D. Figueroa, and E. Bååth. 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biol. Biochem. 39**:** 2485-2495.

Dorrepaal, E., S. Toet, R. S. P. Van Logtestijn, E. Swart, M. J. Van De Weg, T. V. Callaghan, and R. Aerts. 2009. Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. Nature 460**:** 616-619.

Eickhorst, T., and R. Tippkotter. 2008. Improved detection of soil microorganisms using fluorescence in situ hybridization (FISH) and catalyzed reporter deposition (CARD-FISH). Soil Biol. Biochem. 40**:** 1883-1891.

Fang, C. M., P. Smith, J. B. Moncrieff, and J. U. Smith. 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature 433**:** 57-59.

Fouilland, E., and B. Mostajir. 2010. Revisited phytoplanktonic carbon dependency of heterotrophic bacteria in freshwaters, transitional, coastal and oceanic waters. FEMS Microbiol. Ecol. 73**:** 419-429.

Fuhrman, J. A., and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. 39**:** 1085-1095.

Giardina, C. P., and M. G. Ryan. 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature 404**:** 858-861.

Gulis, V., K. Suberkropp, and A. D. Rosemond. 2008. Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams. Appl. Environ. Microbiol. 74**:** 1094-1101.

Howard, D. M., and P. J. A. Howard. 1993. Relationships between CO2 evolution, moisture-content and temperature for a range of soil types Soil Biol. Biochem. 25**:** 1537-1546.

Iovieno, P., and E. Baath. 2008. Effect of drying and rewetting on bacterial growth rates in soil. FEMS Microbiol. Ecol. 65**:** 400-407.

Kirchman, D. L., E. K'nees, and R. E. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49**:** 599-607.

Kirchman, D. L., B. Meon, M. T. Cottrell, D. A. Hutchins, D. Weeks, and K. W. Bruland. 2000. Carbon versus iron limitation of bacterial growth in the California upwelling regime. Limnol. Oceanogr. 45**:** 1681-1688.

Kirchman, D. L., X. a. G. Moran, and H. Ducklow. 2009. Microbial growth in the polar oceans-role of temperature and potential impact of climate change. Nat. Rev. Microb. 7**:** 451-459.

Klein, D. A., and M. W. Paschke. 2004. Filamentous fungi: The indeterminate lifestyle and microbial ecology. Microbial. Ecol. 47**:** 224-235.

Klein, D. A., M. W. Paschke, and T. L. Heskett. 2006. Comparative fungal responses in managed plant communities infested by spotted (*Centaurea maculosa* Lam.) and diffuse (*C. diffusa* Lam.) knapweed. Applied Soil Ecology 32**:** 89-97.

Knorr, W., I. C. Prentice, J. I. House, and E. A. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433**:** 298-301.

López, D., M. A. Fischbach, F. Chu, R. Losick, and R. Kolter. 2009. Structurally diverse natural products that cause potassium leakage trigger multicellularity in Bacillus subtilis. Proc. Natl. Acad. Sci. USA 106**:** 280-285.

Mahecha, M. D., M. Reichstein, N. Carvalhais, G. Lasslop, H. Lange, S. I. Seneviratne, R. Vargas, C. Ammann, M. A. Arain et al. 2010. Global convergence in the temperature sensitivity of respiration at ecosystem level. Science 329**:** 838-840.

Newell, S. Y., and R. D. Fallon. 1991. Toward a method for measuring instantaneous fungal growth in field samples. Ecology 72**:** 1547-1559.

Norton, J. M., and M. K. Firestone. 1991. Metabolic status of bacteria and fungi in the rhizosphere of Ponderosa pine seedlings. Appl. Environ. Microbiol. 57**:** 1161-1167.

Panikov, N. S., P. W. Flanagan, W. C. Oechel, M. A. Mastepanov, and T. R. Christensen. 2006. Microbial activity in soils frozen to below -39 °C. Soil Biol. Biochem. 38**:** 785-794.

Pascoal, C., and F. Cassio. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl. Environ. Microbiol. 70**:** 5266-5273.

Pietikainen, J., M. Pettersson, and E. Baath. 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiol. Ecol. 52**:** 49-58.

Rajapaksha, R., M. A. Tobor-Kaplon, and E. Baath. 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Appl. Environ. Microbiol. 70**:** 2966-2973.

Rogers, S. W., T. B. Moorman, and S. K. Ong. 2007. Fluorescent in situ hybridization and micro-autoradiography applied to ecophysiology in soil. Soil Sci. Soc. Amer. J. 71**:** 620-631.

Rousk, J., and E. Baath. 2007a. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiol. Ecol. 62**:** 258-267.

Rousk, J., and E. Baath. 2007b. Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. Soil Biol. Biochem. 39**:** 2173-2177.

Rousk, J., P. C. Brookes, and E. Baath. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl. Environ. Microbiol. 75**:** 1589-1596.

Rousk, J., L. A. Demoling, A. Bahr, and E. Baath. 2008. Examining the fungal and bacterial niche overlap using selective inhibitors in soil. FEMS Microbiol. Ecol. 63**:** 350-358.

Rousk, J., and N. M. Nadkarni. 2009. Growth measurements of saprotrophic fungi and bacteria reveal differences between canopy and forest floor soils. Soil Biol. Biochem. 41**:** 862-865.

Saito, M. A., T. J. Goepfert, and J. T. Ritt. 2008. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. Limnol. Oceanogr. 53**:** 276-290.

Sampson, D. A., I. A. Janssens, J. C. Yuste, and R. Ceulemans. 2007. Basal rates of soil respiration are correlated with photosynthesis in a mixed temperate forest. Glob Change Biol 13**:** 2008-2017.

Schadt, C. W., A. P. Martin, D. A. Lipson, and S. K. Schmidt. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301**:** 1359-1361.

Six, J., S. D. Frey, R. K. Thiet, and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Amer. J. 70**:** 555-569.

Staroscik, A. M., and D. C. Smith. 2004. Seasonal patterns in bacterioplankton abundance and production in Narragansett Bay, Rhode Island, USA. Aquat. Microb. Ecol. 35**:** 275-282.

Steinbeiss, S., G. Gleixner, and M. Antonietti. 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41**:** 1301-1310.