8.08
Anaerobic Metabolism: Linkages to Trace Gases and Aerobic Processes

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8.08.1 OVERVIEW OF LIFE IN THE ABSENCE OF O_2

8.08.1.1 Introduction

Life evolved and flourished in the absence of molecular oxygen (O_2). As the O_2 content of the atmosphere rose to the present level of 21% beginning about two billion years ago, anaerobic metabolism was gradually supplanted by aerobic metabolism. Anaerobic environments have persisted on Earth despite the transformation to an oxidized state because of the combined influence of water and organic matter. Molecular oxygen diffuses about 10^4 times more slowly through water than air, and organic matter supports a large biotic O_2 demand that consumes the supply faster than it is replaced by diffusion. Such conditions exist in wetlands, rivers, estuaries, coastal marine sediments, aquifers, anoxic water columns, sewage digesters, landfills, the intestinal tracts of animals, and the rumen of herbivores. Anaerobic microsites are also embedded in oxic environments such as upland soils and marine water columns. Appreciable rates of aerobic respiration are restricted to areas that are in direct contact with air or those inhabited by organisms that produce O_2.

Rising atmospheric O_2 reduced the global area of anaerobic habitat, but enhanced the overall rate of anaerobic metabolism (at least on an area basis) by increasing the supply of electron donors and acceptors. Organic carbon production increased dramatically, as did oxidized forms of nitrogen, manganese, iron, sulfur, and many other elements. In contemporary anaerobic ecosystems, nearly all of the reducing power is derived from photosynthesis, and most of it eventually returns to O_2, the most electronegative electron acceptor that is abundant. This photosynthetically driven redox gradient has been thoroughly exploited by aerobic and anaerobic microorganisms for metabolism. The same is true of hydrothermal vents (Tunnicliffe, 1992) and some deep subsurface environments (Chapelle *et al.*, 2002), where thermal energy is the ultimate source of the reducing power.

Although anaerobic habitats are currently a small fraction of Earth's surface area, they have a profound influence on the biogeochemistry of the planet. This is evident from the observation that the O_2 and CH_4 content of Earth's atmosphere are in extreme disequilibrium (Sagan *et al.*, 1993). The combination of high aerobic primary production and anoxic sediments provided the large deposits of fossil fuels that have become vital and contentious sources of energy for modern industrialized societies. Anaerobic metabolism is responsible for the abundance of N_2 in the atmosphere; otherwise N_2-fixing bacteria would have consumed most of the N_2 pool long ago (Schlesinger, 1997). Anaerobic microorganisms are common symbionts of termites, cattle, and many other animals, where they aid digestion. Nutrient and pollutant chemistry are strongly modified by the reduced conditions that prevail in wetland and aquatic ecosystems.

This review of anaerobic metabolism emphasizes aerobic oxidation, because the two processes cannot be separated in a complete treatment of the topic. It is process oriented and highlights the fascinating microorganisms that mediate anaerobic biogeochemistry. We begin this review with a brief discussion of CO_2 assimilation by autotrophs, the source of most of the reducing power on Earth, and then consider the biological processes that harness this potential energy. Energy liberation begins with the decomposition of organic macromolecules to relatively simple compounds, which are simplified further by fermentation. Methanogenesis is considered next because CH_4 is a product of acetate fermentation, and thus completes the catabolism of organic matter, particularly in the absence of inorganic electron acceptors. Finally, the organisms that use nitrogen, manganese, iron, and sulfur for terminal electron acceptors are considered in order of decreasing free-energy yield of the reactions.

8.08.1.2 Overview of Anaerobic Metabolism

Microorganisms derive energy by transferring electrons from an external electron source or *donor* to an external electron sink or *terminal electron acceptor*. Organic electron donors vary from monomers that support fermentation to simple compounds such as acetate and CH_4. The common inorganic electron donors are molecular hydrogen (H_2), ammonium (NH_4^+), manganous manganese (Mn(II)), ferrous iron (Fe(II)), and hydrogen sulfide (H_2S). Energy is harnessed by shuttling electrons through transport chains within a cell until a final transfer is made to a terminal electron acceptor. The common terminal electron acceptors are nitrate (NO_3^-), manganic manganese Mn(IV), ferric iron (Fe(III)), sulfate (SO_4^{2-}), and carbon dioxide (CO_2) (Table 1).

Anaerobic organisms often have the capacity to reduce two or more terminal electron acceptors. In many cases, these alternative reactions do not support growth, as with the fermenting bacteria that reduce Fe(III) (Lovley, 2000b). In other cases, the ability to use multiple electron acceptors is presumably an adaptation for remaining active in an environment where the supply of specific electron acceptors is variable. For example, denitrification permits normally aerobic bacteria to respire in the absence of O_2, albeit at a slower rate.

Table 1 Thermodynamic sequence for reduction of inorganic substances by organic matter[a].

Reaction	*Eh* (V)	ΔG^b
Reduction of O_2		
$O_2 + 4H^+ + 4e^- \rightleftarrows 2H_2O$	0.812	−29.9
Reduction of NO_3^-		
$NO_3^- + 6H^+ + 6e^- \rightleftarrows N_2 + 3H_2O$	0.747	−28.4
Reduction of Mn^{4+} to Mn^{2+}		
$MnO_2 + 4H^+ + 2e^- \rightleftarrows Mn^{2+} + 2H_2O$	0.526	−23.3
Reduction of Fe^{3+} to Fe^{2+}		
$Fe(OH)_3 + 3H^+ + e^- \rightleftarrows Fe^{2+} + 3H_2O$	−0.047	−10.1
Reduction of SO_4^{2-} to H_2S		
$SO_4^{2-} + 10H^+ + 8e^- \rightleftarrows H_2S + 4H_2O$	−0.221	−5.9
Reduction of CO_2 to CH_4		
$CO_2 + 8H^+ + 8e^- \rightleftarrows CH_4 + 2H_2O$	−0.244	−5.6

Source: Schlesinger (1997).
[a] Units are kcal mol^{-1} e^{-1} assuming coupling to the oxidation reaction $\frac{1}{4}CH_2O + \frac{1}{4}H_2O \rightarrow \frac{1}{4}CO_2 + H^+ + e^-$. [b] $\Delta G = -RT \ln(K)$; pH 7.0, 25 °C.

The most fundamental difference between aerobic and anaerobic metabolism is energy yield. Oxidation of glucose yields ~2,900 kJ mol^{-1} under aerobic conditions (Equation (1)) compared to ~400 kJ mol^{-1} under typical methanogenic conditions (Equation (2)):

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \quad (2)$$

The high-energy yield from aerobic metabolism permits a single organism to completely oxidize complex organic compounds to CO_2. In comparison, no single anaerobic microorganism can completely degrade organic polymers to CO_2 and H_2O (Fenchel and Finlay, 1995), and most have highly specialized substrate demands. Under anaerobic conditions, the mineralization of organic carbon to CO_2 is a multistep process that involves a consortium of organisms, each of which conserves a fraction of the potential-free energy that was available in the original organic carbon substrate. As a result, anaerobic organisms are adapted to conserve quantities of energy that often approach the theoretical minimum (20 kJ mol^{-1}) required for metabolism (Valentine, 2001).

The first step in anaerobic decomposition is the breakdown of complex organic molecules to simple molecules such as sugars (Section 8.08.3.1). Next, these are fermented to even simpler molecules such as acetate and H_2 (Section 8.08.3.2). In the final step, fermentation products serve as electron donors for the reduction of inorganic compounds. The low-energy yield of fermentation, SO_4^{2-} reduction, methanogenesis and most other anaerobic pathways make them particularly sensitive to the concentrations of substrates and products. Because fermentation is inhibited by its end products, the process is dependent on end product consumption by nonfermentative organisms. This thermodynamic dependence among anaerobic microorganisms, in which several species must function together in order to consume a single substrate, is known as *syntrophy*, which is a type of mutualism. Mutualism is a particularly important ecological interaction that determines the structure and function of anaerobic communities.

Competition is another important ecological mechanism that affects the structure and function of anaerobic microbial communities. Competition for fermentation products, such as H_2 and acetate, is particularly keen, because they usually limit microbial activity in anaerobic ecosystems. The outcome of competition for these substrates favors those pathways with the highest thermodynamic yield: NO_3^- reduction > Mn(IV) reduction > Fe(III) reduction > SO_4^{2-} reduction > HCO_3^- reduction (i.e., methanogenesis) (Table 1). This hierarchy also tends to apply to the expression of metabolic pathways within a single microorganism that can reduce multiple electron acceptors (Nealson and Myers, 1992). The presence of O_2 suppresses all the anaerobic metabolic pathways primarily because it is a toxin, but also because it has the highest energy yield of all the common terminal electron acceptors. To a first approximation, a single metabolic pathway dominates anaerobic carbon cycling until it is limited by the availability of electron acceptors. However, it is not uncommon for the pathways to coexist because of spatial variation in the abundance of terminal electron acceptors. Coexistence may also occur because the supply of a *competitive* electron donor (e.g., H_2) is large and nonlimiting, or because there is a supply of a *noncompetitive* electron donor that can be used by some organisms, but not others (e.g., Oremland *et al.*, 1982). The outcome of competition for electron donors establishes the redox potential (Yao and Conrad, 1999), and not vice versa as is often suggested.

The thermodynamic yield of the various anaerobic pathways is related to their affinity for substrates. Sulfate-reducing bacteria outcompete methanogens by maintaining H_2 concentrations below the threshold for the process to be thermodynamically feasible (Lovley *et al.*, 1982), and Fe(III) reducers do the same to SO_4^{2-} reducers. In fact, the equilibrium concentration of H_2 can be used to predict the dominant metabolic pathways in a given environment (Lovley and Goodwin, 1988; Lovley *et al.*, 1994a).

8.08.1.3 Anaerobic–Aerobic Interface Habitats

Spatial variation in the abundance of electron donors and acceptors explains large-scale and small-scale patterns of anaerobic metabolism. Sulfate reduction dominates anaerobic carbon metabolism on about two-thirds of the planet because of the high abundance of SO_4^{2-} in seawater (Capone and Kiene, 1988). Fe(III) reduction is important in all anaerobic ecosystems with mineral-dominated soils or sediments, regardless of whether they are marine or freshwater (Thamdrup, 2000). Methanogenesis is important in freshwater environments generally, and it dominates the anaerobic carbon metabolism of bogs, fens, and other wetlands that exist on organic (i.e., peat) soils.

The contribution of the various anaerobic metabolic pathways to carbon metabolism varies temporally and spatially due to changes in the abundance of electron donors and acceptors (Figure 1). Organic carbon is most abundant at the surface of soils and sediments where detritus is deposited, most of which is derived from aerobic photosynthesis. The aerobic zone is also the source of most terminal electron acceptors, some of which diffuse into the anaerobic zone from the atmosphere or water column (e.g., O_2 and SO_4^{2-} in marine ecosystems); others are regenerated at the aerobic–anaerobic interface due to oxidation of NH_4^+, Fe(II), Mn(II), or H_2S. Regeneration at the aerobic–anaerobic interface can supply a large fraction of the terminal electron acceptors consumed in anaerobic metabolism.

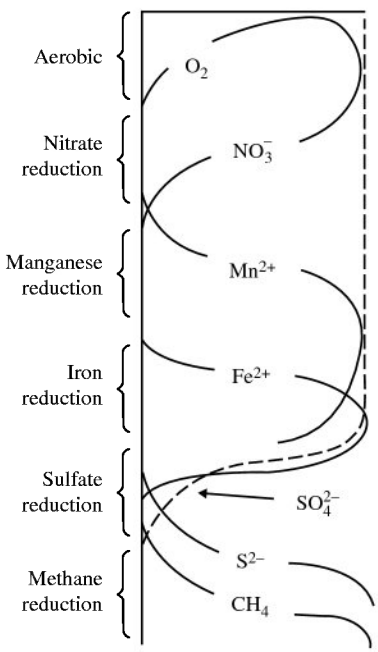

Figure 1 Vertical biogeochemical zones in sediments. The top is the sediment–water interface. Processes on the left represent the use of various electron acceptors (respirations) during the degradation of organic matter. Plots on the right represent the chemical profiles most widely used to delineate the vertical extent of each zone. Rotating the figure 90° to the left shows the sequence of electron acceptors used over time (x-axis) if a sample of oxic sediment were enclosed and allowed to become anaerobic over time.

Oxidant regeneration is stimulated dramatically by the presence of animals and plants. The burrowing activity of animals (i.e., *bioturbation*) is a particularly important agent of Fe(III) and Mn(IV) regeneration in marine sediments and salt marshes (Thamdrup, 2000; Kostka *et al.*, 2000c; Gribsholt *et al.*, 2003; Gribsholt and Kristensen, 2002). Wetland plants promote regeneration by serving as conduits of O_2 infusion deep into the soil profile. Such plants tolerate flooding partly by supplying O_2 to their root system, where some of it leaks into the soil due to *radial* O_2 *loss*. In the absence of physical mixing, burrowing, or radial O_2 loss, the depth of O_2 penetration into saturated soils and sediments is a few millimeters. The influence of plants and animals on anaerobic metabolism is similar in the sense that both effectively increase the aerobic–anaerobic surface area (Mayer *et al.*, 1995; Armstrong, 1964). In addition, plants are a source of labile organic carbon compounds that fuel anaerobic metabolism.

Competition for fermentation products produces a succession of dominant metabolic pathways as distance from the source of electron donors and acceptors increases. Aerobic metabolism dominates the surface of sediments, and methanogenesis the deeper depths, as suggested by their free energy yield (Table 1). There is often very little overlap between each zone, suggesting nearly complete exclusion of one group by another. The same pattern is observed with distance from the surface of a root or burrow, or with distance downstream from an organic pollutant source in rivers and aquifers.

8.08.1.4 Syntax of Metabolism

In ecological and biogeochemical studies, any organism can be described by three basic attributes that define a "feeding" (Greek *trophos*) niche: energy source, electron donor source, and carbon source (Table 2). Organisms that use light energy are *phototrophic*, while those that use chemical energy are *chemotrophic*. Organisms that use inorganic electron donors are *lithotrophic*, while those that use organic electron donors are *organotrophic*. Finally, *autotrophic* organisms assimilate CO_2 for biosynthesis, while *heterotrophic* organisms assimilate organic carbon (e.g., Lwoff *et al.*, 1946; Barton *et al.*, 1991). The modifiers are linked in the order energy–electrons–carbon. For example, plant photosynthesis is technically *photolithoautotrophy*. It is not surprising that

Table 2 Classification terms for microbial metabolism. Each term is composed of modifiers for the source of energy (chemical versus light), the source of electrons (inorganic versus organic), and the source of carbon (inorganic versus organic). The modifiers are linked in the order energy–electrons–carbon. Metabolisms for which organic carbon is both the carbon and energy source are abbreviated as "heterotrophy."

Energy and carbon source	*Electron source*	
	*Inorganic (**litho**trophy)*	*Organic (**organo**trophy)*
I. Chemical energy (***chemo***trophy)		
Carbon source organic (***hetero***trophy)	Chemolithoheterotrophy	Chemoheterotrophy
Carbon source inorganic (***auto***trophy)	Chemolithoautotrophy	Chemoorganoautotrophy
II. Light energy (***photo***trophy)		
Carbon source organic (***hetero***trophy)	Photolithoheterotrophy	Photoheterotrophy
Carbon source inorganic (***auto***trophy)	Photolithoautotrophy	Photoorganoautotrophy

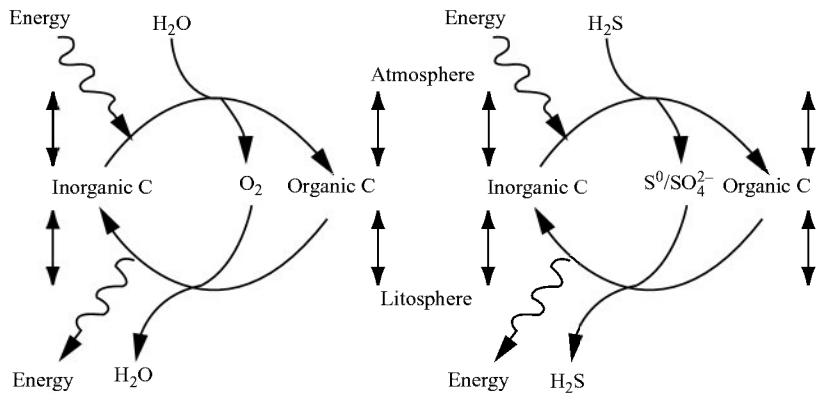

Figure 2 A diagram of the biological carbon cycle. The conversion from inorganic to organic carbon requires light or chemical energy and an electron donor (e.g., H_2O, H_2S, Fe(II)), and is the process of autotrophy. The reverse reaction, in which organic carbon is oxidized to CO_2, releases energy while reducing an electron acceptor (e.g., O_2, SO_4^{2-}/S^0, Fe(III)). This part of the cycle is referred to as heterotrophy.

some organisms do not fit neatly into this classification scheme. *Mixotrophic* organisms alternate between two or more distinct types of metabolism, such as autotrophy and heterotrophy.

In practice, microbiologists use abbreviated terms because certain combinations of physiological traits commonly occur together (Syliva *et al.*, 1998). Because most organisms that metabolize organic compounds can extract electrons, energy, and carbon from them; they are referred to simply as *heterotrophs*. Chemolithoautotrophs are often abbreviated as *chemolithotrophs*, *lithotrophs*, or *autotrophs* because lithotrophic organisms are usually also autotrophic

Microorganisms are also described by more specific substrate requirements or environmental preferences. For example, organisms that require H_2 are *hydrogenotrophic* (i.e., H_2-feeding) and those that thrive in very cold environments are *psychrophilic* (i.e., "cold-loving"). The most important aerobic organisms in oxic–anoxic interfaces are *microaerophiles*, which thrive at very low O_2 concentrations.

8.08.2 AUTOTROPHIC METABOLISM

From a microbial point of view, the carbon cycle is merely an energy cycle (Figure 2). Reduction of CO_2 through a variety of biochemical pathways produces organic carbon, thereby changing the oxidation state of carbon from +IV to between +III and −IV. The main source of energy that drives this process on Earth is quantum energy or light, but there are ecosystems on Earth that are entirely dependent on chemolithoautotrophy. Both forms of autotrophic metabolism are possible under anaerobic conditions. Here, we briefly consider the energy sources that are the ultimate source of reducing power for anaerobic respiration.

8.08.2.1 Phototroph (Photolithoautotrophy) Diversity and Metabolism

Perhaps the most familiar photosynthetic prokaryotes are the cyanobacteria (formerly blue-green algae). These organisms use the Calvin cycle

to assimilate CO_2, releasing O_2 as a waste product in the process (i.e., oxygenic photolithoautotrophy; Table 3). Cyanobacteria include single-cell and filamentous pelagic and benthic species, and they are found across a range of environmental conditions from frigid Antarctic dry valleys to mildly hot springs. The upper temperature limit of photosynthesis is 70–74 °C. The physiological versatility of this group is evident in the capacity of some members to fix N_2, to derive energy from fermentation and to use HS^- as an electron donor (through PS I) for photosynthesis. Cyanobacteria are dominant species in microbial mats and stromatolites and are believed to be responsible for the development of an oxidized (O_2-containing) atmosphere 2.2–2.0 Gyr ago (Des Marais, 2000). In addition, N_2-fixing cyanobacteria are able to produce copious amounts of H_2 during the dark period, which may have had important implications for the development of an O_2-rich atmosphere (Hoehler *et al.*, 2001).

Many phototrophs do not produce O_2 as a waste product. Such *anoxygenic phototrophs* are comprised of purple sulfur, purple nonsulfur, green sulfur, and green nonsulfur bacteria. Although purple sulfur bacteria are typically found in anoxic zones of lakes and sediments, many are capable of photosynthesis under oxic conditions (Van Gemerden, 1993). Most fix N_2 and store S^0 intra- or extracellularly, and some are capable of chemolithoautotrophic growth. Extreme halophilic, sulfidic, and mildly thermophilic environments harbor purple sulfur bacteria. In contrast, purple nonsulfur bacteria typically thrive in less sulfidic, organic rich habitats and are metabolically more diverse. For example, some are photoheterotrophs, which use light as an energy source, but also require organic precursors to synthesize a portion of their organic compounds. Purple bacteria use the Calvin cycle for autotrophic CO_2 fixation (Table 3). In contrast, the green sulfur bacteria are strict anaerobes that store S^0 only outside the cell and predominantly use the reversed citric acid cycle for carbon fixation. Some use ferrous iron as their photosynthetic electron donor (Heising *et al.*, 1999; Widdel *et al.*, 1993). Green nonsulfur bacteria typically grow photoheterotrophically, but also use H_2 or H_2S during autotrophic growth. The upper temperature of anoxygenic photosynthesis is 70–73 °C.

During photosynthesis, the energy from light quanta is converted into chemical energy that can be used to drive biochemical reactions (Warburg and Negelein, 1923). Photosynthetic organisms use a variety of light-harvesting systems, which collectively cover most of the visible spectrum (Stolz, 1991; Samsonoff and MacColl, 2001). Eukaryotic phototrophic organisms contain either Chlorophyll a (Chl*a*) or Chl*b*, which have their maximum absorption at 680 nm and 660 nm, respectively. Among the prokaryotes, aerobic organisms such as cyanobacteria and prochlorophytes typically contain Chl*a*, while anoxygenic phototrophs have a wide variety of chlorophyll

Table 3 Autotrophic reduction–oxidation reactions coupled to CO_2 assimilation. The most common carbon assimilation pathways for each type of metabolism are listed, with alternative modes of CO_2 fixation provided in parentheses. The free energy yield of the reaction is provided for pathways that do not require light.

Metabolism	*Reaction*[a]	$\Delta G^{0\prime}$ (kJ)	*C assimilation pathway*[b]
H_2 oxidation	$H_2 + 0.5O_2 \rightarrow H_2O$	−237	Calvin (RTCA)
CO oxidation	$CO + 0.5O_2 \rightarrow CO_2$	−257	Calvin
S oxidation	$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$	−798	Calvin (AcetylCoA)
S oxidation	$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$	−3,723	Calvin
NH_4^+ oxidation	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H_2O$	−287	Calvin (PEP)
NO_2^- oxidation	$NO_2^- + 0.5O_2 \rightarrow NO_3^-$	−74	Calvin
Fe(II) oxidation	$Fe^{2+} + H^+ + 0.25O_2 \rightarrow Fe^{3+} + 0.5H_2O$	−33	Calvin
Mn(II) oxidation	$Mn^{2+}0.5O_2 + H_2O \rightarrow MnO_2 + 2H^+$	−68	Calvin
Anaerobic phototrophic Fe(II) oxidation (and denitrification)	$4FeCO_3 + 10H_2O + h\nu \rightarrow 4Fe(OH)_3 + (CH_2O) + 3HCO_3^-$		Calvin
Acetogenesis	$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	−105	AcetylCoA
Hydrogenotrophic methanogenesis	$4H_2 + HCO_3^- \rightarrow CH_4 + 3H_2O$	−136	AcetylCoA
Oxygenic phototrophy	$CO_2 + H_2O + h\nu \rightarrow (CH_2O) + O_2$		Calvin
Anoxygenic phototrophy	$2CO_2 + H_2S + 2H_2O + h\nu \rightarrow 2(CH_2O) + SO_4^{2-} + H^+$		Calvin (RTCA, HPC)

[a] $h\nu$ = photon energy that is required to drive the reaction (hence no ΔG reported). [b] Calvin = Calvin cycle, RTCA = reductive or reversed tri-carboxylic acid cycle, PEP = Phosphoenolpyruvate carboxylation, and AcetylCoA = acetyl-CoA pathway, HPC = hydroxy-priopionate cycle.

pigments. The purple bacteria contain either bacteriochlorophyll a Bchl*a* (805 nm and 830–890 nm) or BChl*b* (835–850; 1,020–1,040 nm); green bacteria contain BChl*c* (745–755 nm), BChl*d* (705–740 nm) or BChl*e* (719–726 nm); and heliobacteria contain BChl*g* (670 nm and 788 nm). BChl*a* is also found in some aerobic, heterotrophic phototrophs (e.g., erythrobacters; Yurkov and Van Gemerden, 1993) and anaerobic, heterotrophic phototrophs (e.g., some purple nonsulfur bacteria). A variety of antenna pigments, such as carotenoids and phycobiliproteins, cover the remaining windows in the visible spectrum.

Oxygenic photosynthesis requires two photosystems with different standard potentials and reaction center chlorophylls (Figure 3): PS II ($E^0 = +1.0$ V; P680), which uses H_2O as electron donor, and PS I ($E^0 = +0.3$ V; P700). In contrast, anoxygenic photosynthesis utilizes only a single photosystem: P870 ($E^0 = +0.5$ V) in purple bacteria (Figure 3), P840 ($E^0 = +0.3$ V) in green bacteria and P798 ($E^0 = +0.2$ V) in heliobacteria. Electron donors for anoxygenic phototrophs include a range of reduced S-compounds, H_2 and Fe(II). Oxygenic photosynthesis involves noncyclic electron transfer, light-driven energy generation and light-driven reducing power, whereas in anoxygenic photosynthesis, electron transfer is cyclic and only energy generation is driven by light. In hypersaline environments, bacteriorhodopsin-based phototrophy is found in some halobacteria (Hartman *et al.*, 1980) that can grow in brine (32%, or 5.5 M NaCl). Phototrophic growth in these red-pigmented (bacterioruberin, an antenna pigment) microbes does not involve chlorophyll and takes place under microaerophilic conditions. The phototrophic growth potential is extremely limited. Maximum bacteriorhodopsin absorption is at 570 nm.

8.08.2.2 Chemotroph (Chemolithoautotrophy) Diversity and Metabolism

Inorganic redox reactions provide an alternative to using light as a source of energy and reducing equivalents to assimilate CO_2 (Table 3). Electron donors for chemolithotrophy include H_2 (Bowien and Schlegel, 1981), carbon monoxide (CO_2) (Shiba *et al.*, 1985), H_2S and other reduced sulfur compounds, NH_4^+, and other reduced nitrogen compounds, Fe(II), and Mn(II). It is likely that other reduced elements (e.g., As(IV), Cr(III), Sb(III), Se(-II)/Se(0), U(-IV)) also function as reductants (Battaglia-Brunet *et al.*, 2002;

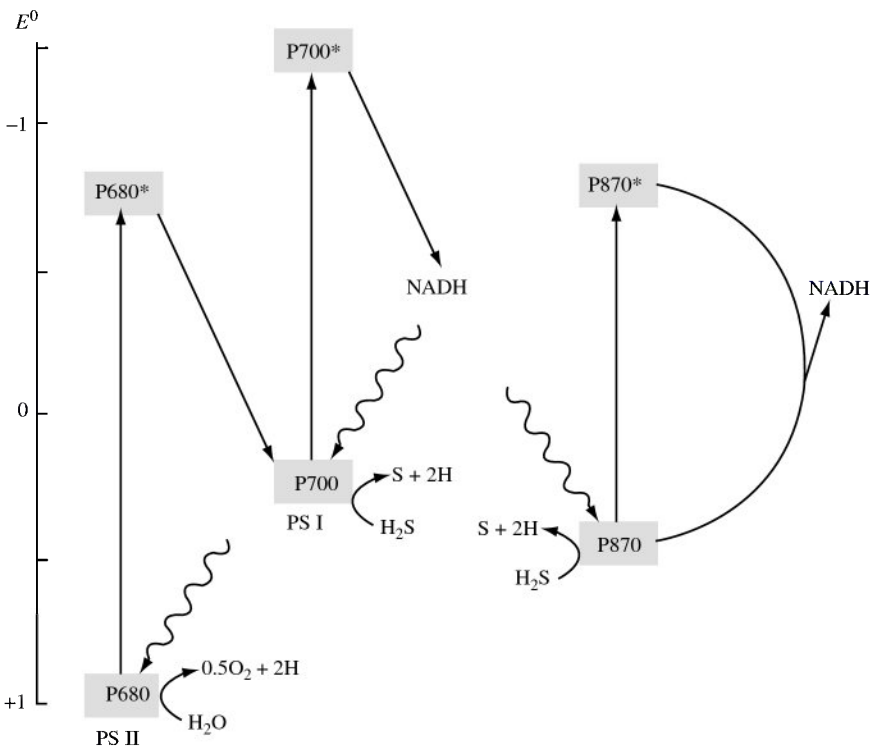

Figure 3 A schematic representation of photosystems and associated standard potentials involved in oxygenic photosynthesis in cyanobacteria (left panel) and anoxygenic photosynthesis (right panel). The flow of electrons in the former is linear, while in the latter it is cyclic.

Dowdle and Oremland, 1998; Ehrlich, 1999, 2002). Several of these pathways are discussed here in the context of the element cycles they influence, including acetogenesis (Section 8.08.3.2.1), hydrogenotrophic methanogenesis (Section 8.08.4.2), NH_4^+ oxidation (Section 8.08.5.3.3), Fe(II) oxidation (Section 8.08.6.5), and sulfur oxidation (Section 8.08.7.9).

8.08.2.3 Pathways of CO_2 Fixation

All microbes, including chemoorganoheterotrophs, possess some ability to engage in reversible carboxylation (i.e., CO_2–C assimilation into an organic compound) and decarboxylation reactions, some of which lead to the incorporation of a significant amount of CO_2 (Wood, 1985). Here, we briefly consider the biochemical pathways that photo- and chemolithotrophic bacteria deploy in order to produce the majority of their biomass. The four major CO_2-fixing pathways are the Calvin cycle, the acetyl-CoA pathway, the reductive tricarboxylic acid (TCA) cycle, and the 3-hydroxypriopionate cycle.

The Calvin cycle or reductive pentose phosphate cycle occurs in all green plants and many microorganisms. Carboxylation is catalyzed by ribulose-bis-phosphate carboxylase–oxygenase (Rubisco). Rubisco also functions as an oxygenase during photorespiration, but its affinity for O_2 is 20–80 times lower than for CO_2. As with most enzymes, Rubisco has a preference for lighter stable isotopes and CO_2 fixation results in the depletion of ^{13}C ($\delta^{13}C$) ranging from −10‰ to −20‰.

The acetyl-CoA or Ljungdahl–Wood pathway is found in anaerobes, including methanogenic bacteria, acetogenic bacteria, and autotrophic sulfate reducers. Two parallel pathways fix CO_2, one of which results in an enzyme-bound carbonyl group, the other one in an enzyme-bound methyl group. Combining the two ultimately yields the acetyl-CoA pathway. The key enzyme in this pathway is carbon monoxide dehydrogenase. In methanogens, the biosynthesis proceeds via the acetyl-CoA pathway as well. Isotope fractionation in the acetyl-CoA pathway yields $\delta^{13}C$ values ranging from −20 to −40‰.

The reductive tricarboxylic acid cycle is basically the reverse of the oxidative tricarboxylic acid cycle that heterotrophs use to generate reducing equivalents (NADH, $FADH_2$) that function as electron donors for energy generation. The 3-hydroxyproprionate cycle was found in the green nonsulfur bacterium *Chloroflexus* (Strauss and Fuchs, 1993) and more recently also in some autotrophic Archea (Mendez *et al.*, 1999). In this pathway, 3-hydroxyproprionate is a key intermediate.

Many other carboxylation reactions exist (Barton *et al.*, 1991). For example, in methylotrophic bacteria, formaldehyde and CO_2 are combined to produce acetyl-CoA in the serine or hydroxypyruvate pathway. In contrast, the ribulose monophosphate cycle, which is another methylotrophic pathway of formaldehyde fixation, does not involve carboxylation steps. In addition to those described above, commonly found carboxylation reactions include those of pyruvate or phosphoenol pyruvate. In view of several relatively recent discoveries of novel CO_2 assimilation pathways (e.g., the hydoxypropionate cycle and anaerobic ammonium oxidation) and growing interest in deep-subsurface microbiology, novel pathways of CO_2 incorporation may be discovered in the near future.

8.08.3 DECOMPOSITION AND FERMENTATION

Most knowledge regarding the anaerobic decomposition of organic materials in depositional environments has been derived from studies of terminal processes in the microbial food web, particularly NO_3^-, Fe(III), Mn(IV), and SO_4^{2-} reduction, and methanogenesis. These processes are far easier to study than those responsible for polymer degradation. Because the degradation products of labile compounds eventually pass through a terminal step, it is generally considered that terminal decomposition exerts a major control on the decomposition pathway by regulating intermediary metabolism and the rate of degradation (Conrad, 1999). However, it is clear that the overall rate of organic matter decomposition is limited by the rate at which polymeric materials are depolymerized. Much information about decomposition can be provided by studies limited solely to intermediary or terminal metabolic events.

Organic matter deposited in sedimentary or wetland habitats is composed of a complex mixture of biopolymers. Some of these compounds, such as proteins, carbohydrates, and lipids are easily degraded by microorganisms (i.e., labile), while other compounds, such as lignin and hemicellulose, are resistant to decomposition (i.e., recalcitrant). Biopolymers are degraded in a multistep process. First, microorganisms simplify polymers to monomers such as amino acids, fatty acids, and monosaccharides (Figure 4). The monomers are further mineralized to CO_2, or to a combination of CO_2 and CH_4.

Under aerobic conditions, the conversion of monomers to fully mineralized products is rather simple because O_2-respiring bacteria can degrade monomers completely to CO_2. However, under anaerobic conditions this process requires

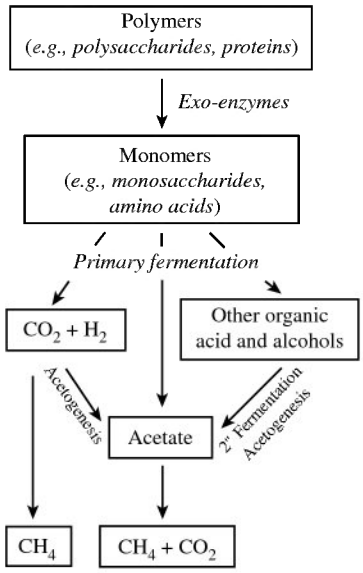

Figure 4 Metabolic scheme for the degradation of complex organic matter, culminating in methanogenesis. Polymers are cleaved via extracellular or cell-surface associated enzymes to monomers that are fermented to organic products, H_2 and CO_2. Methane is formed primarily from the oxidation of H_2 coupled to CO_2 reduction or by the fermentation of acetate. Acetate is formed by primary fermentation, acetogenesis from H_2/CO_2, and from secondary fermentation of primary fermentation products.

a consortium of bacteria that degrade monomers in a series of steps. The first step is primary fermentation to low molecular weight products such as alcohols and volatile fatty acids. Next, primary fermentation products are either mineralized to CO_2 and CH_4, or they undergo secondary fermentation to smaller volatile fatty acids. Finally, the secondary fermentation products are mineralized by respiratory organisms using inorganic terminal electron acceptors, a process that yields CO_2, or CO_2 and CH_4. Secondary fermentation is prevalent under methanogenic conditions.

8.08.3.1 Polymer Degradation

The quality of organic matter degraded in anaerobic environments varies depending on its origin with a gradient of structural complexity occurring from phytoplankton (labile) to vascular plants (recalcitrant) (Wetzel, 1992). Vascular plant detritus is resistant to decomposition because of an abundance of high-molecular-weight structural compounds such as lignocelluloses and complex polysaccharides (Benner *et al.*, 1991), while phytoplankton cells are composed largely of carbohydrates (Benner *et al.*, 1992). Cowie and Hedges (1993) noted that the reactivity of organic matter in sediments was generally amino acids > neutral sugars > total organic carbon > lignin. This pattern was independent of the O_2 content and bioturbation of sediments.

Seasonal variations in decomposition are affected strongly by temperature, but temporal changes in organic matter deposition also affect the degradation rates in sediments. Evidence of this is the observation that carbon mineralization varies strongly across seasons in sediments that are uniformly cold year-around because of seasonal variation in plankton production (Schulz and Conrad, 1995). It is clear that polymer breakdown is often the rate limiting step in organic matter degradation (Glissmann and Conrad, 2002; Reineke, 2001; Wu *et al.*, 2001).

Mineralization of organic macromolecules is initiated by extracellular enzymes because bacteria are unable to hydrolyze substrates that are much larger than about 600 Da (Weiss *et al.*, 1991). Not all bacteria are capable of synthesizing these enzymes, as is often the case with those responsible for terminal decomposition and some intermediary metabolisms. As a result, these terminal organisms depend heavily on the activities of other bacteria for substrates. It is clear that polymer hydrolysis occurs since these compounds are required to support microbial activities in sediments, but some studies have failed to detect polymer hydrolysis potentials sufficient to support *in situ* rates of metabolism (Arnosti, 1998). Such studies underscore the difficulties of examining hydrolytic processes.

The approaches used in studies of polymer hydrolysis include additions of intact plant material to incubating sediments (Battersby and Brown, 1982; Wainwright, 1981), inhibition of carbohydrate consumption with toluene and measurement of carbohydrate increases (Boschker *et al.*, 1995), and the use of labeled materials. The latter approach usually involves estimating the hydrolytic potential of microbial communities by amending sediments with fluorescently labeled precursors that serve as proxy organic macromolecules. A particularly common fluorophore is methylumbelliferyl (MUF) attached to a monomer such as a monosaccharide or an amino acid (Boetius and Lochte, 1994; Boschker and Cappenberg, 1994; Hoppe, 1983; King, 1986; Meyer-Reil, 1986). When these labels are attached to a monomer, they may fail to discern the activities of true extracellular enzymes because they are small enough to enter the bacterial periplasmic space. Thus, labeled compounds may be poor proxies of large-molecular-weight materials (Arnosti, 1998; Martinez and Azam, 1993). In some cases, hydrolytic potential has been assayed using whole polymers that were fluorescently labeled (Arnosti, 1996) or specific polymers have been introduced

into growing cultures of isolated bacteria (Reichardt, 1988).

8.08.3.1.1 Polysaccharides

Studies of polymer use have often focused on the hydrolysis of polysaccharides because carbohydrates make up a large portion of phytoplankton biomass (Parsons *et al.*, 1961) and cellulose is the main polysaccharide in terrestrial ecosystems (Watanabe *et al.*, 1993; Glissman and Conrad, 2002). In general, hydrolytic activity in sediments decreases rapidly with depth in parallel to declining organic matter reactivity and general bacterial metabolic activity (King, 1986). However, Arnosti (1998) found that the potential for hydrolysis of algal-derived polysaccharides, such as pullulan and laminarin, was uniformly rapid throughout the upper ~11 cm of Arctic sediments. Additions of metabolic inhibitors failed to completely arrest hydrolytic activity, indicating that a significant portion of hydrolytic enzymes in sediments are either free in pore waters or attached to particles and active in lieu of bacterial metabolism (Arnosti, 1998; Boschker *et al.*, 1995). Although hydrolytic rate optima have been shown to correlate with environmental factors such as pH or salinity (King, 1986), they often exhibit temperature optima that greatly exceed ambient temperatures (King, 1986; Mayer, 1989; Reichardt, 1988). For example, extracellular enzyme activity in sea-ice bacterial communities exhibited temperature optima near 15 °C and a psychrophilic isolate yielded a protease extract with an optimum activity at 20 °C (Huston *et al.*, 2000).

Relatively slow decomposition in anaerobic environments may partly be due to depressed extracellular enzyme activity caused by low pH, low O_2 concentrations, or other factors (Kang and Freeman, 1999). Freeman *et al.* (2001) determined that depressed extracellular enzyme activity in a peatland soil was due to the high content of phenolic compounds, and concluded that phenols were not degraded because of an O_2 limitation on phenol oxidase activity. This led them to speculate that the vast pool of organic carbon currently sequestered in peatland soils (one-third of all soil carbon) is under the control of a single enzymatic "latch," the activity of which could increase dramatically if drought or drainage were to increase O_2 availability. In other cases, extracellular hydrolase activities may not be affected by environmental factors such as O_2, sulfide, or iron chemistry (King, 1986), and do not necessarily follow substrate concentrations or microbial biomass on an annual basis (Mayer, 1989). Because microbial activity is consistently affected by such factors, these findings reflect the extracellular nature of the hydrolytic enzymes.

8.08.3.1.2 Lignin

Organic matter deposited in near-shore marine and most freshwater habitats is composed primarily (~75% by weight) of lignocellulose, a complex mixture of lignin and the polysaccharides cellulose and hemicellulose (Benner *et al.*, 1985). Lignin is a unique phenolic polymer of nonrepeating units that makes up 25–30% of the biopolymers in vascular plants and is second only to cellulose as the most abundant organic carbon source in the biosphere. It is highly resistant to microbial degradation (Kawakami, 1989) and its association with cellulose and hemicellulose polysaccharides imparts degradation resistance to these polymers as well (Crawford, 1981). Hence, lignin is widely distributed in depositional environments such as soils and peats (Hedges and Oades, 1997; Miyajima *et al.*, 1997; Tsutsuki *et al.*, 1994; Yavitt *et al.*, 1997), riverine sediments (Hedges *et al.*, 1986, 2000; Meyers *et al.*, 1995), and coastal marine sediments (Dittmar and Lara, 2001; Gough *et al.*, 1993; Hedges *et al.*, 1997; Miltner and Emeis, 2001). Lignin's aromatic character makes it the major source of naturally occurring aromatic compounds.

The degradation of lignocelluloses in detritus-based ecosystems like wetlands is crucial to maintain carbon balance since macrophytes are composed of 50–80% lignocellulose (Maccubbin and Hodson, 1980). Early studies suggested that the lignin polymer was essentially inert in the absence of oxygen (Hackett *et al.*, 1977). However, it has since been shown that the complex lignin polymer can undergo anaerobic degradation (Benner *et al.*, 1985, 1986), but the process can be rather slow and tends to yield unmetabolized products (Colberg and Young, 1982; Young and Frazer, 1987). Anaerobic loss of lignin polymers is ~3–30% as complete as aerobic degradation of the same polymers (Benner *et al.*, 1984); the cellulose component is more labile than the lignin component.

Both fungi and bacteria degrade lignocelluloses. Fungi tend to dominate decomposition in upland soils (Orth *et al.*, 1993; Witkamp and Ausmus, 1976), whereas bacteria dominate in most aquatic environments (Benner *et al.*, 1986). The latter authors noted that bacteria were responsible for the degradation of lignin and associated polysaccharides in marine wetlands. Eukaryotes also contributed significantly to lignin degradation in an acidic freshwater wetland, and eukaryotes degraded both lignin and polysaccharides in a slightly alkaline freshwater wetland. The polysaccharide component is usually decomposed several fold more rapidly than lignin, and the lignocellulose of herbaceous plants decomposes more readily than that of woody plants (Benner *et al.*, 1985). The aerobic catabolism of lignin by

fungi and filamentous bacteria utilizes ligninolytic peroxidases (Black and Reddy, 1991; Eriksson *et al.*, 1990), that often require manganese to be active (Brown *et al.*, 1990; Gettemy *et al.*, 1998). Less is known of the enzymes involved in the anaerobic decomposition process.

Under anaerobic conditions, lignin oligomers can be depolymerized to monomers, and lignin monomers can be mineralized to CO_2 (Colberg, 1988; Colberg and Young, 1982; Young and Frazer, 1987). The degradation of the lignin polymers releases aromatic subunits, and many studies have examined the anaerobic pathways by which these monomers are used. In fact, lignin monomers have been used often as lignin model compounds (Pareek *et al.*, 2001; Phelps and Young, 1997). Although it is clear that aromatic rings are readily cleaved aerobically by dioxygenase and peroxidase enzymes, these reactions do not occur anaerobically; aromatic rings are cleaved in the absence of O_2 via a reductive mechanism whereby hydrogenation of the aromatic ring nucleus results in a cyclohexane derivative, the ring of which is then opened (Reineke, 2001; Young and Frazer, 1987). Lignin monomers generally contain hydroxyl, methoxyl, and/or carboxyl groups that are removed from aromatic rings prior to reduction and cleavage of the ring. The O-demethylation (demethyoxylation) of phenylmethylethers has received considerable attention since the methyl product can serve as a C_1 substrate (i.e., not C–C bonds) for bacterial growth (Evans and Fuchs, 1988; Young and Frazer, 1987). Many of the O-demethylating strains of bacteria are acetogenic (Section 8.08.3.2.1) (Frazer, 1995; Kreft and Schink, 1993; Kreft and Schink, 1997; Küsel *et al.*, 2000; Wu *et al.*, 1988), but several other types of anaerobic and facultatively anaerobic bacteria have the ability to demethoxylate aromatic rings, including sulfate reducers, nitrate reducers, fermentative bacteria, and other strains involved in anaerobic syntrophy (Section 8.08.3.2.2) (Cocaign *et al.*, 1991; Krumholz and Bryant, 1985; Liu and Suflita, 1993; Mountfort *et al.*, 1988; Phelps and Young, 1997; Young and Frazer, 1987). Some pure cultures are capable of removing a variety of functional groups from aromatic rings (Küsel *et al.*, 2000). In many instances, the aromatic ring is not cleaved after these groups are removed (Young and Frazer, 1987). During O-demethyoxylation, the released methyl group can be used to methylate sulfide, which leads to the production of the gases methane thiol and dimethylsulfide; the remaining aromatic compound is not further degraded (Bak *et al.*, 1992; Finster *et al.*, 1990, 1992). Bacteria capable of degrading plant-derived aromatic compounds such as ferulic and syringic acids are often capable of degrading xenobiotic aromatics such as chlorinated pollutants.

8.08.3.2 Fermentation

Fermentation is a metabolic process in which organic compounds serve as both electron donors and acceptors. Primary fermentation is the exergonic breakdown of glucose and other monomers to products such as alcohols, fatty acids, H_2, and CO_2. Secondary fermentation further converts these primary products to acetate and other low-molecular weight organic acids (Figure 4). Fermentation differs dramatically from anaerobic respiration because it occurs inside the cell, and energy is generated by organic matter dismutation and substrate-level phosphorylation. By comparison, aerobic and anaerobic respiration requires an external electron acceptor and they proceed by oxidative phosphorylation via electron transport. Fermentation generates very little energy relative to aerobic or anaerobic respiration. However, it is a key component of the anaerobic mineralization process because most nonfermentative organisms cannot use the typical monomers released during polymer hydrolysis. As a result, fermenters can greatly outnumber bacteria dependent on terminal respiration processes in some environments. For example, in SO_4^{2-}-reducing sediments, the SO_4^{2-} reducers typically account for ~5% of the all bacteria present, while most bacteria are involved in polymer hydrolysis and fermentation (Devereux *et al.*, 1996). The situation is different in anaerobic wetland soils, where plant roots release acids and alcohols that can be used directly by SO_4^{2-} reducers. In this case, the SO_4^{2-} reducer populations on roots can account for over 30% of the total microbial community because fermentation is not required (Hines *et al.*, 1999; Rooney-Varga *et al.*, 1997).

In pure culture, fermenting bacteria consume a large variety of organic compounds. The list includes sugars, amino acids, purines, pyrimidines, some aromatics, acetylene, and a broad range of organic acids (C_1–C_{18}). They produce a wide spectrum of fermentation products, but C_1–C_{18} acids and alcohols, H_2, and CO_2 are most prevalent (Schink and Stams, 2002). In methanogenic habitats, all fatty acids longer than two carbons, branched-chain and aromatic fatty acids, and all alcohols longer than one carbon require secondary fermentation prior to use by methanogenic bacteria (Schmitz *et al.*, 2001). Hence, secondary fermentation is essential for complete mineralization to occur during methanogenesis. However, SO_4^{2-}- and metal-reducing bacteria are more metabolically versatile than methanogens and are capable of mineralizing most primary fermentation products directly. Organic mineralization can proceed via

a two-step process in metal oxide and SO_4^{2-}-rich environments like marine sediments where primary fermenters hydrolyze polymers and ferment monomers while metal and SO_4^{2-} reducers utilize the fermentation products (Widdel, 1988). However, if fatty acid oxidizing SO_4^{2-} reducers are absent, then mineralization of fatty acids is possible via secondary fermentation and the use of H_2 by SO_4^{2-}-reducing bacteria (Monetti and Scranton, 1992). The SO_4^{2-}-reducing community does exhibit some other cooperative activities (Section 8.08.7.2.2).

8.08.3.2.1 Acetogenesis

Besides the "classical" fermentation described above, organic monomers can also be degraded to acetate via acetogenesis. This term is somewhat nebulous and has been used to describe any reaction leading to acetate, including fermentation. However, a widely accepted definition holds that acetogens are obligately anaerobic bacteria that use the acetyl-CoA pathway both for the reductive synthesis of acetyl-CoA from CO_2, and as a terminal electron-accepting and energy conserving process (Drake, 1994). If acetate is usually the sole reduced end product, the acetogen is considered to be a homoacetogen. The acetyl-CoA pathway is responsible for the anaerobic dark fixation of CO_2 into organic compounds, and most acetogens are able to use this pathway to reduce CO_2 during the oxidation of H_2 to form acetate autotrophically. In a typical acetate fermentation reaction, glucose is converted to two moles of acetate and two moles of CO_2. However, acetogens can reduce the two CO_2 molecules to acetate yielding a total of three moles of acetate. It is theoretically possible for acetogenic catabolism of glucose to acetate, followed by its degradation to CH_4 and CO_2, to be a major degradative pathway in anaerobic environments because it is highly favorable thermodynamically. However, this joint pathway does not seem to be important *in situ*, and the role of acetogenesis in methanogenic habitats remains unclear (Conrad, 1999).

Acetogenic bacteria are a very diverse group and display a wide range of catabolic capabilities including the utilization of sugars, acids, alcohols, lignin-derived aromatic methoxyl groups, C_1 and C_2 compounds, CO, and methyl halides (Drake, 1994). In addition, acetogens have been isolated that are capable of reverse acetogenesis where acetate is converted to CO_2 and H_2 (Zinder and Koch, 1984). The acetyl-CoA pathway is also present in some SO_4^{2-}-reducing and methanogenic bacteria and is involved in acetate oxidation, disproportionation (i.e., CH_4 production from acetate), autotrophic fixation of CO_2, CO oxidation, and the assimilation of C_1 compounds (Fuchs, 1994).

The autotrophic formation of acetate from H_2/CO_2 can directly compete with autotrophic methanogenesis. Methane formation from H_2/CO_2 is thermodynamically more favorable than acetogenesis, but the latter pathway is dominant in some habitats. Küsel and Drake (1994) noted that flooded soils accumulated acetate for several months without becoming methanogenic. Acetate also accumulates transiently in sediments and wetland peat soils prior to its use by methanogens, suggesting that acetoclastic methanogens grow more slowly than H_2/CO_2 utilizers or are more sensitive to O_2 (Avery *et al.*, 1999; Crill and Martens, 1986; Sansone and Martens, 1982; Shannon and White, 1996). In addition, it appears that acetate accumulates all season in colder high latitude wetlands (Duddleston *et al.*, 2002; Hines *et al.*, 2001), but it is unknown what portion of this acetate is due to fermentation or acetogenesis. In rice paddy soils, up to 40% of the fatty acids can be derived from CO_2 reduction, including virtually all of the acetate formed (Conrad and Klose, 2000). The bacteria physically associated with rice roots exhibited diverse anaerobic metabolisms, including the coexistence of acetogenesis and methanogenesis from CO_2 reduction (Conrad and Klose, 1999; Liesack *et al.*, 2000). This apparent relief of competition may have been due to the abundance of substrates released by roots (Conrad and Klose, 1999; Liesack *et al.*, 2000).

8.08.3.2.2 Syntrophy and interspecies hydrogen transfer

Many anaerobic microorganisms require secondary fermentation products such as acetate for electron donors. However, the ability of bacteria to perform secondary fermentation can be limited if H_2 (a metabolic waste product of the process) accumulates, and causes the reaction to become endergonic. For example, fermentation of organic acids and alcohols such as butyrate, propionate and ethanol does not yield sufficient energy to support growth if the H_2 concentration rises above 10^{-4} atm. Yet, these fermentation reactions are generally exergonic *in situ*, because H_2 is continually removed by H_2-consuming bacteria (Table 4). As a result, H_2 has a short turnover time and usually occurs at very low concentrations (Conrad *et al.*, 1986,1989) even though it is an common intermediate in metabolism (Conrad, 1999). The exchange of H_2 makes these organisms metabolic partners, and allows primary fermentation products to be completely mineralized in anaerobic habitats. Such *syntrophic* relationships (Biebl and Pfennig, 1978) are a symbiotic cooperation between two metabolically different bacteria that depend on each other for the degradation of a substrate, typically for energetic

Table 4 Examples of reactions occurring in methanogenic environments illustrating the effect on energy yield of the consumption of fermentation products. Maintenance of low reactant concentrations allows secondary fermentation reactions that are endergonic under standard conditions to be exergonic (negative ΔG).

Reaction	*Free-energy change* (kJ)	
	$\Delta G^{0,a}$	ΔG^{b}
$Glucose + 4H_2O \rightarrow 2\,acetate^- + 2HCO_3^- + 4H^+ + 4H_2$	−207	−319
$Glucose + 2H_2O \rightarrow butyrate^- + 2HCO_3^- + 3H^+ + 2H_2$	−135	−284
$Butyrate^- + 2H_2O \rightarrow 2\,acetate^- + H^+ + 2H_2$	+48.2	−17.6
$Propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + H^+ + H_2$	+76.2	−5.5
$2\,Ethanol + 2H_2O \rightarrow 2\,acetate^- + 2H^+ + 4H_2$	+19.4	−37
$Benzoate + 6H_2O \rightarrow 3\,acetate^- + 2H^+ + CO_2 + 3H_2$	+47	−18

Source: Zinder (1984).
[a] Standard conditions: solutes, 1 M; gases, 1 atm. [b] Concentrations of reactants typical of anaerobic habitats: fatty acids, 1 mM; glucose, 10 μM; CH_4, 0.6 atm; H_2, 10^{-4} atm; HCO_3^-, 20 mM.

reasons. In many cases, H_2 is the compound transferred to the terminal organism and this process is known as *interspecies H_2 transfer*. Although syntrophy and interspecies H_2 transfer are synonymous in many instances, it should be noted that H_2 transfer is a mechanism of electron transfer and other electron carriers can be involved. Because formate is transferred between organisms similarly to H_2, the term *interspecies H_2 transfer* often refers to both formate and H_2 transfer (Boone *et al.*, 1989; Thiele and Zeikus, 1988). However, in some instances formate transfer is insignificant compared to H_2 transfer and vice versa (Schmidt and Ahring, 1995). In addition, electron transfer can occur via other electron carriers such as acetate (Dong *et al.*, 1994), or amino acids such as cysteine (Cord-Ruwisch *et al.*, 1998).

The classical example of interspecies H_2 transfer was recognized when a presumably pure ethanol-consuming methanogenic culture, *Methanobacillus omelianskii* (Barker, 1940), was found to actually be a co-culture of an ethanol utilizing syntroph (S Strain) and an H_2-consuming methanogen (Strain M.o.H.) (Bryant *et al.*, 1967):

Strain S :

$$2CH_3CH_2OH + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 4H_2$$
$$\Delta G_0{}' = +19\ \text{kJ mol}^{-1}$$

Strain M.o.H. :

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$
$$\Delta G_0{}' = -131\ \text{kJ mol}^{-1}$$

Co-culture :

$$CH_3CH_2OH + CO_2 \rightarrow 2CH_3COO^- + 2H^+ + CH_4$$
$$\Delta G_0{}' = -112\ \text{kJ mol}^{-1}$$

The bacterium responsible for ethanol fermentation will not grow using ethanol without the H_2-scavenging methanogen because the reaction is endergonic at the H_2 concentrations encountered. However, the reaction is exergonic if the H_2 partial pressure is maintained at $<10^{-3}$ atm by the methanogen with a net co-culture.

Primary fermenting bacteria can also profit from the activities of H_2-consuming partners at the end of the degradation chain. The maintenance of low H_2 partial pressures allows primary fermentation to favor the production of H_2 and more oxidized end products like acetate and CO_2. This permits fermentation to be more efficient in general, and leads to additional ATP synthesis (Thauer *et al.*, 1977; Schink and Stams, 2002; Schmitz *et al.*, 2001). The H_2-utilizing population is the primary regulator in the degradation process because its presence dictates which pathways are energetically capable of proceeding (Bryant, 1979; Zeikus, 1977). In an active anaerobic community, the presence of a microbial H_2 sink allows electron and carbon flow to bypass much of the secondary fermentation process, except for the use of long-chained or branched intermediates produced during lipid and amino acid fermentations (Schink, 1997). Therefore, it is conceivable that secondary fermentation could be a rather small portion of anaerobic metabolism if primary fermentation yielded only acetate as the organic product. However, many natural habitats are not as microbially active as those encountered in culture, and secondary fermentation tends to be an important, but rather poorly understood, component of natural anaerobic environments (Conrad, 1999).

Since its discovery around the mid-1960s, a variety of interspecies H_2 transfer reactions have been elucidated. Early studies focused primarily on processes involving methanogenic bacteria because this association was the first described. The small amount of energy available in methanogenic metabolism effectively forces the bacteria into symbiotic relationships that neither partner can function without (Schink, 1997). Although

the original ethanol-utilizing partner (S Strain) of the co-culture comprising "*M. omelianskii*" was lost, other syntrophic ethanol-oxidizing bacteria have been isolated (Ben-Bassat *et al.*, 1981; Schink, 1984). The suite of H_2-releasing compounds used by syntrophs includes alcohols (Bryant *et al.*, 1967; Eichler and Schink, 1986; Schink *et al.*, 1985), fatty acids (McInerney *et al.*, 1981, 1979; Schink, 1985b; Schink and Friedrich, 1994; Zinder and Koch, 1984), aromatic compounds (Dolfing and Tiedje, 1991; Elshahed *et al.*, 2001; Knoll and Winter, 1989; Mountfort *et al.*, 1984), glycolic acid (Friedrich *et al.*, 1991), and amino acids (Nagase and Matsuo, 1982; Nanninga and Gottschal, 1985; Stams and Hansen, 1984; Winter *et al.*, 1987).

Many syntrophic microorganisms can grow in the absence of a H_2-utilizing partner by using slightly more oxidized substrates in a dismutation fermentation reaction (Eichler and Schink, 1986; Elshahed and McInerney, 2001a; Schink, 1997). For example, a bacterium that can only degrade propionate when in co-culture with an H_2-consuming partner can be grown in pure culture on pyruvate (Wallrabenstein *et al.*, 1994), and an ethanol-utilizing syntroph can be grown alone on acetaldehyde analogs like acetylene (Schink, 1985a). The need for physical or chemical removal of H_2 allows for only limited growth of pure culture syntrophs (Mountfort and Kaspar, 1986).

Because the degradation of fatty acids to acetate and H_2 is more endergonic than ethanol oxidation, the H_2 partial pressure must be ~1 atm lower for fatty acids to be degraded under methanogenic conditions than for ethanol degradation (Schink, 1997; Wallrabenstein *et al.*, 1994). The energy yield of fatty acid oxidation is improved when both acetate and H_2 are being removed, thereby "pulling" the reactions to completion. This has been demonstrated in tricultures that degraded either butyrate (Ahring and Westermann, 1988) or propionate (Dong *et al.*, 1994). Fatty acid degradation by a fermenter was enhanced when one methanogenic strain consumed acetate while the other consumed H_2. Thus, the combination of low acetate and low H_2 concentrations improved degradation efficiency via syntrophy. It should be mentioned that acetate can also be consumed *indirectly* by methanogens. This occurs when acetate is first converted to H_2 and CO_2, both of which are then consumed by a hydrogenotrophic methanogen (Zinder and Koch, 1984).

All known propionate oxidizers are also capable of reducing SO_4^{2-}, and the biochemical components may include part of the SO_4^{2-}-reducing apparatus (Schink, 1997). Syntrophic propionate use occurs when SO_4^{2-} is limiting and involves the transfer of H_2 to a methanogenic bacterium. Microscopic studies have shown that syntrophic associations between fatty acid-oxidizers and methanogens can produce structured or layered microcolonies composed of propionate utilizers and H_2-consuming methanogens, and these structures can be surrounded by acetate-utilizing methanogens and other bacteria (Harmsen *et al.*, 1996b). Finding an organized juxtaposition of bacteria is not surprising considering the physically close association required for the transfer of products between syntrophic partners. Further work has shown that acetate and H_2-consuming methanogens tend to segregate into micro-clusters as well (Gonzalez- Gil *et al.*, 2001; Rocheleau *et al.*, 1999).

Anaerobic bacteria can ferment a wide range of amino acids and many do so using interspecies H_2 transfer in association with H_2 utilizers. The classic mode of amino acid fermentation is the Strickland reaction in which a pair of amino acids is consumed, one acting as an oxidant and the other as a reductant. For example, a single bacterium can oxidize alanine to acetate and NH_4^+ while reducing glycine to acetate and NH_4^+:

$$\begin{aligned} &CH_3CH(NH_3^+)COO^- \text{(alanine)} \\ &+ 2CH_2(NH_3^+)COO^- \text{(glycine)} \\ &\rightarrow 3NH_4^+ + 3CH_3COO^- + CO_2 \end{aligned}$$

However, this reaction can be uncoupled in which one bacterium oxidizes alanine to acetate, CO_2, NH_4^+, and H_2, and a second bacterium uses the H_2 to reduce glycine to acetate and NH_4^+:

$$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2 \qquad \Delta G_0' = +2.7 \text{ kJ mol}^{-1}$$

$$CH_2(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + NH_4^+ \qquad \Delta G_0' = -78 \text{ kJ mol}^{-1}$$

Adding these equations, we obtain

$$CH_3CH(NH_3^+)COO^- + 2CH_2(NH_3^+)COO^- + 2H_2O \rightarrow 3CH_3COO^- + 3NH_4^+ + CO_2 \qquad \Delta G_0' = -153 \text{ kJ mol}^{-1}$$

A single bacterium has been isolated that can conduct all three reactions (Zindel *et al.*, 1988). However, H_2 and acetate generated from alanine oxidation can be consumed by other partners such as methanogens (Nagase and Matsuo, 1982), SO_4^{2-} reducers (Nanninga and Gottschal, 1985), or acetogens (Zindel *et al.*, 1988). The Strickland reaction is energetically favorable over the syntrophic fermentation of amino acids (Schink, 1997) and the former may dominate in habitats

rich in amino acids. However, syntrophic pathways may be important where amino acid concentrations are low and methanogenesis is active. A wide variety of bacteria are capable of fermenting various amino acids syntrophically using methanogens as H_2 scavengers with acetate, propionate, and butyrate as common end products (Baena *et al.*, 2000, 1999; Meijer *et al.*, 1999; Stams and Hansen, 1984; Wildenauer and Winter, 1986; Winter *et al.*, 1987). The ratio of organic products formed is a function of the H_2 partial pressure, which underscores the importance of the H_2 scavenger in controlling electron and carbon flow (Stams and Hansen, 1984).

The role of the H_2 scavenger in syntrophic relationships can be accomplished by a variety of H_2-utilizing anaerobes. Methanogens have received the most attention due to the importance of syntrophy in methanogenic habitats since CH_4-producing bacteria are extremely limited in the scope of electron donors used. Anaerobic, SO_4^{2-}-dependent CH_4 oxidation is the most recent example of microbial syntrophy between a methanogen-like organism and a SO_4^{2-} reducer (Section 8.08.4.5). However, this role can be replaced by several other physiologic groups including SO_4^{2-}-, S^0-, metal-, nitrate-, glycine-, and fumarate-reducing, and acetogenic bacteria (Schink, 1997). The ability of an H_2-scavenging reaction to compete for transferred electrons depends on the redox potential of the terminal electron acceptor with nitrate and fumarate $> SO_4^{2-} > CO_2/CH_4 > CO_2$/acetate (Cord-Ruwisch *et al.*, 1988).

Respiring bacteria resort to syntrophy to decompose low molecular weight compounds when exogenous oxidant levels are low. The most widely studied are SO_4^{2-} reducers that utilize fermentation processes coupled to H_2 transfer to methanogens when SO_4^{2-} is depleted (Bryant *et al.*, 1977; Cord-Ruwisch *et al.*, 1986; Harmsen *et al.*, 1996a; van Kuijk and Stams, 1995; Wallrabenstein *et al.*, 1995). Interestingly, SO_4^{2-}-reducing bacteria can fill an opposite role in which H_2 produced during the degradation of methanol and acetate by a methanogenic bacterium is consumed by a SO_4^{2-}-reducing bacterium during SO_4^{2-} reduction (Phelps *et al.*, 1985). This SO_4^{2-}-dependent interspecies H_2 transfer results in a more complete oxidation of organic carbon substrates yielding more CO_2 and less CH_4 than the methanogen would produce alone (Achtnich *et al.*, 1995b). Hence, although SO_4^{2-} reducers and CH_4 producers often compete for substrates, this competition is circumvented in the absence of SO_4^{2-} when fermenting SO_4^{2-} reducers rely on methanogens as H_2 sinks, or when methanogens use SO_4^{2-} reducers as H_2 sinks. Studies using sediment slurries revealed a dual role for SO_4^{2-} reducers in which they act as direct consumers of fatty acids as well as affecting fatty acid degradation by removing H_2 during syntrophy with other fatty acid fermenting species (Monetti and Scranton, 1992). Metal-reducing bacteria such as *Geobacter* sp. are also capable of degrading fatty acids like acetate by interspecies H_2 transfer to nitrate reducing bacteria (Cord-Ruwisch *et al.*, 1998). It appears that the transfer of electrons to the nitrate-reducing partner by the *Geobacter* sp. occurs using cysteine as the electron-transferring agent (Kaden *et al.*, 2002).

Fermenting bacteria can also transfer electrons to oxidized humic acids, allowing for the formation of more oxidized fermentation products than those produced in the absence of humics (Benz *et al.*, 1998). It was shown previously that Fe(III)-respiring bacteria can transfer reducing equivalents from acetate to various humic acid preparations including the quinoid model compound 2,4-anthraquinone disulfonate (AQDS) (Lovley *et al.*, 1996b, 1998). Humic acids act catalytically as electron shuttles by transferring electrons to oxidized iron. It has also been shown that fermenting bacteria can transfer electrons to Fe(III) in this manner, but it appears that these bacteria do not conserve energy via electron transport like iron-respiring bacteria do (Benz *et al.*, 1998). However, the electron sink serves a similar function as a syntrophic partner. For example, propionate fermentation is endergonic in the absence of an H_2-consuming partner, but propionate can be fermented exergonically to acetate without a bacterial partner when electrons are transferred to humic acids (Benz *et al.*, 1998).

8.08.4 METHANE

Methanogenesis is the final step in the anaerobic degradation of organic carbon. The principal steps performed by methanogens are fermentation of acetate to CO_2 and CH_4, and oxidation of H_2 to H_2O. In both cases, the waste product, CH_4, still holds potential energy in the form of reducing equivalents that can support additional anaerobic metabolism. Thus, the true end point of anaerobic organic matter degradation in some ecosystems is the anaerobic oxidation of CH_4 to CO_2 (Section 8.08.4.5). In the presence of O_2, methane is a source of energy for CH_4-oxidizing bacteria (i.e., methanotrophs), some of which are symbionts of deep-sea mussels (Childress *et al.*, 1986; Fiala–Médioni *et al.*, 2002; Kochevar *et al.*, 1992).

The many environmental and economic issues that involve CH_4 have stimulated research in all aspects of methane cycling. Here, we consider the processes that influence CH_4 production and oxidation. The literature on CH_4 emissions is considered only briefly in this article (Section 8.08.4.7).

8.08.4.1 Methane in the Environment

Human activity has increased the atmospheric CH_4 concentration from ~0.7 μL L^{-1} to 1.8 μL L^{-1} (ppmv) since about the 1850s, primarily by stimulating methanogenesis in soils and sediments (Lelieveld *et al.*, 1998; see below). Methane concentrations are currently double the highest level recorded in a 420,000-year ice core (Petit *et al.*, 1999), and it accounts for 20% of human-induced radiative forcing (Prather *et al.*, 2001). Rising CH_4 concentrations during periods of increased solar insolation and interglacial warming have substantially amplified global warming in the past (Petit *et al.*, 1999), and there is concern that this will occur in the future. In the past, changes in the atmospheric CH_4 content were accompanied by changes in the area of northern and tropical wetlands (Blunier *et al.*, 1995; Chappellaz *et al.*, 1993). Because CH_4 hydrate deposits may have been a large and sudden source of atmospheric CH_4 that contributed to past climate change (Nisbet, 1992; Thorpe *et al.*, 1996), the current stability of these vast CH_4 reservoirs is a topic of considerable importance (Kvenvolden, 1999; Wood *et al.*, 2002).

About 70% of the current CH_4 sources are anthropogenic, with roughly equal contributions from fossil fuel-related industries, waste management systems, and enteric fermentation associated with raising livestock (Table 5). Of the natural sources, wetlands are 70% of the total and therefore a major research focus. About 60% of natural wetland sources and most rice paddies occur in the tropical latitudes. Another 35% are in northern latitudes, and 10% are in mid-latitudes. The combined contribution of natural and managed wetlands to global CH_4 emissions is ~32% at present, and perhaps 70% of all sources before the industrial revolution (Lelieveld *et al.*, 1998). Estuaries are <9% of ocean CH_4 sources (Middelburg *et al.*, 2002).

The dynamics of CH_4 in the atmosphere are quite different than CO_2. It is a reactive gas and participates in atmospheric chemical reactions that influence the concentrations of NO, NO_2, CO, and O_3 (Crutzen, 1995). On a molar basis, CH_4 is 3–22 times stronger as a greenhouse gas than CO_2, depending on the period of time over which its impact is considered (Rodhe, 1990; Whiting and Chanton, 2001). Methane concentrations are more responsive than CO_2 to changes in sources or sinks because of a far shorter atmospheric residence time for CH_4 (~10 yr versus >100 yr). These characteristics have inspired the recommendation that efforts to slow the pace of global warming should focus initially on abating CH_4 emissions (Hansen *et al.*, 2000; Fuglestvedt *et al.*, 2000).

Methane is an important compound economically. In the form of natural gas, it is a clean-burning energy source and the major carbon substrate for alkane formation in economic gas reservoirs (Sherwood Lollar *et al.*, 2002). Methane represents an unwelcome loss of metabolic energy from flatulent livestock (Johnson *et al.*, 1993). Although methanogens do not directly degrade organic pollutants, they participate in the process by consuming fermentation products, thereby maintaining an environment that is thermodynamically conducive to the anaerobic degradation of multi-carbon compounds (Lovley, 2000a; Weiner and Lovley, 1998; Section 8.08.3.2.2). It has been demonstrated that methanogens play this role in the degradation of alkanes, a group of particularly stable hydrocarbons that are abundant in fossil fuel deposits (Anderson and Lovley, 2000; Zengler *et al.*, 1999). Aerobic bacteria that depend entirely on CH_4 for their carbon and energy (i.e., methanotrophs) directly degrade organic pollutants such as trichloroethylene (TCE) (R. S. Hanson and T. E. Hanson, 1996).

Table 5 Estimated sources and sinks of methane in the atmosphere in units of 10^{12} g CH_4 yr^{-1}.

	Range	*Likely*
Sources		
Natural		
Wetlands		
Tropics	30–80	65
Northern latitude	20–60	40
Others	5–15	10
Termites	10–50	20
Ocean	5–50	10
Freshwater	1–25	5
Geological	5–15	10
Total		160
Anthropogenic		
Fossil fuel related		
Coal mines	15–45	30
Natural gas	25–50	40
Petroleum industry	5–30	15
Coal combustion	5–30	15
Waste management system		
Landfills	20–70	40
Animal waste	20–30	25
Domestic sewage treatment	15–80	25
Enteric fermentation	65–100	85
Biomass burning	20–80	40
Rice paddies	20–100	60
Total		375
Total Sources		535
Sinks		
Reaction with OH	330–560	445
Removal in stratosphere	25–55	40
Removal by soils	15–45	30
Total Sinks		515
Atmospheric increase	30–35	30

Source : Schlesinger (1997).

8.08.4.2 Methanogen Diversity and Metabolism

Methanogens are strict anaerobes that produce CH_4 as a waste product of energy metabolism. Several characteristics set the methanogens apart from most other microorganisms. They are the largest and most diverse group in the Archea, which is phylogenetically distinct from the other two domains of life, the Bacteria and Eukaryota. Many members of the Archea grow at extremes of temperature, pH, or salinity, although the distribution of methanogens is fairly cosmopolitan. Methanogens have a number of unique coenzymes (Wolfe, 1996) and differ from Bacteria in the construction of their cell walls, a characteristic that makes them insensitive to penicillin and other antibiotics. The methanogens and other Archea can be identified by an electron carrier, F_{420}, that autofluoresces at 420 nm under UV light (Edwards and McBride, 1975). Many methanogens lack cytochromes and other features of electron transport chains such as quinones (Fenchel and Finlay, 1995). It has been suggested that a quinone-like role in electron transfer may be filled by phenazine compounds in the *Methanosarcinales* (Abken *et al.*, 1998; Deppenmeier *et al.*, 1999). In a detailed taxonomic treatment, Boone *et al.* (1993) defined 26 genera and 74 species of methanogens.

Despite a great deal of taxonomic diversity, the methanogens use a limited variety of simple energy sources compared to the other major forms of anaerobic metabolism (Zinder, 1993). The compounds that support energy conservation for growth are H_2, acetate, formate, some alcohols, and methylated compounds. The most important of these are generally H_2 and acetate. About 73% of methanogenic species consume H_2 (Garcia *et al.*, 2000):

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (3)$$

Hydrogenotrophic methanogenesis (also known as *CO_2/H_2 reduction* or *H_2-dependent methanogenesis*) is a chemoautotrophic process in which H_2 is the source of both energy and electrons, and CO_2 is often both an electron sink and the source of cellular carbon. Some hydrogenotrophic methanogens require an additional organic carbon source for growth (Vogels *et al.*, 1988). CO_2/H_2 reduction requires a 4 : 1 molar ratio of H_2 to CO_2, yet H_2 is typically at nM concentrations while CO_2 is at mM concentrations in natural systems. Thus, substrate limitation of hydrogenotrophic methanogenesis must always be caused by a lack of the electron donor, H_2. Roughly 45% of all hydrogenotrophic methanogens can substitute formate for H_2 in reaction (3) (Garcia *et al.*, 2000; Thiele and Zeikus, 1988).

Whereas hydrogenotrophy is wide spread among the methanogens, acetotrophy (also known as *acetate fermentation* or *acetoclastic methanogenesis*) is restricted to just two genera, the *Methanosarcina* and *Methanosaeta* (formerly *Methanothrix*), which comprise ~10% of methanogenic species:

$$CH_3COOH \rightarrow CO_2 + CH_4 \qquad (4)$$

The *Methanosarcina* use a wide variety of substrates and have high potential growth rates, but their affinity for acetate is low (Jetten *et al.*, 1992). In contrast, the *Methanosaeta* specialize in using acetate and have a high affinity for the substrate, but their potential growth rate is low. Despite its limited taxonomic distribution, acetotrophy is the dominant methanogenic pathway in many ecosystems. Acetotrophic methanogenesis can be considered a special case of methylotrophy whereby a portion of the substrate molecule is oxidized to CO_2, while a methyl group on the same molecule is reduced to CH_4 (Fenchel and Finlay, 1995; Hornibrook *et al.*, 2000; Pine and Barker, 1956). In this case, the methyl group is the electron donor and the carboxyl group is the electron acceptor.

About 26% of methanogenic species can use methylated substrates other than acetate, such as methanol, methylated amines, and methylated sulfur compounds (Hippe *et al.*, 1979; Kiene, 1991b). Because the process does not require an external electron acceptor, methylotrophy is a type of fermentation. The contribution of these organisms to CH_4 production in natural ecosystems appears to be minor, but they may contribute substantially to the metabolism of methylated sulfur compounds. Scholten *et al.* (2003) provided thermodynamic constraints on the feasibility of such reactions.

Some methanogens are dependent on a single substrate, such as acetate or methanol, while others are able to grow on two or more alternative substrates. All known formate-oxidizing methanogens are also hydrogenotrophs (Garcia *et al.*, 2000), and at least one methanogen also grows by fermenting pyruvate (Bock and Schönheit, 1995). Members of the *Methanosaetaceae* use acetate but not H_2, whereas the *Methanosarcinaceae* can use both substrates. Both genera fall in the order *Methanosarcinales*.

Methanogens metabolize several substrates that do not support growth. *Methanosarcina barkeri* was able to lower the redox potential by dissimilatory reduction of Fe(III) until the redox potential reached 50 mV, at which point methanogenesis began (Fetzer and Conrad, 1993). In a survey of five methanogenic species drawn from a wide range of phylogenetic and physiological types, all the hydrogenotrophs

could reduce Fe(III) (Bond and Lovley, 2002). The ability to grow on Fe(III) was not investigated. Carbon monoxide is converted to CH_4, but the physiological role this substrate plays is uncertain (Vogels *et al.*, 1988). Rich and King (1999) measured maximum potential uptake velocities of 1–2 nmol CO cm^{-3} sediment h^{-1} in anaerobic soils, but the response to amendments of SO_4^{2-}, Fe(III), and the methanogen inhibitor bromoethanesulfonic acid (BES), suggested that no more than 30% of the oxidation activity was due to methanogens. Methanogens degrade chlorinated pollutants and have been used for bioremediation (Fathepure and Boyd, 1988; Mikesell and Boyd, 1990).

Methanogens grow at temperatures ranging from 4 °C to 100 °C, salinities from freshwater to brine, and pH from 3 to 9. Most grow optimally at temperatures ≥30 °C, but thermophilic methanogens have temperature optima near 100 °C, and a few isolates are adapted to frigid conditions (Franzmann *et al.*, 1997). In a survey of 68 methanogenic species, most species grew best in a pH range from 6 to 8, and none could grow at pH <5.6 (Garcia *et al.*, 2000). Observations of CH_4 production in acidic environments suggests that there are uncultured methanogens that can grow at pH <5.6 (Walker, 1998).

8.08.4.3 Regulation of Methanogenesis

Methane production is regulated mainly by O_2 concentration, pH, temperature, salinity, organic substrates, and nutrient availability. The amount of CH_4 emitted from wetlands is further influenced by a large number of factors that are not addressed in this review, including plant physiology, community composition, and hydrology. In addition to their direct effects on methanogen physiology, these factors influence the process indirectly by regulating the flow of methanogenic substrates from fermenting and syntrophic microorganisms. Methanogenesis can be limited by any single link in the chain of reactions that begins with detrital inputs.

8.08.4.3.1 O_2 and oxidant inhibition

Methanogenesis is inhibited by O_2. This is evident from field studies that show no overlap in the depth distributions of O_2 penetration in soils or sediments and net CH_4 production. Inhibition by O_2 is one reason that water table depth in soils is often a strong predictor of CH_4 emissions (Kettunen *et al.*, 1999; Roulet and Moore, 1995; Sundh *et al.*, 1995), the other reason being its influence on CH_4 oxidation.

Methanogens are not necessarily as oxidant sensitive as it was once believed. Some methanogens are fairly tolerant of O_2 (Kiener and Leisinger, 1983) and have adaptations such as superoxide dismutase (Kirby *et al.*, 1981). Methane production by *Methanosarcina barkeri* starts when the redox potential drops below 50 mV (Fetzer and Conrad, 1993), and CH_4 production has been observed in rice paddy soils at redox potentials >200 mV (Peters and Conrad, 1996; Yao and Conrad, 1999). Despite the fact that they do not form spores or other resting stages, methanogens can survive for long periods of time in largely dry and oxic soils. Mayer and Conrad (1990) observed a rapid increase in CH_4 production within 25 d of flooding an upland agricultural soil and a forest soil. Viable methanogen populations survived in an oxic, air-dried paddy soil for at least 2 yr (Ueki *et al.*, 1997). von Fischer and Hedin (2002) recently developed a ^{13}C pool dilution method that gives a sensitive estimate of *gross* CH_4 production, and found that CH_4 is in fact produced in many dry, oxic soils (mean = 0.15 mg CH_4–C m^{-2} d^{-1}). However, they stopped short of attributing this activity to methanogens because of reports that CH_4 is a minor metabolic product in some eubacteria such as *Clostridia* (Rimbault *et al.*, 1988). Methanogens may be able to survive in small anaerobic microsites imbedded in dry soils, or they may be protected from O_2 by reactive soil minerals. For example, the presence of FeS_2 (pyrite) in paddy soils was shown to enhance the survival of methanogens exposed to O_2 (Fetzer *et al.*, 1993).

The lack of CH_4 production in the presence of O_2 *in situ* may be due to a combination of factors, of which O_2 toxicity is just one. For example, methanogens were more sensitive to desiccation than O_2 exposure in a paddy soil (Fetzer *et al.*, 1993). The oxidized products of denitrification, NO and N_2O, have a toxic effect on methanogens similar to that of O_2 (Klüber and Conrad, 1998). A negative correlation between redox potential and CH_4 production is often observed in the absence of O_2, but this probably reflects competition between methanogens and their competitors for reductants, not a physiological requirement for a certain redox potential.

8.08.4.3.2 Nutrients and pH

Laboratory incubations of wetland soils with nitrogen and phosphorus amendments have shown either no effect or an inhibitory effect on methanogenesis (Bodelier *et al.*, 2000a,b; Bridgham and Richardson, 1992; Wang and Lewis, 1992). A low rate of phosphate supply to rice roots stimulated CH_4 emission (Lu *et al.*, 1999), while phosphate concentrations ≥20 mM specifically inhibited acetotrophic methanogenesis (Conrad *et al.*, 2000).

Methanogens require a somewhat unique suite of micronutrients that include nickel, cobalt, iron,

and sodium (Jarrell and Kalmokoff, 1988). Methanogenesis was stimulated by molybdenum, nickel, boron, iron, zinc, vanadium, and cobalt in a rice paddy soil (Banik *et al.*, 1996), and by a cocktail of nickel, cobalt, and iron in *Sphagnum*-derived peat (Basiliko and Yavitt, 2001). The micronutrient cocktail did not stimulate CO_2 production in the same soils, suggesting that methanogens, rather than fermenters, were directly limited by trace elements. Freshwater methanogens required at least 1 mM Na^+ to drive ATP formation by an Na^+/K^+ pump (Kaesler and Schönheit, 1989). Trace element availability could limit methanogenesis in peatlands that are isolated from groundwater inputs and sea salt deposition. The latter effect could be important in bogs in the interior of continents.

Most methanogenic communities seem to be dominated by neutrophilic species. Some acidic peats have responded to an increase in pH with higher CH_4 production (Dunfield *et al.*, 1993; Valentine *et al.*, 1994), while other peats have not (Bridgham and Richardson, 1992). A substantial portion of the acetate pool may not be available to methanogens at low pH because it is prevented from dissociating (Fukuzaki *et al.*, 1990).

8.08.4.3.3 Temperature

Methanogenesis is often more sensitive to temperature than other biological processes, which typically double in rate with a 10 °C increase in temperature (i.e., $Q_{10} = 2$). A compilation of temperature-response studies using wetlands soils reported an average Q_{10} of 4.1 for CH_4 production and 1.9 for aerobic CH_4 oxidation (Segers, 1998). van Hulzen *et al.* (1999) argued that such high Q_{10} values are often caused by the changing availability of alternative electron acceptors or methanogenic substrates over the course of an experiment. The time required for methanogen competitors (e.g., Fe(III) reducers) to deplete the pool of alternative electron acceptors decreases with increasing temperature. Thus, if CH_4 production is measured cumulatively over a fixed period of time, it can appear to increase at a $Q_{10} > 2$ even though the underlying processes increased at a far lower Q_{10}. This would explain an apparent correlation between Q_{10} and the ratio of CO_2 to CH_4 in anaerobic incubations (Figure 5). It is likely that temperature-sensitive steps during fermentation or acetogenesis contribute to the temperature sensitivity of CH_4 production. It is more difficult to explain high CH_4 Q_{10} values in some pure methanogen cultures (Segers, 1998). Methanogenesis can also be insensitive to temperature if fermentation is limited by other factors such as carbon quality (Yavitt *et al.*, 1988).

Because methanogenesis is severely suppressed by low temperature, CH_4 emissions are often assumed to be negligible during the winter. This is clearly not the case in peatlands where winter CH_4 emissions contribute 2–21% of annual fluxes (Alm *et al.*, 1999; Dise, 1992; Melloh and Crill, 1996). Zimov *et al.* (1997) estimated that 75% of CH_4 emissions from Siberian lakes is emitted in the winter.

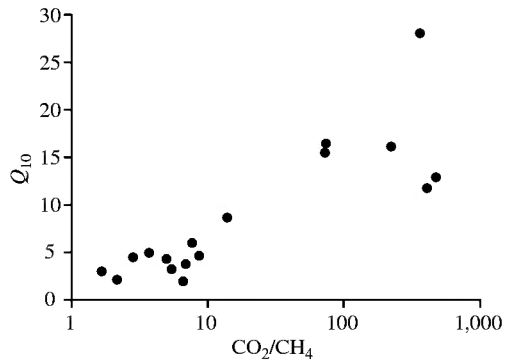

Figure 5 The relationship between the temperature sensitivity (i.e., Q_{10}) of CH_4 production and the ratio CO_2 to CH_4 produced in anaerobic incubations (van Hulzen *et al.*, 1999) (reproduced by permission of Elsevier from *Soil Biol. Biochem.* **1999**, *31*, 1919–1929).

8.08.4.3.4 Carbon quantity and quality

The quantity and quality (i.e., chemical composition) of organic carbon compounds is a master variable regulating methanogenesis. Organic carbon fermentation is the ultimate source of both the electron donors and the electron acceptors required by the two major methanogenic pathways (Section 8.08.4.2). There are inorganic sources of CO_2, but this substrate is abundant compared to H_2 and does not limit hydrogenotrophic methanogenesis. Organic carbon also exerts a strong influence on another key regulator of methanogenesis, namely the supply of inorganic compounds that are toxic to methanogens (O_2, NO_3^-) or more energetically favorable as electron acceptors for competing organisms (NO_3^-, Fe(III), humic acids, SO_4^{2-}). Methanogenesis is most vigorous when the consumption rate of alternative electron acceptors exceeds their supply rate. Because the organisms that consume these alternative substrates are heterotrophic and usually carbon limited, such conditions are most likely to develop in systems with a high supply of labile organic carbon compounds (Section 8.08.8).

A wide variety of evidence suggests that carbon availability limits methanogenesis *in situ*. The fact that methanogenesis is often inhibited by the presence of alternative electron acceptors such as Fe(III) and SO_4^{2-} is evidence of competition for fermentation products and thus widespread carbon limitation of the process (Section 8.08.8).

Methanogenesis is stimulated by plant detritus amendments in rice paddy soils (Dannenberg and Conrad, 1999; Inubushi *et al.*, 1997; Kludze and DeLaune, 1995; Tanji *et al.*, 2003) and peat soils (Valentine *et al.*, 1994), and by amendments of H_2 (Bridgham and Richardson, 1992; Yavitt *et al.*, 1987;Yavitt and Lang, 1990) and dissolved organic carbon (Lu *et al.*, 2000b). Methane *emissions* are stimulated by organic amendments (Watanabe *et al.*, 1995; Bronson *et al.*, 1997), but this effect may be due partly to lower CH_4 oxidation caused by more rapid heterotrophic O_2 consumption at the soil surface.

The chemical composition of organic compounds (i.e., carbon quality) influences methanogenesis by regulating the production of fermentation products (Section 8.08.3). This explains two common observations about changes in methanogenesis with increasing depth in freshwater wetland soil profiles: (i) rates of potential CH_4 production decline with depth below the aerobic zone (Clymo and Pearce, 1995; Kettunen *et al.*, 1999; Megonigal and Schlesinger, 2002; Moore and Dalva, 1997; Sundh *et al.*, 1994; Updergraff *et al.*, 1995; Valentine *et al.*, 1994; Yavitt *et al.*, 1988, 1990) and (ii) methanogenesis is increasingly dependent on hydrogenotrophy (Hornibrook *et al.*, 2000). These depth-dependent patterns reflect a combination of changes in the soil organic carbon quality, and increasing distance from labile carbon substrates deposited near the surface. The mineralization of fresh organic matter is thought to favor acetotrophic methanogenesis (Schoell, 1988; Sugimoto and Wada, 1993). In bogs, common measures of carbon quality, such as lignin content, lignin : N ratio and C : N ratio, show soil organic matter becoming progressively older, more decomposed and recalcitrant with depth (Yavitt and Lang, 1990; Updergraff *et al.*, 1995; Valentine *et al.*, 1994). In a fen, 90% of the CH_4 produced from soil organic matter mineralization came from particles with a diameter >2 mm, which represents recent plant detritus (van den Pol-van Dasselaar and Oenema, 1999). Because the >2 mm soil organic carbon fraction decreased rapidly with depth, 70% of the total CH_4 production occurred in the top 5 cm of the soil profile. In other cases, the depth-dependent decline in potential CH_4 production does not correlate to changes in the quality of the soil organic carbon pool. For example, potential CH_4 production, but not the quality of soil organic matter, decreased strongly with depth in two northern fens (Valentine *et al.*, 1994). Here, the methanogens were apparently using labile carbon compounds that were produced in the root zone and were less abundant with increasing distance from the soil surface. This is consistent with $^{14}CH_4$ measurements that showed microbial respiration in fens, but not in bogs, were based on recently fixed photosynthates that originated in the root zone, and were transported by groundwater through the soil profile (Chasar *et al.*, 2000a).

Soil organic matter quality reflects the chemical characteristics of the dominant plant species, and it is quite poor in peatlands dominated by *Sphagnum* moss. *Sphagnum* tissue has an exceedingly low nitrogen content (Aerts and de Caluwe, 1999; Hobbie, 1996; Johnson and Damman, 1991), which is one reason for lower CH_4 emissions from northern bogs than fens (Bridgham *et al.*, 1995; Dise *et al.*, 1993; Moore and Knowles, 1990). Although *Sphagnum* is abundant in both ecosystem types, fens also support a variety of vascular plant species with comparatively high tissue quality. The dominance of *Sphagnum* is itself a reflection of low nutrient availability, and is ultimately caused by the geomorphologic and hydrologic characteristics of the landscape.

Organic carbon indirectly influences methanogenesis by governing the rate that alternative electron acceptors are consumed. A combination of carbon content and alternative electron acceptor availability explained 90% of the variation in CH_4 production in 10 rice paddy soils (Gaunt *et al.*, 1997). The sediments at Cape Lookout Bight support exceptionally high rates of CH_4 production for a marine sediment because organic carbon inputs are rapid enough to deplete the porewater SO_4^{2-} pool before exhausting the labile organic carbon pool (Martens and Klump, 1984). High sedimentation rates also contribute to SO_4^{2-} depletion at Cape Lookout Bight by increasing the diffusion path length, thus slowing SO_4^{2-} resupply from the water column. Salt marshes tend to have higher CH_4 emissions than marine sediments per unit area (Bartlett *et al.*, 1987) because they receive considerable organic inputs from plants, thus relieving the substrate competition between methanogens and other microorganisms. Blair (1998) demonstrated the effectiveness of combining the labile carbon flux rate and oxidant availability (e.g., O_2 and SO_4^{2-}) into a ratio for modeling the proportion of organic carbon metabolized to CH_4 in marine sediments.

8.08.4.3.5 Plants as carbon sources

Plants and phytoplankton are the most abundant and labile organic carbon sources in ecosystems, supplying photosynthates either in the form of exudates or fresh detritus. Several isotope tracer studies have demonstrated a tight coupling between plant photosynthesis and methanogenesis in pot studies designed to represent rice paddies (Lu *et al.*, 2002; Dannenburg and Conrad, 1999; Minoda and Kimura, 1994; Minoda and Kimura, 1996), freshwater marshes (Megonigal *et al.*, 1999), and arctic tundra (King and Reeburgh, 2002; King *et al.*, 2002). A full cycle of CO_2

assimilation by plants, release into soils, and emission as CH_4 requires as little as 2 h, and up to 6% of the assimilated CO_2 is emitted as CH_4. Photosynthate-derived carbon was estimated to contribute 10–100% of the CH_4–C in rice paddies depending on the growth stage of the crop (Minoda and Kimura, 1994). Lu *et al.* (2000a,b) found that differences in the root exudation rates of three rice cultivars corresponded to variation in dissolved organic carbon in the rhizosphere and CH_4 emissions. It should be noted that isotopic labeling studies alone cannot determine whether there is an *energetic* link between the plants and microbes (Megonigal *et al.*, 1999).

In most instances, it is difficult to apply a radiocarbon label *in situ* because of environmental regulations (for an exception see Wieder and Yavitt, 1994). However, a ^{14}C label was applied worldwide during aboveground nuclear bomb testing, which peaked in 1965. Radiocarbon dates indicate that a significant amount of the pore-water CH_4 that currently exists in peatlands (Chanton *et al.*, 1995; Charman *et al.*, 1994; Martens *et al.*, 1992b), and an aquifer (Harvey *et al.*, 2002) was produced since 1965. In peatlands, pore-water CH_4–C was significantly younger than the bulk soil carbon (Aravena *et al.*, 1993), indicating that recently assimilated organic carbon compounds are carried downward into the soil profile by advection. An interesting exception to this pattern was reported in Siberian lakes where the radiocarbon age of CH_4 indicated that 68–100% of the CH_4–C had been assimilated during the Pleistocene (Zimov *et al.*, 1997). However, the contribution of this "old" carbon fell to 23–46% in the summer with inputs of more recent carbon sources.

There are several additional lines of evidence for a link between methanogenesis and photosynthesis. One is the observation that CH_4 emissions are often strongly related to primary production. Such a relationship was first suggested by Whiting and Chanton (1993), who reported a positive correlation between CH_4 emissions and net ecosystem exchange of CO_2 across North American wetlands distributed from the subarctic to the subtropics (Figure 6(a)). Net ecosystem exchange (NEE) is the difference between gross primary production (GPP) and the respiration of plants (R_p) and heterotrophic organisms (R_h):

$$\mathrm{NEE} = \mathrm{GPP} - R_p - R_h \qquad (5)$$

Similar relationships have been reported for many individual peatland ecosystems (Alm *et al.*, 1997; Chanton *et al.*, 1995; Whiting and Chanton, 1992; Whiting *et al.*, 1991). Methanogenesis accounted for 4% of NEE when averaged across several of these studies (Bellisario *et al.*, 1999). NEE is not a direct measure of photosynthetic activity because it includes R_h, but other studies have shown that the relationship holds when photosynthesis is considered independently. Bridgham *et al.* (2001) observed a positive correlation between GPP and CH_4 emissions in bog and fen mesocosms (Figure 6(b)). Differences in CH_4 emissions that were caused by manipulating temperature and nitrogen availability could be explained by changes in photosynthesis. The effects of plant community type (i.e., bog or fen) and water

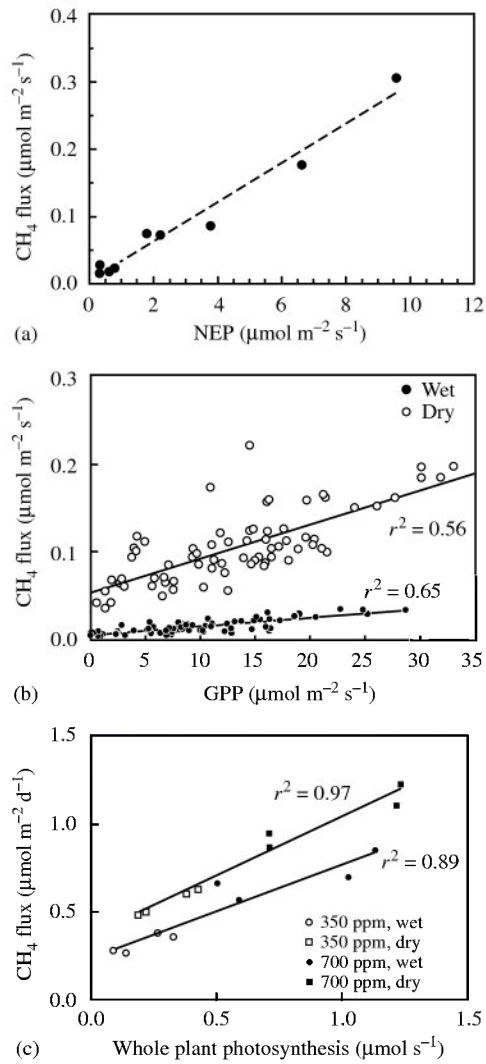

Figure 6 The relationship between wetland CH_4 emissions and various measures of primary productivity: (a) emissions versus net ecosystem production (NEP) in North-American ecosystems ranging from the subtropics to the subarctic; (b) emissions versus GPP in fen peatland mesocosms with high or low water table depths; and (c) emissions versus whole-plant net photosynthesis in marsh microcosms exposed to elevated and ambient concentrations of atmospheric CO_2. (after Whiting and Chanton, (1993); Updergraff *et al.*, (2001); and Vann and Megonigal (2003), respectively).

table depth needed to be accounted for separately. Vann and Megonigal (2003) found that elevated atmospheric CO_2 stimulated CH_4 flux in direct proportion to net photosynthesis in a greenhouse study (Figure 6(c)), but again the regression relationships varied by plant species and water table depth.

A final line of evidence for a direct link between methanogenesis and photosynthesis is a report that CH_4 emissions from a group of peatlands were correlated to δ^{13}C-CH_4 (Chanton *et al.*, 1995). The positive slope of this relationship indicated that the highest fluxes occurred at sites where labile carbon was relatively abundant, which favors acetotrophic methanogenesis and ^{13}C enrichment (Section 8.08.4.4.1). Similar observations have been reported from other types of wetland and aquatic ecosystems (Chanton and Martens, 1988; Martens *et al.*, 1986; Tyler *et al.*, 1994). Collectively, these studies suggest that wetland methanogens depend on labile, high-quality organic carbon supplied by plants in the form of root exudates or detritus.

8.08.4.4 Contributions of Acetotrophy versus Hydrogenotrophy

Acetotrophy and hydrogenotrophy are the dominant methanogenic pathways *in situ*, although trimethylamines and methylated sulfur species may be important in some marine sediments (Oremland *et al.*, 1982). Because acetotrophic methanogenesis is associated with high rates of total CH_4 production, it is useful to understand the factors that govern the relative dominance of these two pathways. The theoretical contribution of hydrogenotrophic methanogenesis to CH_4 production during anaerobic degradation of carbohydrates is 33%, and this value is often observed in freshwater ecosystems (see Conrad (1999) for a partial compilation). However, the relative contributions of the two pathways can vary considerably across ecosystems, seasons, and depths. An early generalization was that acetotrophic methanogenesis is dominant in freshwater ecosystems, while hydrogenotrophic methanogenesis is dominant in marine systems (Whiticar, 1999). This is a reasonable first approximation because SO_4^{2-} reducers readily outcompete methanogens for the limited acetate supply in marine systems. However, there are examples of freshwater systems dominated by hydrogenotrophic methanogenesis (Lansdown *et al.*, 1992), marine systems with a relatively large contribution from acetotrophic methanogenesis (Martens *et al.*, 1986), and wide variation in time and space within a given site (Martens *et al.*, 1986; Sugimoto and Wada, 1993).

8.08.4.4.1 Organic carbon availability

The availability of labile organic carbon is perhaps a more appropriate basis than salinity on which to generalize about the relative importance of the two primary methanogenic pathways. Freshwater wetlands that are dominated by acetotrophic methanogenesis also support high rates of primary productivity, and thus high rates of organic carbon mineralization. Labile carbon pools are typically high in rice paddy soils, which are highly productive and dominated (>50%) by acetotrophic methanogenesis (Conrad, 1999). By comparison, *Sphagnum* bog peatlands have exceptionally low nutrient availability, low primary productivity, and highly recalcitrant soil organic matter pools. Isotopic evidence and direct rate measurements indicate that these systems are dominated by hydrogenotrophic methanogenesis (Bellisario *et al.*, 1999; Chasar *et al.*, 2000a; Hines *et al.*, 2001; Lansdown *et al.*, 1992). Despite deep accumulations of nearly pure organic soils in bogs, the size of the labile organic carbon pool is small because of poor carbon quality (Section 8.08.4.3.4). Whereas bogs are isolated from groundwater, fen peatlands receive substantial groundwater inputs and are characterized by comparatively high nutrient availability, herbaceous plant biomass, labile carbon availability, CH_4 emissions, and acetotrophic methanogenesis. This is particularly evident at the soil surface.

The size of the labile carbon pool is an important predictor of methanogenic pathways in marine sediments (Blair, 1998). Sediments with low carbon availability are dominated by hydrogenotrophic methanogenesis, while carbon-rich systems such as Cape Lookout Bight support relatively more acetotrophic methanogenesis (Martens *et al.*, 1986). The labile portion of the organic carbon pool in marine sediments is determined by several factors such as chemical composition, adsorption by mineral surfaces, and burial efficiency (Hedges and Keil, 1995). Poor organic carbon quality is expected in coastal sediments that receive large inputs of terrestrially derived, highly degraded particulate organic matter from rivers (Hedges *et al.*, 1994; Martens *et al.*, 1992a).

Stable isotopes provide a nondestructive alternative to radiolabeled substrates for inferring the relative importance of methanogenic pathways. Whiticar *et al.* (1986) recognized that acetotrophic methanogenesis yields CH_4 that is ^{13}C-enriched ($\delta^{13}C = -65‰$ to $-50‰$) compared to hydrogenotrophy ($\delta^{13}C = -110‰$ to $-60‰$). The reason for this difference is a larger $^{12}C/^{13}C$ fractionation during CH_4 production by hydrogenotrophy ($\alpha_c = 1.055–1.090$) than acetotrophy ($\alpha_c = 1.04–1.055$). Similarly, CH_4 from acetotrophy is deuterium-depleted

(δD = −400‰ to −250‰) compared to hydrogenotrophy (δD = −250‰ to −170‰). Subsequent studies have suggested somewhat broader ranges for these fractionation factors (Tyler, 1991). Plots of δD versus δ^{13}C can be used to infer the extent of aerobic or anaerobic oxidation of CH_4 (Alperin *et al.*, 1988; Coleman *et al.*, 1981). If oxidation has a substantial influence on the CH_4 pool, the relationship between δD and δ^{13}C is positive. In the absence of oxidation, and provided that the relative contributions of the methanogenic pathways varies in time or space, the relationship between δD and δ^{13}C is negative. In such cases, the variability in δ^{13}C-CH_4 can often be interpreted as a shift in the contribution of acetotrophy and hydrogenotrophy to total methanogenesis (Burke *et al.*, 1988, 1992). There are several factors other than the methanogenic pathways and oxidation that can affect isotope fractionation factors (Happell *et al.*, 1993; Avery and Martens, 1999; Bergamaschi, 1997; Tyler, 1992), and these make it difficult to interpret stable isotope data in terms of absolute rates. Additional fractionations occur when CH_4 passes through plants, which must be accounted for when interpreting the stable isotope ratios of CH_4 emitted from wetlands (Chanton *et al.*, 1999a,b; 2002; Chanton and Dacey, 1991; Harden and Chanton, 1994).

Plots of δ^{13}C-CH_4 versus δ^{13}C-$\sum CO_2$ in peatland soils typically indicate a progressive transition from acetotrophic methanogenesis at the surface to hydrogenotrophic methanogenesis at depth (Chasar *et al.*, 2000b; Hornibrook *et al.*, 1997; 2000), that coincides with increasingly recalcitrant soil organic matter pools and distance from plant-derived labile carbon at the soil surface (Section 8.08.4.3.4). In a survey of the δD and δ^{13}C content of CH_4 in gas bubbles collected from lake sediments in western Alaska, there was a larger contribution from acetotrophic methanogenesis at vegetated sites than those that were unvegetated (Martens *et al.*, 1992b). Miyajima *et al.* (1997) reported that ^{13}C-CH_4 enrichment increased in proportion to increasing leaf litter decomposition rate; decomposition rate was in turn negatively related to lignin content (Figure 7). Thus, this study made a direct link between carbon quality and the relative contributions of hydrogenotrophic and acetotrophic methanogenesis to overall CH_4 production. This study was also notable because it was a rare investigation of anaerobic metabolism in a *tropical* peatland. In a variety of shelf and slope marine sediments, δ^{13}C-CH_4 is positively related to the flux of labile organic carbon to the sediment surface (Boehme *et al.*, 1996) and to the rate of pore-water SO_4^{2-} depletion (Figure 8). This relationship indicates that acetotrophic methanogenesis makes a relatively large contribution to CH_4 production in marine sediments when the labile carbon flux is high enough to deplete pools of O_2, Mn(IV), Fe(III) and SO_4^{2-}. However, the relationship did not hold for deep-sea sediments (Blair, 1998).

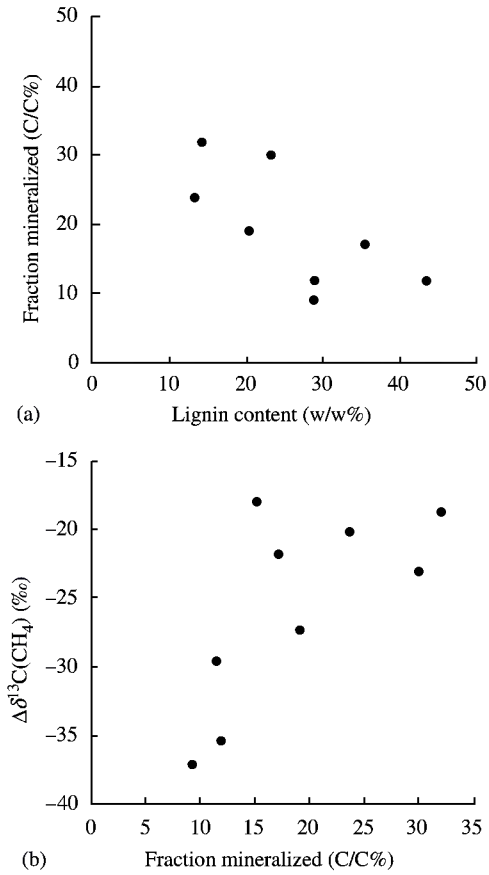

Figure 7 Influence of (a) lignin content on leaf litter decomposition rates, and (b) leaf litter decomposition rates on methanogenic pathway as reflected in the δ^{13}C of CH_4. In (b), the *y*-axis is the difference in δ^{13}C-CH_4 and δ^{13}C of total mineralized carbon ($CH_4 + CO_2$) (Miyajima *et al.*, 1997) (reproduced by permission of Elsevier from *Geochim. Cosmochim. Acta,* **1997**, *61*, 3739–3751).

The contribution of acetotrophy to methanogenesis can be quite stable in time (Avery and Martens, 1999; Burke *et al.*, 1992; Hines *et al.*, 2001), or it can vary strongly with the seasons. A mid-latitude bog was dominated by hydrogenotrophic methanogenesis for 10 months of the year, then switched to acetotrophic methanogenesis (Avery *et al.*, 2002). Because the switch was accompanied by a large increase in CH_4 emissions, acetotrophy contributed ~50% of total annual emissions. Seasonal shifts in methanogenic pathways were observed in several peatlands located at mid-latitudes (Kelley *et al.*, 1992; Lansdown *et al.*, 1992; Shannon and White, 1996). By comparison, no such switch occurred in a group of bogs and fens located at high latitudes (Duddleston *et al.*, 2002; Hines *et al.*, 2001).

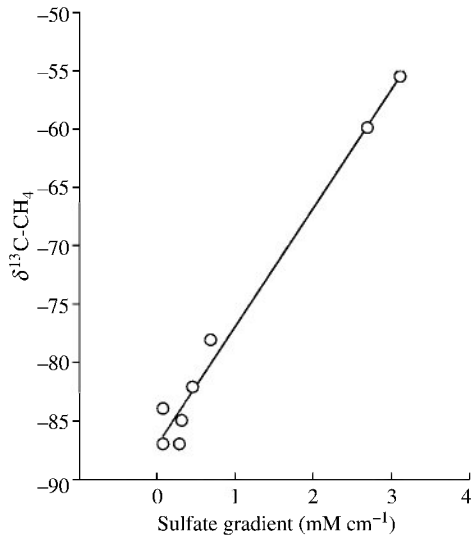

Figure 8 The relationship between that rate at which SO_4^{2-} is consumed down-core in shallow marine sediments and the δ^{13}C-CH_4. The r^2 fit of the regression line is 0.98 (Blair, 1998) (reproduced by permission of Elsevier from *Chem. Geol.*, **1998**, *152*, 139–150).

These sites were completely dominated by hydrogenotrophic methanogenesis despite an accumulation of acetate in the pore water. Incubating these soils at 24 °C for 5 months did not trigger acetotrophic CH_4 production, even though acetate continued to accumulate during the period. Apparently, the methanogens in these systems are limited by factors other than temperature, such as microbial community composition, pH, or perhaps trace nutrient availability (Section 8.08.4.3.2).

Chapelle *et al.* (2002) recently described a subsurface microbial community in which hydrogenotrophic methanogens were >90% of the 16S ribosomal DNA sequences and geothermal H_2 was the primary energy source. Since geothermal H_2 is likely to be an abundant energy source for microbial metabolism on other planets, hydrogenotrophy may be an important pathway of anaerobic metabolism elsewhere in the universe.

8.08.4.4.2 Temperature

Temperature is one of several factors that influence the contributions of acetotrophic and hydrogenotrophic methanogenesis to overall CH_4 production. The most comprehensive work on this topic has been done by Conrad and colleagues in rice paddy soils and lake sediments. They have repeatedly observed that the contribution from hydrogenotrophic methanogenesis declines at low temperatures, while the contribution of acetotrophic methanogenesis shows the opposite pattern (Figure 9(a); Chin and Conrad, 1995; Chin *et al.*, 1999a; Conrad *et al.*, 1987; Fey and Conrad, 2000; Schultz and Conrad, 1996; Schultz *et al.*, 1997; Yao and Conrad, 1999). Although changes in the relative contributions of the two pathways coincide with shifts in the structure of methanogenic communities (Chin *et al.*, 1999b; Fey and Conrad, 2000), they are probably not a direct response of methanogens to temperature. Rather, the methanogens appear to be responding to changes in substrate availability (Figure 9(b)), suggesting that the actual source of the observed temperature limitation was organisms that produce or consume H_2 and acetate. Indeed, H_2 amendments stimulated hydrogenotrophic methanogenesis at low (15 °C) temperature in a paddy soil, but not cellulose amendments, which would have required fermentation to methanogenic substrates (Schultz *et al.*, 1997). This result is consistent with temperature limitation of syntrophic bacteria, which produce H_2 by fermentation (Section 8.08.3.2.2). It is more difficult to explain an increase in acetate concentrations at low temperatures. One proposal is that low temperature favors the fermentation of multicarbon substrates directly to acetate (Conrad, 1996; Fey and Conrad, 2000), but this has not been demonstrated. Acetogenesis was shown to be <10% of the acetate sources at low temperature (Rothfuss and Conrad, 1993; Thebrath *et al.*, 1992). This body of work demonstrates that building a mechanistic understanding of the temperature responses of methanogens will require comprehensive studies that include fermenting, syntrophic, and homoacetogenic bacteria.

Physiological differences in the two methanogenic genera that can use acetate may influence temperature-driven changes in methanogenic pathways (reviewed by Liesack *et al.*, 2000). The members of the *Methanosaetaceae* that have been isolated to date use acetate exclusively and have a relatively low threshold for the substrate (typically <100 μM), while members of the *Methanosarcinaceae* use both acetate and H_2/CO_2, and have a relatively high threshold for acetate (typically between 200 and 1200 μM) (Jetten *et al.*, 1992; Großkopf *et al.*, 1998). The relative abundance of the two genera in paddy soils appears to be a function of both temperature and acetate concentration (reviewed in Liesack *et al.*, 2000). When acetate concentrations were above the threshold of both genera, the *Methanosaeta* were dominant, presumably because they were more tolerant of suboptimal (15 °C) temperature than the *Methanosarcina* (Chin *et al.*, 1999a,b). The *Methanosarcina* became dominant at high (30 °C) temperature, which is consistent with the fact that they can also use H_2. The reverse of this pattern was observed when acetate levels were maintained below the threshold concentration for both genera. In this case, the *Methanosaeta* were dominant at the high

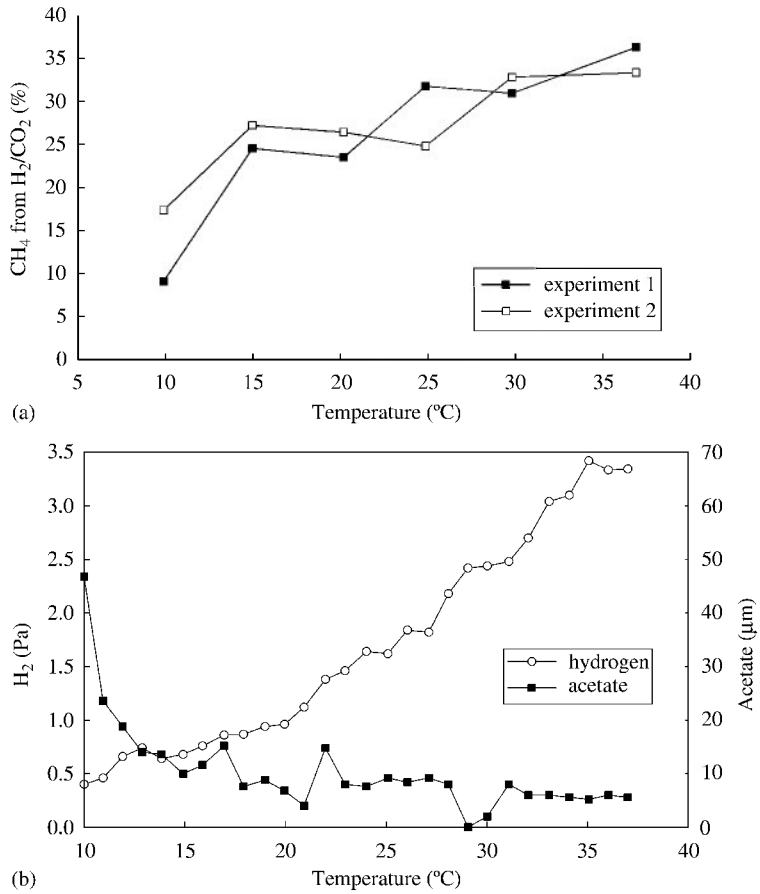

Figure 9 The influence of temperature on: (a) methanogenic pathways and (b) methanogenic substrates (after Fey and Conrad, 2000).

temperature because acetate concentrations were below the threshold concentration of the *Methanosarcina* (Fey and Conrad, 2000).

The extensive work on methanogenic pathways in profundal lake sediments and rice paddy soils does not appear to apply to a broader sample of anaerobic ecosystems. The proportions of CH_4 produced from hydrogenotrophic and acetotrophic methanogenesis did not vary seasonally in a temperate tidal freshwater estuarine sediment (Avery and Martens, 1999; Avery *et al.*, 1999) or a northern peatland soil (Hines *et al.*, 2001) despite changes of 25 °C or more. Temperature alone could not explain transient changes in the methanogenic pathways in a mid-latitude (45° N) peatland (Avery *et al.*, 1999, 2002). Clearly, detailed mechanistic studies on temperature regulation of fermentation and methanogenesis need to be done in a wider variety of anaerobic ecosystems (e.g., Kotsyurbenko *et al.*, 1996).

8.08.4.5 Anaerobic Methane Oxidation

Anaerobic CH_4 oxidation in marine sediments was first observed around the mid-1970s (Barnes and Goldberg, 1976; Reeburgh, 1976; Martens and Berner, 1977). The process was inferred from pore-water SO_4^{2-} and CH_4 profiles because it imparted the CH_4 profile with a strong "concave up" shape (Martens and Berner, 1977). Further geochemical evidence for this process included conversion of $^{14}CH_4$ to $^{14}CO_2$ under anoxic conditions, and a zone of relatively ^{13}C-enriched CH_4 that coincided with a zone of ^{13}C-depleted CO_2 (citations in Valentine, 2002). Although anaerobic CH_4 oxidation was shown to be microbially mediated long ago (Reeburgh, 1982), recent advances in molecular biology and stable isotope techniques have provided compelling new biological insights on the process. Nevertheless, there are no pure cultures or co-cultures of organisms that oxidize CH_4 anaerobically, and several competing hypotheses about the metabolic pathways involved are still considered viable explanations of the existing data (reviewed by Hinrichs and Boetius, 2002; Valentine, 2002).

Three primary mechanisms have been offered to explain anaerobic CH_4 oxidation in marine sediments. The process may be carried out by a single organism that couples methanotrophy to SO_4^{2-} reduction. Anaerobic methane oxidation coupled to sulfate reduction is thermodynamically

favorable under conditions found in marine sediments (Martens and Berner, 1977):

$$CH_4 + SO_4^{2-} \rightarrow HS^- + HCO_3^- + H_2O \quad (6)$$

A second possibility yields the same net reaction but is driven by a syntrophic relationship between an organism that oxidizes CH_4 to CO_2/H_2 and an SO_4^{2-}-reducing bacterium that consumes H_2, thereby maintaining a thermodynamically favorable environment for the reaction (Hoehler *et al.*, 1994). In this case, anaerobic CH_4 oxidation is analogous to hydrogenotrophic methanogenesis in reverse:

$$CH_4 + 2H_2O \rightarrow CO_2 + 4H_2 \quad (7)$$

$$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + HCO_3^- + 4H_2O \quad (8)$$

The ability to run metabolic pathways in forward or reverse, thereby swapping end products for substrates, has been documented previously in anaerobic organisms. For example, the homoacetogen nicknamed *Reversibacter* oxidizes acetate to CO_2/H_2 when the H_2 concentration is low, and vice versa (Lee and Zinder, 1988; Zinder and Koch, 1984). However, attempts to reverse methanogenesis in pure cultures by varying the H_2 concentration have not been successful (Valentine *et al.*, 2000). Thermodynamic and kinetic considerations suggest that acetogenic methanogenesis running in reverse is feasible only in high-CH_4 environments such as CH_4 seeps, as are pathways that require interspecies transfer of acetate (Valentine, 2002). Likewise, pathways the require interspecies transfer of H_2, formate or methanol are not favorable for anaerobic CH_4 oxidation under high- or low-CH_4 conditions (Sørensen *et al.*, 2001). Nauhaus *et al.* (2002) were unable to stimulate SO_4^{2-} reduction in sediments collected from a CH_4 seep by adding H_2, formate, acetate, or methanol.

A modification of the reverse methanogenesis hypothesis was offered by Valentine and Reeburg (2000). This reaction would yield twice the amount of energy normally associated with anaerobic CH_4 oxidation (reaction (6)), making it more thermodynamically favorable:

$$2CH_4 + 2H_2O \rightarrow CH_3COOH + 4H_2 \quad (9)$$

In this case, the methanogens operating in reverse produce acetate and H_2, which are then consumed by SO_4^{2-}-reducing bacteria:

$$SO_4^{2-} + 4H_2 \rightarrow S^{2-} + 4H_2O \quad (10)$$

$$SO_4^{2-} + CH_3COOH \rightarrow H_2S + 2HCO_3^- \quad (11)$$

All of these mechanisms conform to the observation that CH_4 is consumed and S^{2-} is produced in a molar ratio of 1 : 1 in marine sediments (Nauhaus *et al.*, 2002).

The fact that CH_4 is highly depleted in ^{13}C has proven useful for constraining the nature of the microorganisms that oxidize methane anaerobically. Because the $\delta^{13}C$ of CH_4 ranges from about −50‰ to −110‰ PDB (Whiticar *et al.*, 1986), the fate of CH_4-derived carbon can be traced through food webs by means of its distinctive isotopic ratio. Such work has tended to support some form of reverse methanogenesis. Most isotope studies have been done in sediments overlaying CH_4 hydrate deposits or seeps where the high porewater CH_4 concentration favors rapid anaerobic CH_4 oxidation. At such sites, Archea-specific lipids are abundant and highly depleted in ^{13}C (often <−100‰), indicating they could only be derived from CH_4 (Bian *et al.*, 2001; Elvert *et al.*, 1999, 2000; Hinrichs *et al.*, 1999, 2000; Orphan *et al.*, 2001a; Peckmann *et al.*, 1999; Pancost *et al.*, 2000, 2001; Thiel *et al.*, 1999, 2001). Interestingly, fatty acids that are characteristic of SO_4^{2-}-reducing bacteria are also highly ^{13}C-depleted (up to −75‰) suggesting that SO_4^{2-} reducers are consuming an organic intermediate produced by methane oxidizers (Boetius *et al.*, 2000b; Hinrichs *et al.*, 1999; Orphan *et al.*, 2001a,b; Thiel *et al.*, 2001). If so, this is evidence that anaerobic CH_4 oxidation is performed by an Archea–Bacteria consortium.

Perhaps the most striking evidence of a consortium between Archea and Bacteria is their spatial arrangement in hydrate seep sediments. Using fluorescent phylogenetic stains, Boetius *et al.* (2000b) observed clusters of ~100 Archeal cells surrounded by a 1–2 cell-thick shell of sulfate-reducing bacteria (Figure 10). Orphan *et al.* (2001b) further showed that highly depleted $\delta^{13}C$

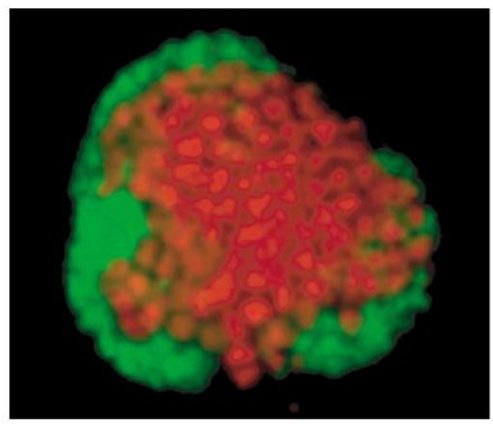

Figure 10 *In situ* identification of microbial aggregates consisting of Archea (red) in the center and SO_4^{2-}-reducing bacteria (green) on the periphery. Microorganisms were labeled with rRNA-targeted oligionucleotide probes. (source Boetius *et al.*, 2000b).

ratios coincided with the Archeal core of these aggregates. Collectively, the evidence supports a syntrophic model of anaerobic CH_4 oxidation in which methanogen-like Archea oxidize CH_4, and SO_4^{2-}-reducing bacteria consume end products, thereby making the reaction thermodynamically favorable. It remains to be determined whether the Archeal member is a methanogen capable of both CH_4 production and oxidation, or an obligate methanotroph. Highly ^{13}C-depleted Archeal lipids have also been observed in solitary organisms, suggesting a microbial consortium is not necessarily a requirement for anaerobic CH_4 oxidation (Orphan *et al.*, 2002).

Little is known about the organisms that oxidize methane anaerobically except for the broad outlines of their phylogeny. Sequences of 16S rRNA cloned from several CH_4 seep sediments are dominated by two groups of previously unknown Archeal genes, ANME-1 and ANME-2 (Boetius *et al.*, 2000b; Hinrichs *et al.*, 1999; Orphan *et al.*, 2001a). The ANME-1 group typically occurs as single filaments or monospecific mats, and does not appear require a bacterial partner (Orphan *et al.*, 2002). They have been recovered from hydrothermal vents (Takai and Horikoshi, 1999; Teske *et al.*, 2002), methane hydrates (Lanoil *et al.*, 2001), and shallow marine sediments (Thomsen *et al.*, 2001). Massive ANME-1 "reefs" were recently discovered in the Black Sea (Michaelis *et al.*, 2002). The cores of anaerobic methane-oxidizing aggregates have so far been composed of ANME-2 type Archea. This group is closely related to known methanogens in the *Methanosarcinales*, an order with members that ferment acetate to CH_4, but may also oxidize small amounts of acetate to CH_4 (Zehnder and Brock, 1979). The proposition that some members of the anaerobic consortium are capable of oxidizing CH_4 to acetate and H_2 (reaction (9)) is attractive because it provides a mechanism to explain the presence of highly ^{13}C-depleted lipids in SO_4-reducing bacteria (Valentine and Reeburg, 2000). It is known that acetate is consumed by members of the same SO_4-reducing group found in CH_4 seep sediments (i.e., *Desulfosarcinales*).

Judging by the microbial diversity of methane seeps, anaerobic CH_4 oxidation may not be fully explained by any single mechanism proposed so far. Many of the SRB associated with anaerobic CH_4 oxidation environments are unknown and unique (Thomsen *et al.*, 2001), and biomarker data suggest a great deal of taxonomic diversity among both Archea and Bacteria in sediments that support anaerobic CH_4 oxidation (Orphan *et al.*, 2001a; Thiel *et al.*, 2001). Specific inhibitors intended for methanogens, SO_4^{2-} reducers, and acetate oxidizers have sometimes influenced rates of anaerobic CH_4 oxidation (Hoehler *et al.*, 1994) and had no effect at other times (Alperin and Reeburgh, 1985). Anaerobic CH_4 oxidation occurs in freshwater environments where SO_4^{2-} concentrations are low (Smith *et al.*, 1991; Miura *et al.*, 1992; Murase and Kimura, 1994a,b; Smemo and Yavitt, 2000; Grossman *et al.*, 2002), suggesting that electron acceptors other than SO_4^{2-} may play a role in the process. Thermodynamic considerations suggest that anaerobic CH_4 oxidation pathways may vary between sites as a function of CH_4 partial pressure (Valentine, 2002). Further progress will undoubtedly require isolating or enriching the responsible organisms.

Very little information exists about the ecological conditions that govern anaerobic CH_4 oxidation. Manipulation of a CH_4 seep sediment demonstrated a broad temperature optimum of 4–16 °C, and rates that varied predictably with temperature (Nauhaus *et al.*, 2002). The process is widespread in marine sediments and water columns provided there is a sufficient supply of labile organic carbon to permit substantial methanogenesis (Section 8.0.8.4.3.4), or some other CH_4 source exists. Anaerobic CH_4 oxidation is the primary sink for CH_4 in the world oceans (D'Hondt *et al.*, 2002), and it accounts for a large fraction of the SO_4^{2-} reduced in some marine sediments (Table 6), particularly in the narrow zone where CH_4 and SO_4^{2-} profiles intersect. Sulfate reduction rates as high as 140 mmol m^{-2} d^{-1} have been reported above decomposing CH_4 hydrate deposits (Boetius *et al.*, 2000b), suggesting that anaerobic oxidation significantly reduces CH_4 flux to the water column from these vast carbon reservoirs (Gornitz and Fung, 1994).

8.08.4.6 Aerobic Methane Oxidation

Methane is subject to aerobic oxidation by methanotrophic bacteria when it diffuses across an anoxic–oxic interface before escaping to the atmosphere (King, 1992):

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad (12)$$

Methanotrophs occur at the oxic–anoxic interface of methanogenic habitats, in symbiotic association with animals (Kochevar *et al.*, 1992), and *inside* wetland plants (Bosse and Frenzel, 1997). Although methanotrophs dominate aerobic CH_4 oxidation, NH_4-oxidizing bacteria may account for a small amount of the CH_4 oxidation activity in soils and sediments (Bodelier and Frenzel, 1999).

8.08.4.6.1 Methanotroph diversity

Methanotrophs are obligate aerobes that use CH_4 as a sole carbon and energy source (reviewed by R. S. Hanson and T. E. Hanson, 1996). They are a subset of the methylotrophic bacteria, all of which oxidize compounds lacking C–C bonds

Table 6 Selected estimates of the proportion of SO_4^{2-} reduction in marine sediments that is mediated by anaerobic CH_4 oxidation.

Site	*Peak contribution to SO_4^{2-} reduction (%)*	*Depth-integrated contribution to SO_4^{2-} reduction (%)*	*Citation*
Aarhus Bay, Denmark	47–52	9	Thomsen *et al.* (2001)
Kattegat, Denmark	61	10	Iverson and Jørgensen (1985)
Skagerrak	89	10	Iverson and Jørgensen (1985)
Upwelling Zone, Namibia	100		Niewöhner *et al.* (1998)
Amazon Fan Sediment	50–85		Burns (1998)
Norsminde Fjorde	10–30		Hansen *et al.* (1998)
Big Soda Lake, Nevada		2	Iversen *et al.* (1987)
Kysing Fjord		<0.1	Iversen and Blackburn (1981)
Hydrate Ridge, Oregon[a]	100	100	Boetius *et al.* (2000b)

[a] Inferred by comparing SRR above decomposing CH_4 hydrates to nearby nonhydrate sites.

(i.e., C_1 compounds) (Murrell and Kelley, 1993). The ability to metabolize C_1 compounds is a feature that methylotrophs have in common with methanogens. Indeed, the two groups share many homologous genes for C_1 metabolism, despite the large evolutionary distance between the *Archea* and the *Proteobacteria* (Chistoserdova *et al.*, 1998).

Methanotrophic species are separated into two types that differ with respect to phylogeny, ultrastructure, lipid composition, biochemistry, and physiology. The type I methanotrophs belong to the family *Methylococcaceae* in the gamma proteobacteria; the type II methanotrophs are in the family *Methylocystaceae* in the alpha proteobacteria. There is growing evidence that the two groups also differ ecologically. Amaral and Knowles (1995) used opposing gradients of CH_4 and O_2 to determine that a type I methanotroph preferred a somewhat lower CH_4 concentration than a type II methanotroph. An often-cited ecological distinction between the two groups is that only the type II methanotrophs are N_2 fixers (R .S. Hanson and T. E. Hanson, 1996). This is consistent with a report that a type II methanotroph out-competed a type I methanotroph in a nitrogen-limited chemostat culture (Graham *et al.*, 1993), and in a field study where NH_4^+ fertilization increased the proportion of type I methanotrophs in planted rice paddy soils (Bodelier *et al.*, 2000b). In the latter study, the abundance of type II methanotrophs did not change in response to nitrogen fertilization, suggesting that only the type I methanotrophs were nitrogen limited. However, a survey of several type I and type II methanotrophic strains for N_2-fixation genes (*nif*H) and nitrogenase activity demonstrated that the capacity for N_2 fixation occurs in both groups (Auman *et al.*, 2001). Thus, it is no longer valid to assume that type I methanotrophs lack the capacity for N_2 fixation. The extent to which nitrogenase activity is expressed by type I methanotrophs *in situ* remains to be determined.

All known methanotrophs have a low affinity for CH_4 ($K_m > 1$ μM) and none can maintain growth on CH_4 at atmospheric concentration (for a compilation of kinetic data see Conrad, 1996). Although there are no pure cultures of "high affinity" CH_4 oxidizers, these organisms are known to exist in upland soils where they account for ~10% of the annual global CH_4 sink (Table 5). High-affinity CH_4 oxidizers evidently occur in wetland soils as well because there have been reports of net CH_4 consumption from the atmosphere in response to falling water table depth (Happell and Chanton, 1993; Harriss *et al.*, 1982; Pulliam, 1993; Roulet *et al.*, 1993; Shannon and White, 1994), low temperature (Megonigal and Schlesinger, 2002), and low soil organic matter (Giani *et al.*, 1996). The capacity for both types of oxidation kinetics in saturated soils and sediments has been assumed to be due to the presence of mixed methanotrophic populations (Bender and Conrad, 1992). This was confirmed in a recent study that demonstrated the power of combining lipid biomarker analysis with isotope pulse-labeling (Boschker *et al.*, 1998). Bull *et al.* (2000) pulse-labeled a forest soil with $^{13}CH_4$, and followed its incorporation into phospholipid fatty acids (PLFAs). At the soil surface, where ambient CH_4 levels prevail, the label was incorporated into novel organisms with PLFAs similar to type II methanotrophs. A buried soil horizon with higher CH_4 levels (100 μL L^{-1}) indicated the presence of both type I and type II methanotrophs. Another promising technique is "stable-isotope probing" in which a microbial community is exposed to an isotopically labeled substrate (e.g., $^{13}CH_4$), thereby changing the mass of organisms that assimilate the substrate. The

labeled and unlabeled microorganisms are separated by density-gradient centrifugation and DNA sequenced (Radajewski *et al.*, 2000). This technique demonstrated that novel proteobacteria contributed to high-affinity CH_4 oxidation in a peat soil (Morris *et al.*, 2002).

Until recently, all cultured methanotrophs required a pH $\geq$5 and most were neutrophilic (R. S. Hanson and T. E. Hanson, 1996). Yet, CH_4 oxidation at pH $<$5 has been demonstrated in acidic *Sphagnum*-bogs (Born *et al.*, 1990; Dunfield *et al.*, 1993; Fechner and Hemond, 1992; Yavitt *et al.*, 1990). Relatively recently, acidophilic methanotrophic bacteria have been isolated and two species have been described with an optimum activity between pH 4.5 and 5.5 (Dedysh *et al.*, 1998a,b, 2000, 2002). This demonstrates that some methanotrophs are adapted to the ambient pH conditions of their environment.

The relative abundance of various methanotrophic species or types has been investigated in peatlands, rice paddies, lakes and elsewhere with PLFA analyses, community DNA analyses (McDonald and Murrell, 1997), and fluorescent *in situ* hybridization (FISH). A suite of FISH probes has been developed for methanotrophs with a range of specificity for types, genera and species (Amann *et al.*, 1990b; Bourne *et al.*, 2000; Dedysh *et al.*, 2001; Eller *et al.*, 2001; Gulledge *et al.*, 2001; Rosselló-Mora *et al.*, 1995). The use of such probes in an acidic northern peatland showed that $<$1% of the methanotrophic community was type I. Although the remainder could tolerate low pH, $<$5% were known acidophiles (Dedysh *et al.*, 2003). A single genus, *Methylocystis*, contributed 60–95% of all detectable methanotrophic cells.

8.08.4.6.2 Regulation of methanotrophy

Methane oxidation rates can be limited by O_2, CH_4, or nitrogen. Limitation by O_2 is most likely to occur in continuously flooded or submerged systems that are strongly methanogenic. Methane oxidation is limited by O_2 flux to the sediment surface in lakes (Frenzel *et al.*, 1990) and flooded wetlands (King, 1990a,b; King *et al.*, 1990). For this reason, the presence of algal mats or submerged aquatic vegetation can cause diurnal variations in CH_4 oxidation, with the highest rates during daylight due to photosynthetic O_2 production (King, 1990b). Heilman and Carlton (2001) reported a particularly dramatic example of diel variation in CH_4 emissions driven by O_2 release from submerged aquatic macrophytes. In this case, the sediment was a net CH_4 sink in daylight and a CH_4 source at night. However, they concluded that O_2 was inhibiting methanogenesis rather than stimulating methanotrophy. Methanotrophy itself can account for a substantial fraction of the O_2 demand in some systems; it accounted for 30% of O_2 consumption in Lake Erie (Adams *et al.*, 1982) and $>$9% of O_2 consumption in Lake Constance (Frenzel *et al.*, 1990).

Root-associated methanotrophy has been demonstrated to be O_2-limited in the rhizosphere of emergent aquatic macrophytes such as *Phragmites australis* (van der Nat and Middelburg, 1998), *Sparganium eurycarpum* (King, 1996a), and *Typha latifolia* (Lombardi *et al.*, 1997). Plant species with high rates of root O_2 loss also support high rates of rhizosphere CH_4 oxidation (Calhoun and King, 1997), further suggesting the process is generally O_2 limited. Differences in root O_2 loss rates among plant species should cause species-specific differences in rhizosphere CH_4 oxidation rates if the process is O_2 limited. Indeed, soil-free root CH_4 oxidation potentials vary among plant species (Sorrell *et al.*, 2002). Because $>$75% of the CH_4 efflux from wetlands may pass through plants (Chanton and Dacey, 1991; King, 1996a; Shannon and White, 1994), O_2 limitation in the rhizosphere should translate into O_2 limitation on an ecosystem basis.

By comparison with uncultivated plants, the case for O_2 limitation in the rice rhizosphere is not so clear. Linear correlations between rhizosphere CH_4 oxidation and CH_4 concentration suggested that oxidation is CH_4-limited (Bosse and Frenzel, 1998; Gilbert and Frenzel, 1995; Gilbert and Frenzel, 1998). This result is consistent with a study by Denier van der Gon and Neue (1996) in which the atmospheric O_2 concentration was doubled to 40% around rice shoots, but CH_4 oxidation increased by only 20%. However, kinetic and theoretical considerations have also suggested that O_2 limits rhizosphere CH_4 oxidation in rice (Bosse and Frenzel, 1997; van Bodegom *et al.*, 2001).

To the extent that O_2 limitation exists, O_2 competition is an important factor regulating CH_4 oxidation efficiency. Based on kinetic modeling, van Bodegom *et al.* (2001) concluded that methanotrophs were only able to out compete heterotrophic bacteria for O_2 when O_2 concentrations were $<$10 μM. The competitive advantage of the methanotrophs at low O_2 concentrations was due to their high affinity for O_2. There are other microaerophilic bacteria with a high affinity for O_2 (e.g., Fe(II)-oxidizing bacteria, Section 8.08.6.5.2) that compete with methanotrophs for O_2, as well as abiotic oxidation reactions involving Fe(II) or H_2S. The continued development of simulation models, such as the one offered by van Bodegom *et al.* (2001), will help integrate the effects of the multiple factors affecting rhizosphere CH_4 oxidation.

Methane availability is likely to limit oxidation in systems that are weakly methanogenic, such as marine sediments or dry-end wetlands that have

exposed soils and subsurface water tables. Methane-limited methanotrophy was recently demonstrated in a pair of tidal forested wetlands that were aerobic in the top 5–10 cm (Megonigal and Schlesinger, 2002; Figure 11). Indirect evidence for CH_4-limited methanotrophy in peatland ecosystems is the observation that potential CH_4 oxidation tends to peak near the water table boundary, where CH_4 concentrations are high and O_2 concentrations are low (Kettunen *et al.*, 1999; Sundh *et al.*, 1995; Sundh *et al.*, 1994). Such a relationship should be common in bog-type peatlands that are typically dry at the soil surface, have relatively low potential CH_4 production rates, and are dominated by nonvascular plants. Methanotrophy in bogs can also be limited by high rates of diffusivity through the soil profile due to low bulk density (Freeman *et al.*, 2002).

Methane oxidation can be limited by nitrogen due to enzyme-level inhibition or unmet methanotroph nitrogen demand. The oxidation of CH_4 by methanotrophic bacteria and NH_4^+ by ammonium-oxidizing bacteria is initiated by similar enzymes—methane monooxygenase (MMO) and ammonium monooxygenase (AMO). The membrane form of MMO is evolutionarily related to AMO (Holmes *et al.*, 1995); there is also a soluble form of MMO. MMO and AMO fortuitously oxidize a variety of additional compounds (Hanson and Hanson, 1996), including NH_4^+ (MMO) and CH_4 (AMO). Bodelier and Frenzel (1999) determined that the contribution of nitrifiers to CH_4 oxidation was negligible in rice paddy microcosms; however, methanotrophs contributed substantially to NH_4^+ oxidation. Competition between NH_4^+ and CH_4 for the active site on MMO is one explanation for the common observation that nitrogenous fertilizers inhibit CH_4 oxidation (Steudler *et al.*, 1989; Conrad and Rothfuss, 1991; Crill *et al.*, 1994; King, 1990a), and it is consistent with fact that inhibition often varies with the relative concentrations of CH_4 and NH_4^+ (Bosse *et al.*, 1993; Boeckx and Van Cleemput, 1996; Dunfield and Knowles, 1995; King and Schnell, 1994a,b; Schnell and King, 1994; van der Nat *et al.*, 1997). A second explanation for depressed CH_4 oxidation is NO_2^- toxicity (King and Schnell, 1994b; Kravchenko, 1999). Both of these factors should be relatively unimportant in anaerobic environments because CH_4 concentrations are high and nitrification rates are low due to limited O_2 availability. Indeed, nitrogen fertilization unexpectedly stimulated CH_4 oxidation in rice paddy soils (Bodelier *et al.*, 2000b), where both CH_4 concentrations and plant nitrogen demand are typically high. These authors used a combination of radioisotope labeling and PFLA analysis to determine that type I methanotrophs were nitrogen-limited in the absence of fertilizer application. Using the same technique, Nold *et al.* (1999) observed that NH_4^+ additions decreased CH_4 incorporation into methanotroph lipids in a lake sediment. Although CH_4 oxidation is clearly influenced by fertilization, the variety of different responses that have been observed and the number of mechanisms that have been proposed for these effects makes it difficult to generalize about the process (Gulledge *et al.*, 1997).

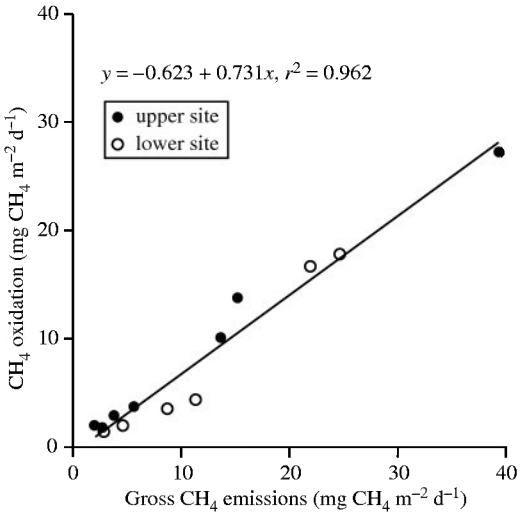

Figure 11 Relationship between rates of gross CH_4 emission and CH_4 oxidation measured over a 13-month period in two tidal freshwater wetlands (source Megonigal and Schlesinger, 2002).

In a recent compilation of the wetland CH_4 oxidation literature, Segers (1998) concluded that pH is not an important factor governing aerobic CH_4 oxidation, salt concentrations of ~40 mM inhibit the process, and aerobic CH_4 oxidation responds to temperature with $Q_{10} = \sim 2$. The Q_{10} for CH_4 oxidation can be <2 when there is a phase-transfer limitation on CH_4 diffusion (e.g., King and Adamsen, 1992).

8.08.4.6.3 Methane oxidation efficiency

The amount of CH_4 emitted to the atmosphere is just a fraction of that produced because of methanotrophy. Globally, it accounts for ~80% of gross CH_4 production (Reeburgh, 1996; Reeburgh *et al.*, 1993). The wetland CH_4 oxidation sink has been estimated at 40% (King, 1996b) to 70% (Reeburgh *et al.*, 1993) of gross CH_4 production, or roughly 100–400 Tg yr^{-1}. Methane oxidation efficiency is generally higher in wetland forests than marshes, perhaps because forests occupy drier positions on the landscape and develop a relatively deep oxic

zone at the soil surface (Megonigal and Schlesinger, 2002). It follows that wetland forests are more likely than marshes to be CH_4 limited. In some cases, CH_4 oxidation efficiency is 100% and the site becomes a net CH_4 sink (Harriss *et al.*, 1982).

The path taken by a CH_4 molecule en route to the atmosphere affects the chance it will be oxidized. Oxidation is perhaps most likely when CH_4 diffuses across a soil or sediment surface because of the potential for a wide aerobic zone, and a correspondingly long residence time in the aerobic zone. One of the highest CH_4 oxidation rates that has been reported was measured in a landfill soil that had high pore space CH_4 concentrations overlaid by >1.5 m of aerobic topsoil (Whalen *et al.*, 1990). In most cases, the potential for CH_4 oxidation in the rhizosphere will be limited by a narrow aerobic zone of perhaps 1–2 mm, or an aerobic zone may be absent altogether (e.g., King *et al.*, 1990; Roura-Carol and Freeman, 1999). However, there is a notable exception in which O_2 from roots can penetrate a few centimeters into the soil (Pedersen *et al.*, 1995). Methane oxidation efficiency in the rhizosphere is likely to be lower than at the soil surface because the residence time of CH_4 in the rhizosphere is relatively short. For example, King (1996a) determined that 43% of the CH_4 crossing the soil surface was oxidized compared to 27% of the CH_4 crossing the rhizosphere in a freshwater marsh. Methane bubbles (i.e., ebullition) bypass the CH_4 oxidation zone altogether. Ebullition occurs when the CH_4 partial pressure exceeds a critical threshold. It is a significant process in aquatic sediments (Chanton and Martens, 1988; Martens and Klump, 1980; Zimov *et al.*, 1997) and is generally under-appreciated as a source of CH_4 emissions from wetlands (Romanowicz *et al.*, 1993). Because plants efficiently evacuate CH_4 to the atmosphere by passive diffusion or mass flow through stems (Chanton and Dacey, 1991), they improve CH_4 oxidation efficiency by decreasing ebullition (Bosse and Frenzel, 1998; Grünfeld and Brix, 1999). Understanding the proportion of CH_4 efflux that occurs by these three pathways is essential for modeling the response of natural wetlands, rice paddies and landfills to management or climate change (Bogner *et al.*, 2000).

A variety of techniques have been used to estimate the proportion of gross production that is consumed by CH_4 oxidation (i.e., CH_4 oxidation efficiency), most of which involve some degree of manipulation. A common approach is to measure methanotrophy in the presence and absence of O_2. However, this approach will underestimate the process if it simultaneously stimulates CH_4 production. An alternative approach is to apply a specific inhibitor of methanotrophy. This technique was used by de Bont *et al.* (1978) to estimate methanotrophy in the rice rhizosphere. They applied acetylene gas to the shoot, and allowed it to diffuse through the stem, along with O_2, into the root zone where it blocked methanotrophy. Acetylene has the unfortunate property of causing irreversible inhibition, but it is far less expensive and equally effective as some alternative inhibitors (King, 1996a). Methylfluoride (CH_3F) is an attractive inhibitor because the effects are reversible, so that it can be used many times on the same site (Epp and Chanton, 1993; Oremland and Culbertson, 1992a,b). However, CH_3F inhibits acetotrophic methanogenesis and may therefore underestimate CH_4 oxidation. This is not necessarily a problem when the gas is applied to shoots (King, 1996a; Megonigal and Schlesinger, 2002), but the concentration and duration of application should be considered beforehand (Lombardi *et al.*, 1997). Recently, difluoromethane was shown to reversibly inhibit CH_4 oxidation without severely affecting hydrogenotrophic or acetotrophic methanogenesis (Miller *et al.*, 1998). Stable isotope ratios offer a nonintrusive means of estimating CH_4 oxidation (e.g., Popp and Chanton, 1999).

8.08.4.7 Wetland Methane Emissions and Global Change

The influence of global-scale changes in climate, nutrient availability and land use on CH_4 efflux from freshwater wetlands is of much current interest because they are one-third of all CH_4 sources to the atmosphere. Methane emissions from wetlands *per se* are not considered in detail here because the topic falls outside the primary scope of this review and many excellent reviews already exist (Matthews and Fung, 1987; Aselmann and Crutzen, 1989; Bouwman, 1991; Neue, 1993; Bartlett and Harriss, 1993; Bubier and Moore, 1994; Bridgham *et al.*, 1995; Denier van der Gon and Neue, 1995; Vourlitis and Oechel, 1997; Minami and Takata, 1997; Le Mer and Roger, 2001). In a compilation of 48 published studies of wetland CH_4 emissions (Le Mer and Roger, 2001), median rates were 0.43 kg CH_4 ha^{-1} d^{-1} for peatlands, 0.72 kg CH_4 ha^{-1} d^{-1} for other nonagriculture freshwater wetlands, and 1.0 kg CH_4 ha^{-1} d^{-1} for rice paddies. This ranking matches that for primary production and is consistent with the large body of evidence that suggests methanogenesis is closely coupled to the photosynthetic carbon supply (Sections 8.08.4.3.4 and 8.08.4.3.5). In this section, we briefly consider the influence of global change on CH_4 emissions.

Rising atmospheric CO_2 will influence CH_4 emissions from wetlands even in the absence of

changes in climate. Temperate and tropical wetland ecosystems, including rice paddies, consistently respond to elevated CO_2 with an increase in photosynthesis and CH_4 emissions (Dacey *et al.*, 1994; Megonigal and Schlesinger, 1997; Ziska *et al.*, 1998), although one exception has been reported (Schrope *et al.*, 1999). One study reported a strong linear relationship ($r^2 = 0.87–0.97$) between photosynthesis and CH_4 emissions, suggesting that the elevated CO_2 response is due to an increase in the supply of labile carbon compounds (Vann and Megonigal, 2003). By comparison, the responses of northern peatland ecosystems have been inconsistent, with several studies reporting negligible effects on photosynthesis, primary production or CH_4 emissions (Berendse *et al.*, 2001; Hutchin *et al.*, 1995; Hoosbeek *et al.*, 2001; Kang *et al.*, 2001; Saarnio *et al.*, 1998, 2000). A likely reason for the absence of a photosynthetic response to elevated CO_2 in these systems is nitrogen limitation. In a study of tussock tundra, Oechel *et al.* (1994) found that the elevated CO_2 treatment did not enhance photosynthesis for more than a few months unless it was crossed with a 4 °C increase in air temperature. They suggested that warming relieved a severe nutrient limitation on photosynthesis by increasing nitrogen mineralization. Thus, the effects of elevated CO_2 alone are expected to interact with nitrogen deposition and temperature. The influence of elevated CO_2 may be particularly important in tropical wetlands in which nitrogen mineralization and methanogenesis are not temperature-limited. These systems account for about 60% of all CH_4 emitted from natural wetlands (Table 5).

Temperature changes are anticipated to be the largest at latitudes above 45° N that hold 57% of all wetland areas and 33% of the global soil carbon pool. Northern hemisphere wetlands contribute about 10% of all CH_4 emissions (Table 5). Tropical areas emit amounts of CH_4 comparable to northern-hemisphere wetlands (Aselmann and Crutzen, 1989; Matthews and Fung, 1987). This region is also expected to warm in the coming decades (Kattenberg *et al.*, 1996), but efforts to quantify the contribution of southern hemisphere peatlands to global carbon storage and fluxes are just beginning (Thompson *et al.*, 2001). Increasing temperature stimulates the decomposition and fermentation of soil organic matter (Section 8.08.4.3.3), and many studies have reported temperature-dependent increases in CH_4 emissions as a result (Bartlett and Harriss, 1993). However, the most important impacts of rising temperature on CH_4 emissions are likely to be realized through changes in the species composition and metabolic activity of plants (Schimel, 1995). A temperature-induced increase in nitrogen mineralization is expected to stimulate plant productivity in northern bogs, and simultaneously lengthen the growing season (Myneni *et al.*, 1997). Verville *et al.* (1998) concluded that plant community composition had a larger effect on CH_4 emissions than direct changes in temperature in wet tussock tundra. A similar conclusion was reached in a study of global change variables in a fen peatland (Granberg *et al.*, 2001). These results are consistent with evidence that the influence of plants on substrate availability (Section 8.08.4.3.5), CH_4 ventilation, and methanotrophy (Section 8.08.4.6.3) vary strongly among plant species.

The effects of global warming on hydrology are relatively uncertain compared to temperature. The long-term effects of a falling water table will be a decrease in CH_4 emissions (e.g., Nykänen *et al.*, 1998) caused by lower CH_4 production, lower CH_4 oxidation or both. Freeman *et al.* (2002) concluded that reduced CH_4 emissions following a simulated drought in a peatland were due mainly to its effect on methanogenesis, and only secondarily to methanotrophy. They proposed that CH_4 diffusion through the unsaturated soil surface was too rapid to permit an increase in CH_4 oxidation efficiency; this is plausible in peat soils because they typically have a low bulk density at the surface, but is unlikely to apply to mineral soil wetlands unless they are on sands. Methane emissions often recover slowly following drought because of reduced methanogen populations and regeneration of alternative electron acceptors.

Human activity has substantially increased the hydrologic import of oxidized nitrogen and sulfur into wetland ecosystems. Emissions of sulfur are expected to double over the next 50 years (Rodhe, 1999), and anthropogenic N_2 fixation will also increase dramatically (Section 8.08.5.1). This raises the possibility that methanogenesis will be suppressed due to substrate competition between methanogens and denitrifiers or SO_4^{2-} reducers. It is well known that methanogenesis is low in SO_4^{2-}-rich marine sediments, and SO_4^{2-} amendments to rice paddies suppress CH_4 production and emissions. However, even the low doses of SO_4^{2-} in acid rain can suppress methanogenesis (Dise and Verry, 2001). Gauci *et al.* (2002) suggested that 1990 levels of SO_4^{2-} deposition depressed CH_4 emissions from northern wetlands by 5–17%. The effects of nitrogen fertilization on CH_4 emissions are variable and depend on the integrated responses of plants, methanogens and methanotrophs, which can offset one another (Bodelier *et al.*, 2000b). Several studies have shown a net increase in CH_4 emissions with nitrogen fertilization (Banik *et al.*, 1996; Lindau *et al.*, 1991).

It is important to test how various factors that force global changes interact because the results may be nonadditive or counterintuitive. Granberg *et al.* (2001) evaluated CH_4 emissions from a fen

in response to crossed treatments of elevated air temperature, nitrogen deposition, and sulfur deposition. They found that SO_4^{2-} additions depressed CH_4 emissions at ambient temperature, but not at elevated temperature. The reason for this unexpected result was that pore-water SO_4^{2-} concentrations did not increase at elevated temperature, presumably because of stronger SO_4^{2-} sinks such as plant uptake.

Global changes in climate and nutrient availability are expected to alter the existing source–sink balance of several radiatively active trace gases including CO_2, CH_4, and N_2O. Elevated atmospheric CO_2 and falling water tables have the potential to increase carbon sequestration in biomass if forests replace herbaceous communities (Trettin and Jurgensen, 2003). However, an increase in primary production will translate into an increase in CH_4 emissions due to improved substrate supply (Whiting and Chanton, 2001). Falling water tables will decrease CH_4 emissions, but may increase N_2O emissions (Martikainen *et al.*, 1995). At present, it is uncertain whether feedbacks between climate change and wetland metabolism will exacerbate or mitigate radiative forcing. This can be determined only by accounting for changes in the current fluxes of CO_2, CH_4, and N_2O, allowing for differences in their global warming potential (e.g., Liikanen *et al.*, 2002).

8.08.5 NITROGEN

All forms of anaerobic metabolism are influenced directly or indirectly by O_2, but this is particularly true of nitrogen metabolism. Many of the organisms that anaerobically transform nitrogen perform best under aerobic conditions, resorting to anaerobic metabolism in order to cope with low O_2 availability (Tiedje, 1988). This physiological versatility is favored by the fact that nitrogen oxides can replace O_2 as terminal electron acceptors with only a small loss of energy yield (Table 1), and it is the reason that nitrogen-based anaerobic metabolism is so cosmopolitan. Denitrification occurs in even the driest ecosystems on Earth (Peterjohn, 1991). Whereas most of this review is concerned with saturated soils and sediments as venues for anaerobic metabolism, the section on nitrogen includes work in upland soils. Here we focus on anaerobic metabolism involving nitrogen, but it should be recognized that aerobic metabolism is important to the physiological ecology of many of the organisms concerned.

Research on nitrogen-based anaerobic metabolism has addressed denitrification to the exclusion of several other pathways. Recent advances make it clear that some less-studied pathways make a larger contribution to NO_3^- consumption or N_2 production than previously believed (Zehr and Ward, 2002). Furthermore, completely new metabolic pathways have been discovered in the past decade that will require a re-evaluation of nitrogen sinks in many ecosystems.

Although processes are the focus of this review, there have been significant strides made in quantifying N_2 fixation, denitrification and other nitrogen transformations at large scales (e.g., van Breemen *et al.*, 2002). There exist several recent compilations and reviews of nitrogen oxide emissions (Olivier *et al.*, 1998) and denitrification rates (Barton *et al.*, 1999; Herbert, 1999), including one focused on studies that used the ^{15}N isotope pairing technique (Steingruber *et al.*, 2001).

8.08.5.1 Nitrogen in the Environment

Microbial transformations of nitrogen have a dramatic influence on the structure and function of ecosystems because nitrogen availability often establishes the upper limit of productivity, particularly in terrestrial and marine ecosystems. Widespread nitrogen limitation is somewhat ironic because the atmosphere is a vast reservoir of N_2, yet no eukaryote can use nitrogen in this form. Plants require nitrogen in the form of NO_3^- (nitrate), NH_4^+ (ammonium) or organic-N, and most animals ultimately derive nitrogen from plants (except perhaps rock-eating snails (Jones and Shachak, 1990)!). These compounds have single or double covalent bonds that require far less energy to sever than the triple bonds of N_2, and they are referred to collectively as *fixed*, *combined* or *biologically available* nitrogen. Prokaryotes are unique in their ability to convert N_2 to fixed nitrogen (i.e., perform N_2 fixation), and they participate in nearly every other important nitrogen transformation (Figure 12).

Among several factors that favor persistent nitrogen limitation is the fact that prokaryotes also convert fixed nitrogen back to N_2 in the process of denitrification (Vitousek and Howarth, 1991), a dominant form of anaerobic microbial metabolism. The global inventory of fixed nitrogen is negligible compared to the atmospheric N_2 reservoir, because denitrification has roughly balanced N_2 fixation over eons. Denitrification is also a source of nitrous oxide (N_2O) and nitric oxide (NO), which are far less benign gases. Nitrous oxide is a greenhouse gas that is ~300 times more effective at radiative forcing than CO_2 on a mole basis (Ramaswamy *et al.*, 2001), and accounts for ~6% of the radiative forcing since ca. 1750. Because its lifetime in the atmosphere is very long, N_2O mixes in to the stratosphere where it promotes O_3 destruction. Nitric oxide is an ingredient in smog and promotes tropospheric O_3 production, which is a danger to human health. Emissions of N_2O and NO have increased in the

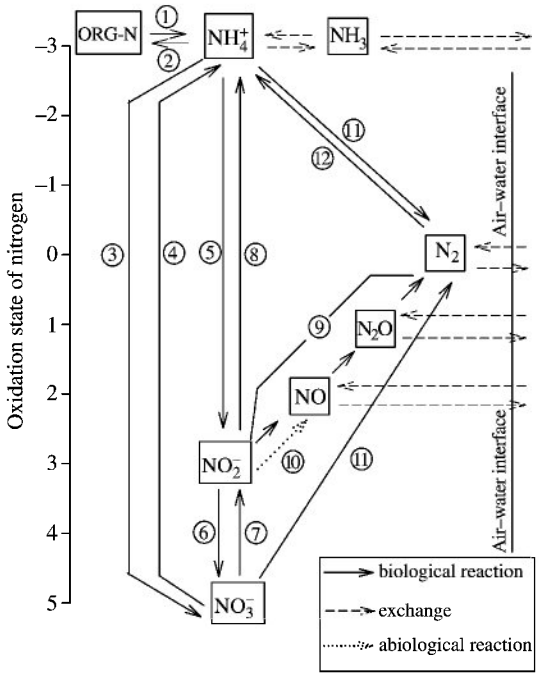

Figure 12 Major reduction–oxidation reactions involving nitrogen. The reactions are numbered as follows: (1) mineralization, (2) ammonium assimilation, (3) nitrification, (4) assimilatory or dissimilatory nitrate reduction, (5) ammonium oxidation, (6) nitrite oxidation, (7) assimilatory or dissimilatory nitrate reduction, (8) assimilatory or dissimilatory nitrite reduction, (9) denitrification, (10) chemodenitrification, (11) anaerobic ammonium oxidation, and (12) dinitrogen fixation (after Capone, 1991) (reproduced by permission of ASM Press from *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*, **1991**).

past decades from aerobic and anaerobic sources due to the widespread use of fertilizers and fossil fuel combustion.

Human activity has roughly doubled the rate of N_2 fixation (Vitousek *et al.*, 1997), and thereby enhanced microbial nitrogen transformations (Galloway *et al.*, 1995). The enhanced metabolism of N_2-fixing bacteria through cultivation of legumes was an intended outcome of this activity, but many other effects have been unintended. Transport of nitrogen through water, air, and sediments has increased the productivity of adjacent ecosystems, resulting in terrestrial and aquatic eutrophication (Meyer-Reil and Köster, 2000). For example, nitrogen export from the Mississippi River basin has been linked to the expansion of anaerobic sediments in the Gulf of Mexico (Rabalais *et al.*, 2002). Elevated NO_3^- levels are a direct human health threat in newborn infants and adults deficient in glucose–phosphate dehydrogenase (Comly, 1945; Payne, 1981). Denitrification is an effective means of removing fixed nitrogen from sewage effluents and is used widely in wastewater treatment facilities. Wetland and aquatic ecosystems often provide the same service for the cost of protecting these areas as a resource (Bowden, 1987; Groffman, 1994).

8.08.5.2 Nitrogen Fixation

In a sense, all biological N_2 fixation is anaerobic because the nitrogenase enzyme is strongly inhibited by O_2. As a result, many aerobic diazotrophs (N_2-fixing microorganisms) have specialized structures (i.e., heterocysts) for keeping the site of N_2 fixation O_2-free. In the absence of such structures, N_2 fixation varies with O_2 concentrations, often peaking at night when oxygenic photosynthesis is absent (Bebout *et al.*, 1987; Currin *et al.*, 1996).

Dinitrogen fixation is an energetically expensive reaction and is inhibited by micromolar levels of NH_4^+, which requires comparatively little energy to assimilate. The energetic demands of the process is one reason why many diazotrophs are either photosynthetic themselves or occur in symbiotic relationships with plants. However, diazotrophy is certainly not restricted to these groups; it occurs widely among heterotrophic and chemoautotrophic microorganisms, and both aerobes and anaerobes (Lovell *et al.*, 2001).

A provocative link between diazotrophy and methanogenesis was recently proposed by Hoehler *et al.* (2001), who reported that cyanobacteria (formerly blue-green algae) in a coastal mudflat were a "hot spot" of H_2 production, presumably due to a side-reaction of the nitrogenase enzyme system. In some cases, H_2 accumulated underneath the mats to levels that were high enough to support methanogenesis, despite >50 mM SO_4^{2-}. However, the more important link to anaerobic metabolism was the possibility that H_2 production by diazotrophs promoted the oxidation of the primordial Earth. The loss of diazotroph-produced hydrogen to space may have promoted the accumulation of oxidants, thereby initiating the transition from an anaerobic to an aerobic planet before there were significant amounts of atmospheric O_2.

8.08.5.3 Respiratory Denitrification

The term *denitrification* is often used in the general literature to describe any process that converts nitrogen oxides (NO_3^- or NO_2^-) to reduced nitrogen gases (N_2O or N_2). In the context of microbial metabolism the term is used more narrowly to describe a specific respiratory pathway. Denitrification is the most common form of anaerobic respiration based on nitrogen. Energy is conserved by coupling electron transport phosphorylation to the reduction of nitrogen oxides

located outside the cell. Because nitrogen is not assimilated into the cell, the process is *dissimilatory*. Respiratory denitrification is more energetically favorable than Fe(III) reduction, SO_4^{2-} reduction or methanogenesis (Table 1), and it tends to be the dominant form of anaerobic carbon metabolism when NO_3^- or NO_2^- are available. Many microorganisms also reduce nitrogen oxides without conserving energy for growth, in which case the process is nonrespiratory. Nonrespiratory nitrogen oxide reduction may be assimilatory (i.e., assimilated into the cell) or dissimilatory. Dissimilatory NO_3^- reduction to NH_4^+ is a potentially important sink for NO_3^- in most ecosystems (Section 8.08.5.4).

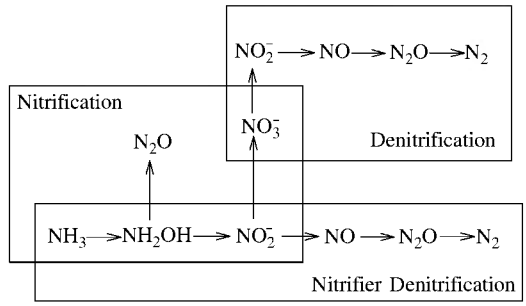

Figure 13 Relationships between three pathways of inorganic nitrogen oxidation and reduction (Wrage *et al.*, 2001) (reproduced by permission of Elsevier from *Soil Biol. Biochem.* **2001**, *33*, 1723–1732).

8.08.5.3.1 Denitrifier diversity and metabolism

Denitrifying bacteria are aerobes that substitute NO_3^- (or NO_2^-) for O_2 as the terminal electron acceptor when there is little or no O_2 available (Payne, 1981; Firestone, 1982). Aerobic respiration yields more free energy than NO_3^- respiration, and it is favored metabolically because O_2 inhibits key denitrification enzymes. Although some organisms can denitrify at O_2 concentrations up to 80% of air saturation (Robertson and Kuenen, 1991), the process is most rapid *in situ* under anaerobic conditions provided there is a supply of NO_3^-. The critical O_2 concentration where denitrifiers switch to mostly anaerobic respiration is roughly $\leq 10\ \mu mol\ L^{-1}$ (Seitzinger, 1988; Tiedje, 1988). Denitrification activity survives well in aerobic soils, even without new enzyme synthesis or cell growth (Smith and Parsons, 1985). It persists in habitats that lack O_2 or NO_3^- presumably because denitrifiers can maintain themselves with a low level of fermentation (Jørgensen and Tiedje, 1993).

Denitrifying bacteria use all three energy sources available to bacteria including organic carbon compounds (organotrophs), inorganic compounds (lithotrophs), and light (phototrophs). The dominant populations of denitrifiers appear to be organotrophs such as *Pseudomonas* and *Alcaligenes*. Others are able to ferment or use H_2, sulfur, or NH_4^+ as energy sources. Some denitrifiers are also N_2 fixers that grow in association with plants, including members of the genus *Rhizobium* (Tiedje, 1988), although it is not clear if many of these strains are capable of growing solely on NO_3^- as an electron acceptor. Many denitrifiers are chemolithotrophs, including the obligate chemolithotroph *Nitrosomonas europaea* (Poth and Focht, 1985; Poth, 1986), which is better known as a *nitrifying* microorganism.

In its most familiar form, denitrification requires organic carbon for the electron donor and NO_3^- or NO_2^- for the terminal electron acceptor. A variety of intermediate compounds are produced as the terminal electron acceptor is reduced stepwise to N_2 (Figure 13), including the solute nitrite (NO_2^-), and the gases nitric oxide (NO) and nitrous oxide (N_2O) (Ye *et al.*, 1994). With a few exceptions, a single organism can complete the entire series of reductions from NO_3^- to N_2 (Tiedje, 1988). Nonrespiratory denitrifiers tend to produce N_2O rather than N_2 (Tiedje, 1988). Because these intermediate compounds are required for metabolism, it is reasonable to assume that denitrifiers can also consume them. Consumption of external NO and N_2O creates many variations on the basic denitrification scheme. Nitrite, NO, and N_2O can serve as terminal electron acceptors rather than intermediates, and they can also replace N_2 as terminal products. It was recently observed that nitrogen dioxide gas (NO_2) is a terminal electron acceptor leading to N_2 production by the nitrifying bacterium *Nitrosomonas eutrophica* (Schmidt and Bock, 1997).

Respiratory denitrification is widespread among various physiologic and taxonomic groups of bacteria (Tiedje, 1988; Zumft, 1997), and may be considered the most versatile form of anaerobic metabolism. The only prokaryotic group that does not include denitrifying members is the *Enterobacteriaceae* (Zumft, 1997). Interestingly, most members of this group are capable of dissimilatory nitrate reduction to ammonium (Tiedje, 1994), a process that competes with denitrification for NO_3^-. With a few exceptions (Zumft, 1997), denitrification is absent among the gram-positive bacteria and obligate anaerobes.

The use of 16S rDNA gene sequences to detect the presence of denitrifying bacteria in microbial communities is limited by the high phylogenetic diversity of this group. Priemé *et al.* (2002) characterized the heterogeneity of gene fragments coding for two types of nitrate reductase (*nirK* and *nirS*) in an upland soil and a wetland soil.

They concluded that both soils, but particularly the wetland soil, had a high richness of *nir* genes, most of which have not yet been found in cultivated denitrifiers. This contrasts with a similar survey by Rösch *et al.* (2002) in which denitrification was not a genetic trait of most of the uncultured bacteria in a hardwood forest soil.

Fusarium oxysporum and a few other fungi have the capacity to denitrify NO_3^- or NO_2^- to N_2O (Shoun and Tanimoto, 1991; Usuda *et al.*, 1995). Their denitrifying enzyme system is coupled to the mitochondrial electron transport chain where it produces ATP (Kobayashi *et al.*, 1996). They do not grow under strictly anaerobic conditions, but require a minimal amount of O_2 (Zhou *et al.*, 2001). *F. oxysporum* can also produce N_2 using NO_2 as an electron acceptor (Tanimoto *et al.*, 1992). Zhou *et al.* (2002) have reported that *F. oxysporum* is capable of at least three types of metabolism depending on O_2 availability. Aerobic respiration is favored when O_2 is abundant, denitrification occurs under microaerobic conditions, and NO_3^- is reduced to NH_4^+ during fermentation under anoxic conditions. There have been virtually no field studies of NO_3-based metabolism by these organisms. However, Laughlin and Stevens (2002) selectively inhibited fungal activity using cycloheximide and observed a decrease in N_2O fluxes of 89% in a grassland soil; the antibiotic streptomycin decreased N_2O flux by 23%. Because fungi represent a large portion of the microbial biomass in soils (Ruzicka *et al.*, 2000), they may be an important source of N_2O in some terrestrial ecosystems.

8.08.5.3.2 Regulation of denitrification

Foremost on the long list of factors that regulate denitrification are those that influence the availability of O_2, NO_3^-, and organic carbon, the primary substrates that are metabolized by denitrifying bacteria. Some factors (e.g., nitrification rate) influence substrate pools directly, while other factors (e.g., soil water content) influence the transfer of substrates to sites where denitrification occurs. These factors have been reviewed previously (Cornwell *et al.*, 1999; Herbert, 1999; Seitzinger, 1988). Although it is convenient to consider the factors that regulate denitrification individually, the ability to predict *in situ* rates requires an understanding of their complex interactions (Parton *et al.*, 1996; Strong and Fillery, 2002).

Molecular oxygen (O_2) is the physiologically preferred terminal electron acceptor for denitrifying bacteria, and its presence represses denitrification enzyme synthesis and activity. Although some microorganisms are capable of performing denitrification in the presence of O_2, it occurs only under anaerobic or microaerobic conditions *in situ*. The presence of water is nearly a prerequisite for denitrification activity because it slows O_2 diffusion by a factor of 10^4 compared to air. In upland soils, abundant air-filled pore spaces permit rapid gas exchange and O_2 concentrations are typically 21% to depths of 1 m or more (Megonigal *et al.*, 1993). Water reduces the O_2 supply by blocking a fraction of the pores, thereby increasing the effective distance (i.e., tortuosity) an O_2 molecule travels to a given microsite (Renault and Sierra, 1994; Figure 14). As the soil water content increases, some sites are blocked completely so that there is no air-filled pathway to the atmosphere. This explains why denitrification rates in soils are often positively related to water content (Drury *et al.*, 1992; Groffman and Tiedje, 1991). For example, denitrification enzyme activity in a temperate hardwood forest was higher in wet years than dry years (Bohlen *et al.*, 2001).

Soil texture is a good predictor of denitrification rates at the landscape scale (Groffman, 1991), in part because it captures the interaction between water content and soil porosity with respect to gas and solute diffusion path length. At a given soil water content, the small pores found in clayey soils are more likely to be blocked than the relatively large pores found in loam and sand soils. Soil texture also influences temporal variability in soil water content because it largely establishes the water infiltration rate and water holding capacity (de Klein and van Logtestijn, 1996; Sexstone *et al.*, 1985). A particularly useful expression for soil water content is *percent water-filled porosity* (Williams *et al.*, 1992), which is the ratio of volumetric water content to total soil porosity. Based on a compilation of denitrification studies in agricultural and forest soils, Barton *et al.* (1999) determined that denitrification increases dramatically above 65% water-filled porosity on average, with higher values for sandy soils (74–83%) than clayey soils (50–74%).

Carbon availability largely controls O_2 consumption rates, either directly by fueling aerobic heterotrophic respiration, or indirectly by supporting the anaerobic production of reductants, such as Fe(II), that subsequently react with O_2. Even small amounts of organic carbon can produce anaerobic conditions in soils that are partially or completely saturated. In upland soils, anaerobic sites occur inside soil aggregates. Højberg *et al.* (1994) used O_2 and N_2O microsensors to demonstrate simultaneous O_2 consumption and denitrification in the surface of soil aggregates (Figure 15). Anaerobic microsites are particularly pronounced near sources of organic carbon such as roots or detritus. For example, Parkin (1987) found that a single leaf constituting 1% of the soil mass supported

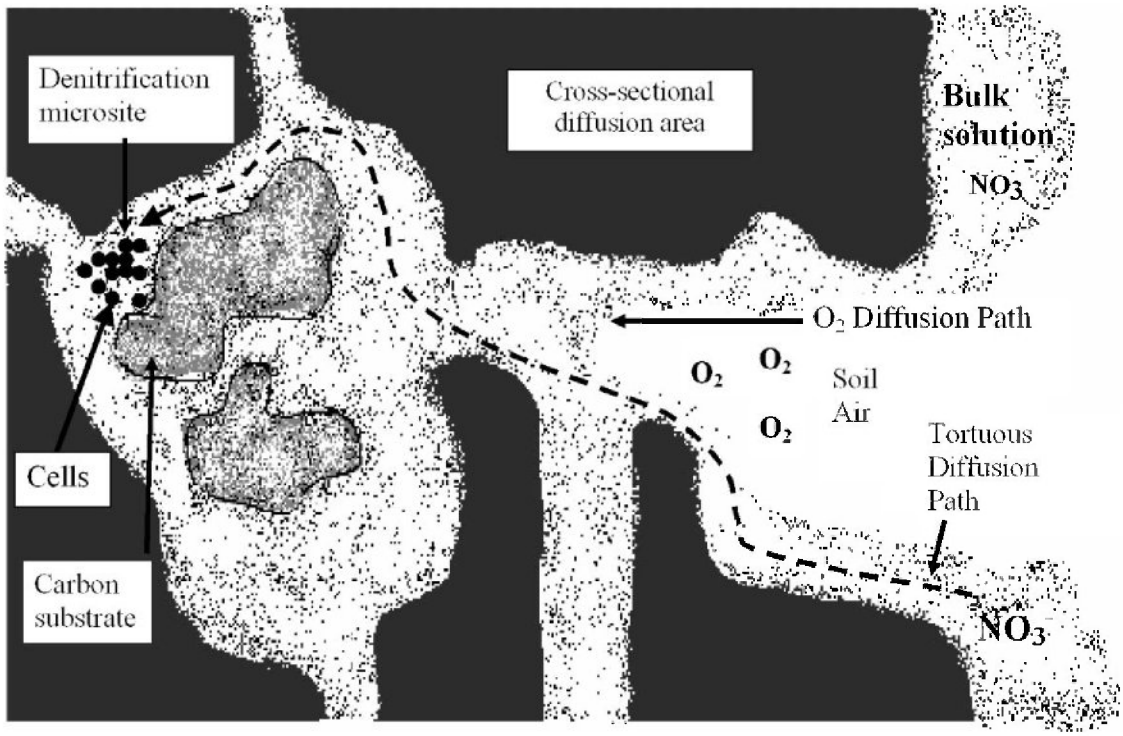

Figure 14 The physical and chemical factors that regulate substrate diffusion to denitrifying bacteria (Strong and Fillery, 2002) (reproduced by permission of Elsevier from *Soil Biol. Biochem.* **2002**, *34*, 945–954).

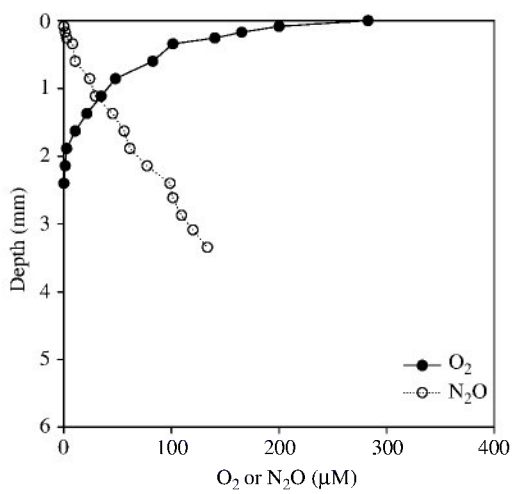

Figure 15 Profiles of O_2 and N_2O with depth from the surface of a soil aggregate. The profiles were determined with microelectrodes (source Højberg *et al.*, 1994).

85% of the denitrification. Such "hot-spots" are one explanation for the notoriously high spatial variability in N_2O production measured in upland soils (Parkin, 1987) and, to a lesser extent, in estuarine sediments (Kana *et al.*, 1998). Although microsites in upland soils are a small fraction of the total soil volume, they contribute perhaps 70% of global N_2O emissions (Conrad, 1996).

Some forms of organic carbon support higher denitrification rates than others due to differences in carbon quality. For example, fresh pine needles supported higher denitrification rates than senescent pine needles in a riparian wetland (Schipper *et al.*, 1994), presumably because the former had more labile compounds such as soluble carbohydrates. van Mooy *et al.* (2002) interpreted depth-dependent changes in the amino acid content of sinking particulate organic carbon in the Pacific Ocean as evidence that denitrifiers preferred to metabolize nitrogen-rich amino acids. If this is the case, calculations of denitrification in the eastern tropical North Pacific Ocean may be 9% higher than previous estimates that were based on typical Redfield ratios.

Denitrification can be limited by carbon availability when O_2 is absent and NO_3^- is abundant. Additions of glucose stimulated denitrification in 11 of 13 agricultural soils that were presumably fertilized (Drury *et al.*, 1991). Similar observations have been made in water columns (Brettar and Rheinheimer, 1992), marine sediments (Slater and Capone, 1987), river sediments (Bradley *et al.*, 1995), aquifers (Smith and Duff, 1988; Obenhuber and Lowrance, 1991), wastewater treatment wetlands (Ingersoll and Baker, 1998), and forested wetlands (DeLaune *et al.*, 1996). Tiedje (1988) proposed that the major influence of carbon on *in situ* denitrification is to promote anaerobic conditions.

Due to a high demand for nitrogen by all organisms, the NO_3^- pool in upland ecosystems is commonly small. In wetland and aquatic ecosystems, NO_3^- availability may be limited further by anaerobic conditions and low nitrification rates. Nitrate addition studies have demonstrated that NO_3^- availability limits denitrification in a variety of aquatic ecosystems (Lohse *et al.*, 1993; Nowicki, 1994). In upland soils, NO_3^- limitation can be an indirect effect of low soil water content, which increases the length of the diffusion pathway between aerobic sites where nitrification takes place and anaerobic microsites (Figure 14). Thus, low soil water content suppresses denitrification in upland soils by simultaneously increasing the O_2 supply and decreasing the NO_3^- supply. Seitzinger (1994) concluded that NO_3^--limitation of denitrification in eight riparian wetlands was an indirect response to limited organic carbon availability. Organic carbon governed mineralization rates and, therefore, the NH_4^+ supply to nitrifying bacteria. The relationship between NO_3^- concentration and denitrification rate often approximates a Michaelis–Menten function. Half-saturation concentrations generally range from 27 μM to 53 μM for stirred marine sediments (Seitzinger, 1988); the values for intact soils can be 30-fold higher because of diffusion limitation (Schipper *et al.*, 1993; Strong and Fillery, 2002).

8.08.5.3.3 Nitrification–denitrification coupling

Microbial metabolism and anthropogenic activity are the primary sources of NO_3^- used by denitrifying bacteria. Nitrate is a waste product of *chemoautotrophic nitrification*, a series of two dissimilatory oxidation reactions, each performed by a distinct group of bacteria. In the first step, NH_4^+ is oxidized to NO_2^- by *Nitrosomonas europaea* and other "Nitroso-" genera including *Nitrosococcus* and *Nitrosospira*. Although $\geq 95\%$ of the total $NH_3 + NH_4^+$ pool is in the form of NH_4^+ at pH ≤ 8, these organisms are also known as *ammonia oxidizers* because NH_3 is used at the enzymatic level. In the second step, NO_2^- is oxidized to NO_3^- by nitrite-oxidizing bacteria belonging to "Nitro-" genera such as *Nitrobacter* and *Nitrospira*. The two genera constitute the *Nitrobacteriaceae* (Buchanan, 1917). Ammonium is also oxidized by microorganisms that specialize in other types of metabolism. The most important of these are methanotrophic bacteria (Section 8.08.4.6.1), which were estimated to mediate 44–85% of the NH_4^+ oxidation in a rice paddy soil (Bodelier and Frenzel, 1999). In addition, methanotrophic bacteria produce and consume NO (Ren *et al.*, 2000), a common nitrification product (Section 8.08.5.3.5). Heterotrophic oxidation of NH_4^+ or organic-N to NO_2^- and NO_3^- has been observed in fungi, but bacteria are generally considered the dominant *in situ* source of NO_3^- in most ecosystems. The factors regulating nitrification in marine sediments were reviewed by Henriksen and Kemp (1988).

Because nitrification occurs only under aerobic (or microaerobic) conditions and denitrification under anaerobic conditions, the two processes are spatially separated. However, if the sites where these processes occur are sufficiently close together, NO_3^- transport and consumption are very rapid and the overall process is considered to be *coupled nitrification-denitrification*. On the basis of a literature review, Seitzinger (1988) concluded that nitrification is generally the major source of NO_3^- for denitrification in river, lake, and coastal sediments. The same is likely to be true of nonagricultural soils that are largely dependent on mineralization and atmospheric deposition for fixed nitrogen.

Denitrification can be uncoupled from nitrification when NO_3^- is delivered to soils and sediments from outside sources such as overlying water, fertilizers, and atmospheric deposition. Fertilizers are a source of NO_3^- to groundwater (Spalding and Exner, 1993), which in turn is a source of NO_3^- to riparian forests (Hill, 1996), tidal marshes (Tobias *et al.*, 2001a), and estuarine sediments (Capone and Bautista, 1985). Surface water runoff carries NO_3^- directly to rivers, lakes, estuaries and oceans (Jordan and Weller, 1996; Joye and Paerl, 1993). Because of high NO_3^- loading, denitrification rates in estuarine sediments are often directly proportional to the NO_3^- concentration in the overlying water column (Kana *et al.*, 1998; Nielsen *et al.*, 1995; Pelegrí *et al.*, 1994), indicating that denitrification is controlled by the NO_3^- diffusion rate across the aerobic sediment surface.

The response of denitrification to certain environmental factors varies according to how strongly the process is coupled to nitrification. An increase in O_2 penetration into sediments can stimulate coupled nitrification–denitrification by enhancing NO_3^- availability (Rysgaard *et al.*, 1994), but simultaneously depress water column-supported denitrification because of the increased distance that NO_3^- must diffuse to reach the anaerobic layer (Cornwell *et al.*, 1999). Risgaard-Petersen *et al.* (1994) reported that a photosynthesis-induced increase in O_2 penetration into an estuarine sediment doubled the coupled nitrification–denitrification rate, but reduced the amount of water column-supported denitrification by 50%. Because the contribution of coupled nitrification–denitrification to total denitrification at this site was small (~16%), the net effect of deeper O_2 penetration was to reduce

denitrification. An increase in O_2 penetration in North Sea sediments had an entirely different outcome (i.e., higher overall denitrification) because the sediment, rather than the water column, was the dominant NO_3^- source supporting denitrification (Lohse *et al.*, 1993).

8.08.5.3.4 Animals and plants

Animals and plants influence denitrification in wetland and aquatic ecosystems by altering the availability of O_2, NO_3^-, and carbon. In organic-rich sites, nitrification rates tend to be limited by the depth of O_2 penetration (Kemp *et al.*, 1990). Benthic invertebrates enhance pools of O_2 and NO_3^- by digging burrows and irrigating them with overlying water (Pelegrí *et al.*, 1994). Macrofaunal tubes and burrow walls support higher potential rates of nitrification than nearby surface sediments, and contribute >25% of benthic nitrification is some coastal ecosystems (Blackburn and Henriksen, 1983; Kristensen *et al.*, 1985). The degree to which infauna enhance potential nitrification varies among worm species due to variations in irrigation behavior (Mayer *et al.*, 1995). Nitrification and denitrification are coupled in burrow wall sediments just as they are in surface sediments (Sayama and Kurihara, 1983). Animals also enhance denitrification by concentrating organic matter into faecal pellets, thereby creating "hot-spots" of O_2 demand.

Aquatic plants influence sediments in much the same manner as animals. Reddy *et al.* (1989) applied $^{15}N\text{-}NH_4^+$ to the saturated root zone of three emergent aquatic macrophytes and recovered ~25% of the label as $^{15}N_2$ over 18 d. Since $^{15}N_2$ was not recovered from the unplanted controls, it was apparent that coupled nitrification–denitrification was stimulated by the rhizosphere. Similar observations have been reported for submerged aquatic plants (Caffrey and Kemp, 1991; Christensen and Sørensen, 1986) and microalgae (Law *et al.*, 1993). Risgaard-Petersen and Jensen (1997) reported that denitrification was enhanced in the presence of a submerged aquatic macrophyte by six-fold. This impressive effect was due in part to the fact that O_2 was released well below the sediment surface, creating a zone of nitrification capped above and below by zones of denitrification. The result was a highly efficient coupling between the two processes. The influence of plants on anaerobic metabolism is apparently species- or site-specific because studies of other marine angiosperms have found no effects on denitrification (Rysgaard *et al.*, 1996). In addition to introducing O_2, plants introduce organic carbon in the form of root exudates (Lynch and Whipps, 1990) and detritus, and compete with microorganisms for NH_4^+ (Lin *et al.*, 2002). In upland systems, plants are more likely to have an overall negative effect on denitrification due to competition for NH_4^+ and NO_3^-, and consumption of water (Tiedje, 1988).

8.08.5.3.5 N_2O and NO fluxes

Classical coupled nitrification–denitrification requires NH_4^+, organic carbon, aerobic conditions, and anaerobic conditions. It involves three distinct populations of microorganisms, some of which are heterotrophic and others autotrophic. As a result, regulation of the process is rather complex and the relative proportions of NO_3^-, NO, N_2O, and N_2 as end products varies widely with environmental and ecological conditions.

Several factors can cause the reduction of NO_3^- to N_2 to be incomplete, resulting in the pooling of NO and N_2O. Denitrification and nitrification enzymes are inhibited by H_2S (Joye and Hollibaugh, 1995) and O_2. The sensitivity of denitrifying enzymes to O_2 inhibition is inversely proportional to the oxidation state of the nitrogen substrate (Figure 12), increasing in the order: NO_3^- reductase < NO_2^- reductase < NO reductase < N_2O reductase (Dendooven and Anderson, 1994; McKenney *et al.*, 1994). Pooling can be caused by an excess of NO_3^- relative to organic carbon, or an imbalance in the kinetics of the various steps. Some organisms lack key enzymes in the sequence and release NO or N_2O as waste products (Tiedje, 1982). Reduction to N_2 appears to be favored over N_2O at circumneutral pH (Šimek *et al.*, 2002).

Nitrogenous gas emission from soils varies strongly with soil water content (Williams *et al.*, 1992). The water content at which efflux from soils peaks generally increases in the order: NO > N_2O > N_2. Yang and Meixner (1997) reported that NO fluxes peaked at ~20% water-filled pore space in a grassland soil, which is in qualitative agreement with other studies (Otter *et al.*, 1999; Potter *et al.*, 1996). Nitrous oxide emissions peak at higher levels of soil moisture than NO emissions (Drury *et al.*, 1992), and N_2 emissions are highest in saturated soils. This pattern is caused by differences in the O_2 sensitivity of denitrifying enzymes, and by the influence of soil water content on gas diffusion. High soil water content restricts the diffusion of gases and enhances the diffusion of solutes (Section 8.08.5.3.2). Because nitrifying bacteria require both a gas (O_2) and a solute (NH_4^+), the optimal availability of substrates occurs where soils are wet, but not saturated (Williams *et al.*, 1992). Such conditions favor N_2O production because nitrification and denitrification can be simultaneously producing N_2O (Stevens *et al.*, 1997). The influence of soil water on NO and N_2O

emissions is complicated by that fact that these gases are also consumed by microorganisms. Water promotes microbial consumption of NO and N_2O by restricting diffusion to the atmosphere, thus increasing their residence time in the soil (Davidson, 1992; Skiba *et al.*, 1997).

It is difficult to distinguish between nitrification and denitrification as sources of NO and N_2O. Selective inhibition of nitrification by 10 Pa C_2H_2 (acetylene) and denitrification by 10 kPa C_2H_2 (Davidson *et al.*, 1986) has widely been used, but it suffers from a number of problems. These include the possibility of C_2H_2-insensitive denitrifiers (Dalsgaard and Bak, 1992), and NO scavenging by O_2 (Bollmann and Conrad, 1997; McKenney *et al.*, 1997). Techniques based on ^{15}N or ^{13}N tracing present different problems (Boast *et al.*, 1988; Arah, 1997), but are favored in more recent studies. The contribution of nitrification to NO and N_2O production is highest under the conditions that favor nitrification, namely, moderate O_2 partial pressure and high NH_4^+ concentrations (Avrahami *et al.*, 2002). Nitrification is generally considered to be the dominant source of NO upland in soils, but there are studies that suggest the opposite (reviewed by Ludwig *et al.*, 2001). The dominant source may vary with environmental factors such as pH (Remde and Conrad, 1991).

Nitric oxide and N_2O are also produced abiotically by chemical decomposition of NO_2^- (Hooper and Terry, 1979). The reaction is favorable at low pH and yields mainly NO (van Cleemput and Baert, 1984), although N_2O can also be produced (Martikainen and De Boer, 1993). Abiotic production of NO and N_2O is assumed to be relatively unimportant in most ecosystems (e.g., Webster and Hopkins, 1996).

8.08.5.4 Dissimilatory Nitrate Reduction to Ammonium (DNRA)

Nitrate reduction studies have focused overwhelmingly on denitrification at the expense of other NO_3^- sinks such as dissimulatory NO_3^- reduction to NH_4^+ (also known as DNRA or *nitrate ammonification*). The ecological implications of reducing NO_3^- to NH_4^+, versus N_2 are vastly different because NH_4^+ is more readily retained in the ecosystem, and it is a form that is readily assimilated by biota. Thus, DNRA contributes to eutrophication by reducing the quantity of fixed nitrogen that is returned to the atmosphere as N_2.

There are two pathways by which NO_3^- can be reduced to NH_4^+. In assimilatory NO_3^- reduction, NO_3^--N is taken up by microorganisms or plants, reduced to NH_4^+, and assimilated into organic nitrogen compounds such as amino acids. The process is inhibited by low concentrations ($0.1\ \mu L\ L^{-1}$) of NH_4^+ or organic nitrogen (Rice and Tiedje, 1989), and not regulated by O_2. These factors have the opposite effect on dissimilatory NO_3^- reduction to NH_4^+. Assimilatory NO_3^- reduction by microorganisms is usually assumed to be negligible due to inhibition by NH_4^+.

8.08.5.4.1 *Physiology and diversity of DNRA bacteria*

Dissimilatory nitrate reduction to ammonium is an anaerobic pathway that is insensitive to NH_4^+ and yields energy. The first step of the process is termed *nitrate respiration* because it is coupled to electron transport phosphorylation that generates ATP:

$$NO_3^- + H_2 \rightarrow NO_2^- + H_2O \qquad (13)$$

Nitrate respiration is widely found among microorganisms, some of which further reduce NO_2^- to NH_4^+:

$$NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O \qquad (14)$$

This second step is not coupled to energy production except in a few species that are not expected to be abundant *in situ*, such as *Campylobacter*, *Deulfovibrio*, and *Wolinella* (Tiedje, 1988). The overall DNRA reaction transfers $8e^-$ and yields 600 kJ mol^{-1} NO_3^- (Tiedje, 1994):

$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O \qquad (15)$$

The second step in which NO_2^- is reduced to NH_4^+ is ecologically significant because it ensures that the nitrogen can be retained in the ecosystem for plant uptake, microbial assimilation or adsorption to cation exchange sites; otherwise, NO_2^- would accumulate and be subjected to denitrification. Because NO_2^- reduction to NH_4^+ does not normally yield energy, it presumably has other physiological or ecological advantages. Perhaps its most likely role is to serve as an electron sink for the regeneration of NADH from $NADH_2$ (Bonin, 1996).

DNRA bacteria can be aerobic, facultatively anaerobic or obligately anaerobic. As a group they are unlike the denitrifying bacteria in that most species are fermentative (Bonin, 1996; Tiedje, 1988). DNRA bacteria are abundant in aerobic soils and other environments that do not favor DNRA activity *per se*. This suggests that they can compete with other fermentative bacteria or aerobes for carbon substrates (Tiedje, 1988). Much less is understood about the diversity and physiology of DNRA bacteria than denitrifiers, despite the fact that they are sometimes the larger of the two dissimilatory NO_3^- sinks (Table 7).

Table 7 Examples of the contribution of dissimilatory nitrate reduction to ammonium (DNRA) to total dissimulatory nitrate reduction in various freshwater, marine, and terrestrial ecosystems. The ranges are inclusive of all sites and treatments reported for which appropriate data could be drawn, including those that manipulated substrate levels.

Ecosystem	*%DNRA*[a]	*Method*[b]	*Citation*
Marine sediment	79–93	ABT + estimate of DNRA	Bonin (1996)
Marine sediment	18–97	^{15}N assay + ABT	Bonin *et al.* (1998)
Marine sediment	98	^{15}N assay + ABT	Gilbert *et al.* (1997)
Marine sediment	33	^{15}N assay	Goeyens *et al.* (1987)
Marine sediment	46–68	^{15}N assay + ABT	Sørensen (1978)
Marine sediment	0–18	^{15}N assay + ABT	Kaspar *et al.* (1985)
Estuarine sediment	5–30	^{15}N assay + ABT	Kaspar (1983)
Estuarine sediment	0–85	^{15}N assay	Christensen *et al.* (2000)
Estuarine sediment	73–82	ABT + estimate of DNRA	Jørgensen and Sørensen (1985)
Estuarine sediment	66–75	ABT + estimate of DNRA	Jørgensen and Sørensen (1988)
Estuarine sediment	6–75	^{15}N assay + ABT	Jørgensen (1989)
Estuarine sediment	10–61	^{15}N assay	Koike and Hattori (1978)
Estuarine sediment	2–3	^{15}N assay	Pelegrí *et al.* (1994)
Mangrove soil	3–100	^{15}N assay	Riviera-Monroy (1995)
Brackish marsh soil	7–70	^{15}N assay	Tobias *et al.* (2001a)
Brackish marsh soil	1–7	^{15}N assay	Tobias *et al.* (2001b)
River sediment[c]	6–10	^{15}N assay	Kelso *et al.* (1997)
Aquifer sediment	22–60	^{15}N assay	Bengtsson and Annadotter (1989)
Rice paddy soil	1–56	^{15}N assay	Buresh and Patrick (1978)
Rice paddy soils	4–12	^{15}N assay	Yin *et al.* (2002)
Boreal forest	40–93	^{15}N assay + ABT	Bengtsson and Bergwall (2000)
Calcareous clay soil	4–19	^{15}N assay	Fazzolari *et al.* (1998)
Clay loam soil	1–2	^{15}N assay	Chen *et al.* (1995)
Silt loam soil	1–77	^{15}N assay + ABT	deCatanzaro *et al.* (1987)
Silt loam soil[c]	11–30	^{15}N assay	Stanford *et al.* (1975)
Wet tropical forest	71–83	^{15}N assay	Silver *et al.* (2001)

[a] Percent DNRA calculated as (NO_3^- reduction to NH_4)/(NO_3^- reduction to $NH_4 + N_2 + N_2O$). [b] ABT is the acetylene block technique.
[c] Used last reported incubation time point.

8.08.5.4.2 DNRA versus denitrification

DNRA and denitrification occur simultaneously and can be expected to compete for carbon and NO_3^-. Tiedje *et al.* (1982) proposed that the partitioning of NO_3^- to N_2 versus NH_4^+ is a function of the carbon : NO_3^- ratio. They reasoned that a combination of abundant electron donors (i.e., carbon) and limited electron acceptors (i.e., NO_3^-) should favor organisms that use electron acceptors most efficiently. In this case, DNRA has a competitive advantage because it transfers 8 moles of electrons per mole of NO_3^- reduced, while denitrification transfers five moles of electrons. Nitrite respiration alone transfers two moles of electrons. Thus, denitrifying bacteria should out compete DNRA bacteria for organic carbon when carbon is limiting. Several studies support the generalization that high labile carbon availability and/or low NO_3^- availability favor DNRA over denitrification (Bonin, 1996; Fazzolari *et al.*, 1998; King and Nedwell, 1985; Nijburg *et al.*, 1997; Yin *et al.*, 2002). In wetlands, the presence of emergent aquatic macrophytes appeared to stimulate DRNA activity (Nijburg *et al.*, 1997; Nijburg and Laanbroek, 1997), perhaps because of increased carbon availability due to root exudates. Nonetheless, competition between the two groups of organisms has not been adequately studied and there are exceptions to this generalization that suggest the influence of other factors.

A series of laboratory and field studies showed that low temperatures favor denitrifying bacteria while high temperatures favor DNRA bacteria in temperate salt marsh and estuarine sediments of the Colne estuary, UK (King and Nedwell, 1984; Ogilvie *et al.*, 1997a,b). It is not yet clear whether this effect can be generalized to other systems. Field estimates of NO_3^- partitioning to the two processes in a North Sea estuary were best explained when it was assumed that DNRA activity was favored by *both* high and low temperatures (Kelly-Gerreyn *et al.*, 2001). Temperature effects may explain why the relative importance of DNRA versus denitrification decreased from May to October in a temperate tidal marsh despite a three-fold increase in dissolved carbon concentrations (Tobias *et al.*, 2001a).

DNRA activity has been assumed to be important only under highly reduced conditions

($E_h = -200$ mV; Buresh and Patrick, 1981). However, there is growing evidence that DNRA can be important in relatively oxidized environments. DNRA accounted for ~75% of NO_3^- reduction in a humid tropical rainforest where the soil O_2 content was 15% (Silver *et al.*, 2001). DNRA activity was stimulated by the amphipod *Corophium volutator* (Pelegrí *et al.*, 1994), an organism that increases redox potential through burrowing. Fazzolari *et al.* (1998) suggested that DNRA bacteria are less O_2 sensitive than denitrifying bacteria. Clearly, highly reduced conditions are not a prerequisite for DNRA to be an important NO_3^- sink.

DNRA has an abiotic equivalent reaction that can proceed at rates comparable to the biotic reaction in the presence of "green rust" (Hansen *et al.*, 1996) or trace metals such as Cu(II) (Ottley *et al.*, 1997). Green rusts are Fe(II)–Fe(III) precipitates that form in nonacid, Fe(II)-rich soils and sediments (Hansen *et al.*, 1994). A similar reaction may have made a significant contribution to the NH_4^+ inventory on the prebiotic Earth (Summers and Chang, 1993).

In the past, DNRA has been considered to be inconsequential in nonmarine ecosystems (Tiedje, 1988). There is now evidence that the process can be an important, and even dominant, NO_3^- sink in a wide variety of freshwater aquatic and terrestrial systems (Table 7). Strong relationships between DRNA activity, carbon : NO_3^- ratio, O_2 and temperature provide a basis for formulating hypotheses on the range of conditions where DRNA activity should be important. Many aspects of the microbial ecology of DNRA bacteria are ripe for study, such as the factors that determine the outcome of competition with denitrifying bacteria. It is likely that some of the NO_3^- consumption attributed to denitrifying bacteria is actually due to NO_3^--respiring bacteria that do not express DNRA activity. Because the two groups differ in key aspects of their physiology, this difference in NO_3^- reduction pathway could have implications for understanding NO_3^--dependent anaerobic carbon metabolism.

8.08.5.5 Alternative Pathways to N_2 Production

Alternative pathways for denitrification have been proposed that do not involve the classical enzyme systems, but their existence has only recently been confirmed or investigated in detail. In the absence of O_2, a number of elements and compounds have the potential to oxidize NH_4^+, including Mn(II), MnO_2, NO_3^-, NO_2^- and NO_2. Because anaerobic oxidation of NH_4^+ directly to N_2 bypasses the multiple oxidation steps required for coupled nitrification–denitrification (Figure 12), the substrate and environmental controls on anaerobic NH_4^+ oxidation are likely to be quite different from those for the classical sequence of reactions. The contribution of these processes to overall denitrification is largely unknown, but the limited evidence suggests they may be quantitatively important in both natural ecosystems and wastewater treatment processes.

8.08.5.5.1 Anammox

Ammonium oxidation linked to NO_2^- (nitrite) reduction was first recognized in a wastewater treatment system and patented under the process name *anammox* for *an*aerobic *amm*onium *ox*idation (Mulder *et al.*, 1995). The discovery was motivated by observations of simultaneous NH_4^+ and NO_3^- losses balanced by N_2 production under anaerobic conditions. The existence of a "missing" chemolithotrophic organism capable of coupling NH_4^+ oxidation to either NO_3^- or NO_2^- reduction had been predicted nearly two decades earlier based on thermodynamic considerations (Broda, 1977). The anammox reaction was initially proposed to involve NO_3^- (Mulder *et al.*, 1995):

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \quad (16)$$

However, it was subsequently determined that NO_2^- is the oxidant in anammox (van de Graaf *et al.*, 1995):

$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O \quad (17)$$

Reactions (16) and (17) were distinguished using an ^{15}N isotope pairing technique in which the NH_4^+ pool was enriched with ^{15}N while the NO_3^- and NO_2^- pools remained dominated by ^{14}N. With the NH_4^+ pool dominated by ^{15}N, reaction (16) yields 75% $^{29}N_2$ (i.e., $^{14}N-^{15}N$) and 25% $^{30}N_2$, whereas reaction (17) yields 100% $^{29}N_2$ (van de Graaf *et al.*, 1995, 1997). The anammox process also produces small amounts of NO_3^- that are thought to provide reducing equivalents for CO_2 assimilation (Jetten *et al.*, 1999).

The microorganisms responsible for anaerobic NH_4^+ oxidation have proven extremely difficult to isolate and no pure cultures exist. However, two organisms have been enriched from wastewater treatment plants that perform the anammox reaction (Egli *et al.*, 2001; Strous *et al.*, 1999a; Toh *et al.*, 2002), and aspects of their physiology have been described (Strous *et al.*, 1999b). Both the archetype strain "*Candidatus* Brocadia anammoxidans" and its relative "*Candidatus* Kuenenia stuttgartiensis" are deeply branching members of the order *Planctomycetales*, a major division of the Bacteria. Members of this division share several unusual features such as internal compartmentalization similar to the Eukaryotes

(Lindsay *et al.*, 2001). To add further intrigue to these organisms, the NH_4^+ oxidizing members of the order have a specialized compartment, the anammoxosome, composed of "ladderane" lipids that have never before been observed in nature (Sinninghe Damsté *et al.*, 2002). The anammoxosome lipid membrane is highly impermeable and may be specialized to protect the cell from toxic intermediates generated by the anammox process. These include hydrazine (N_2H_4), an active ingredient in rocket fuel (DeLong, 2002), and hydroxalamine (NH_2OH). The organisms are chemolithoautotrophic and grow exceptionally slowly in enrichment culture, dividing once every nine days under optimal conditions (Egli *et al.*, 2001; Strous *et al.*, 1999a). The function of a highly impermeable anammoxosome membrane may be to maintain an electrochemical gradient in spite of low rates of catabolism (Sinninghe Damsté *et al.*, 2002). Anammox bacteria are reversibly inhibited by O_2 concentrations as low as 2 μM and NO_2^- concentrations between 5 mM and 10 mM (Jetten *et al.*, 1999).

The nature of interactions between anammox bacteria and other microorganisms in the nitrogen cycle is a matter of speculation (Schmidt *et al.*, 2002). If anammox bacteria consume NO_2^- supplied directly by autotrophic NH_3-oxidizers, they are probably competing with NO_2^--oxidizers for a limited substrate (i.e., NO_2^-). In this case, N_2 production is bypassing the NO_3^- link between nitrifiers and denitrifiers in the classical nitrogen cycle (Zehr and Ward, 2002). It is clear that NO_3^--N is readily consumed in the anammox process (Mulder *et al.*, 1995; Dalsgaard and Thamdrup, 2002), although it is first reduced to NO_2^-. Nitrification is presumably the NO_3^- source in this case, and anammox is coupled to nitrification in a manner analogous to coupled nitrification–denitrification. A key difference between anammox and denitrification is that anammox produces twice the amount of N_2 per mole of NO_3^-. Thus, anammox may enhance N_2 production in soils and sediments where the supply of oxidized nitrogen substrates is limited by nitrification rates.

Few studies have quantified the contribution of anammox to nitrogen cycling, and most of these were done in wastewater reactors under conditions that do not exist in most ecosystems (e.g., Dong and Tollner, 2003). The contribution of anaerobic NH_4^+ oxidation to overall N_2 production in natural systems is largely unknown aside from a pair of studies in marine sediments (but see very recent contributions from Dalsgaard *et al.* (2003) and Kuypers *et al.* (2003). In one study, sediments were taken from three sites across the Baltic–North Sea transition that differed in sediment organic carbon content (Thamdrup and Dalsgaard, 2002). Isotope pairing showed that up to 67% of total N_2 production was due to anaerobic NH_4^+ oxidation. Moreover, a 1 : 1 stoichiometry between consumption of $^{15}NH_4^+$ and $^{15}NO_3^-$, and nearly 100% production of $^{29}N_2$, suggested that it was an anammox-like process. A subsequent study confirmed that that anaerobic NH_4^+ oxidation required NO_2^- rather than NO_3^- (Dalsgaard and Thamdrup, 2002).

In the Baltic–North Sea transition study of Thamdrup and Dalsgaard (2002), anammox contributed 33–67% of total N_2 production at continental shelf sites, but just 2% of total N_2 production at the site in a coastal bay (Figure 16). The difference between the bay site and the closest continental shelf site was due primarily to an increase in the denitrification rate, rather than a decrease in anaerobic oxidation. Denitrification declined in parallel with declining sediment organic carbon content from the coastal bay seaward. Absolute rates of anaerobic NH_4^+ oxidation were in the range 30–99 μM d^{-1}, and were lowest at the site where the percent contribution was highest. This pattern is consistent with evidence that anammox organisms are autotrophic, whereas denitrifiers are heterotrophic.

Anaerobic NH_4^+ oxidation may be most significant in ecosystems where denitrification is limited by carbon availability rather than NH_4^+, NO_3^-, or the presence of O_2. This may include large areas of the continental shelves and slopes (Seitzinger and Giblin, 1996), O_2-deficient waters such as the Black Sea, and eutrophic soils and sediments. Anammox-specific 16S rRNA gene sequences (Schmidt *et al.*, 2002) and lipids (Sinninghe Damsté *et al.*, 2002), and ^{15}N isotope pairing provide a suite of powerful techniques for surveying ecosystems for anammox bacteria and anaerobic NH_4^+ oxidation activity.

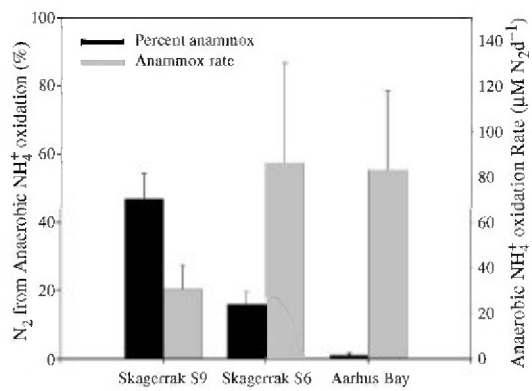

Figure 16 Summary of experiments from three sites on the continental shelf that quantified the absolute rates of anaerobic ammonium oxidation (i.e., anammox) and its contribution to total N_2 production (i.e., anammox + denitrification). The sites constitute a transect with Aarhus Bay closest to shore and Skagerrak S9 furthest from shore. (after Dalsgaard and Thamdrup, 2002).

8.08.5.5.2 Nitrifier denitrification

NH_3-oxidizing bacteria are typically characterized as a NO_2^- source for other bacteria that ultimately produce NO_3^- (Henriksen and Kemp, 1988; Schlesinger, 1997). However, it is important to recognize the metabolic versatility of NH_3-oxidizers (i.e., nitrifiers) and to consider the other roles they may play in nitrogen cycling. Some nitrifying bacteria produce N_2 from NH_4^+ using O_2 or NO_2 (nitrogen dioxide) as oxidants. The process has been named *nitrifier denitrification* to indicate that it involves autotrophic NH_3-oxidizers with an enzyme system similar to that of the heterotrophic denitrifying bacteria (Poth and Focht, 1985; Wrage *et al.*, 2001). In fact, there may be an evolutionary linkage between denitrifying NH_3-oxidizers and denitrifiers, as suggested by a high degree of similarity in their nitrite reductase gene sequences (Casciotti and Ward, 2001). During nitrifier denitrification, NH_3 is oxidized to NO_2^-, as in typical nitrification, then reduced to NO, N_2O, or N_2 (Figure 13). Thus, the process couples NH_3 oxidation and denitrification within a single organism, such as *Nitrosomonas europaea* or *N. eutropha* (Ritchie and Nicholas, 1972; Bock *et al.*, 1995).

The most recent metabolic pathway described in *Nitrosomonas* is anaerobic NH_3 oxidation in which O_2 is replaced by nitrogen dioxide (NO_2) or its dimeric form, N_2O_4:

$$NH_3 + N_2O_4 \rightarrow NO_2^- + 2NO + 3H^+ \quad (18)$$

This pathway has been described in *N. eutropha* and a few other related species (Schmidt and Bock, 1997; Schmidt *et al.*, 2002). It is superficially similar to aerobic NH_3 oxidation in that hydroxylamine is an intermediate, NO_2^- is a product, and a portion of the NO_2^- may be reduced further N_2:

$$NO_2^- + 4H^+ \rightarrow 0.5N_2 + 2H_2O \quad (19)$$

Schmidt and Bock (1997) demonstrated that anaerobic nitrifier denitrification supports growth.

The anaerobic and aerobic nitrifier denitrification pathways differ in that NO is an end product under anaerobic conditions rather than an intermediate compound. In addition, nitrogen dioxide-dependent NH_3 oxidation by *N. eutropha* does not require ammonium monooxygenase (Schmidt *et al.*, 2002), demonstrating that the two pathways are enzymatically different. In the absence of NH_3, *N. eutropha* can use H_2 or simple organic compounds as electron donors (Abeliovich and Vonhak, 1992; Bock *et al.*, 1995). In contrast to the anammox process, which is strictly anaerobic, O_2 does not inhibit NO_2-dependent NH_3 oxidation and N_2 production can occur even under aerobic conditions (Zart and Bock, 1998). However, Shrestha *et al.* (2002) observed N_2 production only under conditions of low O_2 concentration or anaerobiosis in *N. europaea*.

Very few studies have attempted to establish whether these alternative metabolic pathways in NH_3-oxidizing bacteria contribute to N_2O or N_2 production *in situ*. Webster and Hopkins (1996) estimated that nitrifier denitrification contributed 29% of the N_2O produced in a dry soil and 3% in a wet soil, but an earlier study concluded that their contribution to N_2O production was negligible (Robertson and Tiedje, 1987). In a review of the topic, Wrage *et al.* (2001) proposed the highest contributions from nitrifying denitrifiers are likely to occur under conditions of low carbon and nitrogen content. The contribution of nitrifier denitrification to anaerobic metabolism and N_2 production will ultimately be limited by the low availability of NO_2 (nitrogen dioxide) in anoxic soils and sediments.

Autotrophic microorganisms are of interest in wastewater treatment because they can denitrify without an organic carbon supplement (Jetten *et al.*, 1999; Verstraete and Philips, 1998). The microbial ecology of these artificial systems is better understood than natural systems at present. The anammox bacterium *B. anammoxidans* and the nitrifier *N. eutropha* were able to coexist in a laboratory-scale reactor (Schmidt *et al.*, 2002). Indeed, the specific anaerobic NH_3 oxidation activity of *N. eutropha* was 10 times higher than in co-culture than pure culture. Rather than the two groups producing N_2 simultaneously, their metabolism is more likely to be coupled in a nitrification–denitrification reaction that bypasses nitrite-oxidizing bacteria (Schmidt *et al.*, 2002). Wastewater reactors that favor the anammox process contain aerobic and anaerobic NH_3-oxidizers, but not nitrite oxidizers such as *Nitrobacter*. This suggests that the nitrite oxidizers cannot compete effectively with the NH_3 oxidizers for O_2, nor can anaerobic NH_4^+ oxidizers compete with NH_3 oxidizers for NO_2^- (Schmidt *et al.*, 2002). The discovery of anammox-like metabolism in marine sediments (Thamdrup and Dalsgaard, 2002) raises many ecological questions about the nature of interactions between these groups of organisms in natural ecosystems.

8.08.5.5.3 Abiotic and autotrophic denitrification

Denitrification can be supported by electron donors other than organic carbon such as Fe(II), Mn(II) and H_2S, and they can proceed by both abiotic and biotic pathways. Abiotic reduction of NO_3^- to N_2 coupled to Fe(II) oxidation may occur at low rates in the pH range 2–7 (Postma, 1990):

$$10Fe^{2+} + 2NO_3^- + 14H_2O \rightarrow 10FeOOH + N_2 + 18H^+ \quad (20)$$

The reaction is quite sensitive to temperature (Sørensen and Thorling, 1991), and is catalyzed by freshly precipitated Fe(III) oxides and Cu^{2+} (Buresh and Moraghan, 1976; Postma, 1990). In contrast to the abiotic reaction, chemolithoautotrophic denitrification coupled to NO_3^- proceeds rapidly under the low temperature, circumneutral pH conditions that are typical of the Earth's surface (Weber *et al.*, 2001) (Section 8.08.6.5.2).

Two pathways have been proposed that form N_2 from abiotic reactions between fixed nitrogen and mangenese, and both are thermodynamically feasible in typical marine pore water. Reduction of NO_3^- by Mn^{2+} (i.e., dissolved Mn(II)) was proposed by Aller (1990):

$$2NO_3^- + 5Mn^{2+} + 4H_2O \rightarrow N_2 + 5MnO_2 + 8H^+ \quad (21)$$

Because Mn^{2+} reacts rapidly with O_2, reaction (21) may be restricted to anoxic zones in manganese-rich sediments. Although this reaction is similar to a microbially mediated reaction involving Fe(II) (reaction (26)), there is no evidence at present to suggest it is performed biotically. Luther *et al.* (1997) proposed a mechanism for producing N_2 from NH_4^+ that should be favored in aerobic zones where MnO_2 is rapidly regenerated from Mn^{2+}:

$$2NH_3 + 3MnO_2 + 6H^+ \rightarrow N_2 + 3Mn^{2+} + 6H_2O \quad (22)$$

The reaction was demonstrated in anaerobic marine sediments amended with freshly synthesized MnO_2 and NH_4^+ (Luther *et al.*, 1997). However, it could not be detected with a sensitive ^{15}N-labeling technique in an unamended, manganese-rich sediment (Thamdrup and Dalsgaard, 2000). The reaction may be favorable only with certain forms of MnO_2 (Hulth *et al.*, 1999) or high levels of NH_4^+.

Chemoautotrophic denitrification coupled to H_2S, S^0, or $S_2O_3^{2-}$ occurs in some bacteria of the genus *Thiobacillus* such as *T. denitrificans* (Hoor, 1981; Section 8.08.7.9). Nitrate reduction by such a pathway increased with FeS additions and followed Michaelis–Menten kinetics in a marine sediment (Garcia-Gil and Golterman, 1993).

8.08.6 IRON AND MANGANESE

Iron and manganese oxides are the most abundant components of Earth's surface that can serve as anaerobic terminal electron acceptors in microbial metabolism, yet it was recognized only recently that microorganisms play a key role their cycling. Despite early reports that suggested biological Fe(III) reduction was important in wet rice paddy soils (Kamura *et al.*, 1963; Takai *et al.*, 1963a,b), the process was thought to be either unimportant or dominated by abiotic mechanisms (Lovley, 2000c). Similarly, biological Fe(II) oxidation was assumed to be negligible except under acidic (pH $\leq$ 3) conditions. It is now clear that microorganisms mediate both Fe(III) reduction and Fe(II) oxidation across a broad range of pH, temperature, and salinity conditions.

Dissimilatory Fe(III) reduction is the dominant form of anaerobic carbon metabolism in many ecosystems, and may be one of the earliest forms of microbial metabolism to evolve on Earth or elsewhere (Lovley, 2000c). In contrast to metabolism based on oxygen, nitrogen, or carbon terminal electron acceptors, metabolisms involving iron, manganese, and sulfur (Section 8.08.7) are regulated strongly by both geochemical and biochemical phenomena. Geochemistry is fundamental to understanding the use of metals in anaerobic metabolism, and several of the most intriguing questions about iron and manganese bacteria concern their interactions with mineral surfaces or their influence on geochemistry. Previous reviews have addressed many of the salient interactions between iron cycling and other biogeochemical processes (Figure 17).

Because Mn(IV) reduction generally makes a small contribution to carbon metabolism, it is not considered in detail in this review. However, there are many similarities between the cycles of the two metals that we allude to in the text. In fact, many Fe(III)-reducing bacteria are more aptly described as metal-reducing bacteria because they also reduce Mn(IV) and a variety of other metals. A recent and excellent review of both Fe(III) and Mn(IV) cycling was provided by Thamdrup (2000), and Tebo *et al.* (1997) reviewed microbial Mn(IV) oxidation.

8.08.6.1 Iron and Manganese in the Environment

Fe(III) and Mn(IV) respiration influences the cycling of many other elements that are of concern to environmental scientists. Fe(III)- and Mn(IV)-reducers interfere with the metabolism of SO_4^{2-}-reducers and methanogens by competing for organic carbon. Because anaerobic metabolism is often organic carbon limited and Fe(III) reduction yields more free energy than methanogenesis, Fe(III)-reducing bacteria suppress freshwater emissions of CH_4, an important greenhouse gas (Section 8.08.4). Roden and Wetzel (1996) concluded that CH_4 emissions from a freshwater wetland were reduced by 70% due to Fe(III) cycling.

Some Fe(III)-reducing bacteria oxidize organic pollutants (Anderson *et al.*, 1998; Heider *et al.*, 1999; Lovley, 2000a; Lovley and Anderson, 2000; Gibson and Harwood, 2002).

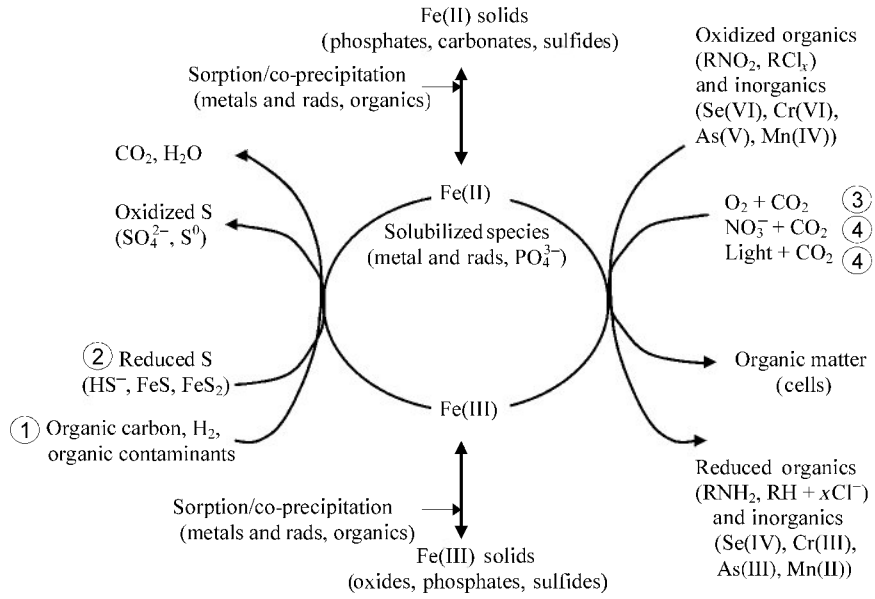

Figure 17 Substrates and processes coupled to Fe reduction–oxidation. Circled numbers refer to recent reviews of the role of microbial processes in various iron transformations. (1) Lovley and Anderson (2000), Thamdrup (2000), Johnson (1998), Blake and Johnson (2000), Küsel *et al.* (1999), and Peine *et al.* (2000). (2) Thamdrup *et al.*, (1993), Lovely and Phillips (1994a, Schipper and Jørgensen (2002), Blake and Johnson (2000), Pronk and Johnson (1992). (3) Emerson (2000), Johnson (1998), Blake and Johnson (2000), Edwards *et al.* (2000b), and Roden *et al.* (in press). (4) Straub *et al.* (2001). (after Tebo and He, 1999; Roden *et al.*, in press).

Benzene degradation is stimulated in the laboratory by substances that enhance Fe(III) reduction (Lovley *et al.*, 1994b, 1996b), and benzene-degrading sediments are enriched in *Geobacter* species (Anderson *et al.*, 1998; Rooney-Varga *et al.*, 1999), the only genus of microorganism known to couple Fe(III) reduction to the oxidation of aromatic compounds. Because petroleum-contaminated sediments are often anaerobic, Fe(III)-reducing bacteria are attractive candidates for bioremediation.

Some Fe(III)-reducing organisms, particularly members of the *Geobacteraceae*, have the ability to reduce heavy metal pollutants such as U(VI) (Holmes *et al.*, 2002). Because reduction changes uranium from a soluble to an insoluble form, such organisms may be used to remove uranium from polluted waters (Lovley, 1997; Lovley and Phillips, 1992a). Fe(III)-reducing bacteria can reduce a long list of metals including gold, silver, chromium, cobalt, selenium, and technetium (Lovley, 1997).

Iron and manganese have a strong influence on the availability of trace metal pollutants through precipitation–dissolution reactions (Burdige, 1993; Cornell and Schwertmann, 1996). Trace metals form surface complexes or co-precipitate with Fe(III) and Mn(IV) oxides, and they are released upon Fe(III) and Mn(IV) reduction (Zachara *et al.*, 2001). For example, processes that oxidize Fe(II) retain arsenic in sediments (Raven *et al.*, 1998; Senn and Hemond, 2002), and vice versa (Cummings *et al.*, 1999; Harvey *et al.*, 2002; Zachara *et al.*, 2001). Fe(III) oxides complex and co-precipitate phosphorus (Gunnars *et al.*, 2002; Bjerrum and Canfield, 2002), perhaps the biosphere's ultimate limiting nutrient (Tyrrell, 1999), and P is released upon Fe(III) reduction (Smolders *et al.*, 2001).

8.08.6.2 Iron and Manganese Geochemistry

Iron and manganese total 5% of the continental crust, with iron contributing 98% and manganese the remainder (Weaver and Tarney, 1984). They are subject to rapid changes in redox state mediated by both geochemical and biological processes. Iron atoms in near-surface Earth environments cycle between an oxidized or *ferric* state, Fe(III), and a reduced or *ferrous* state, Fe(II). Manganese exists in three redox states: Mn(II), Mn(III), and Mn(IV). In this review, the abbreviation *Mn(IV)* is understood to represent Mn(IV) and Mn(III).

Oxidized iron in equilibrium with even the most unstable of iron minerals has a concentration of about 10^{-8} M in seawater at pH 8 (Stumm and Morgan, 1981), and oxidized forms of manganese are nearly insoluble at neutral pH. Due to their poor solubility, these elements are conserved in soils and sediments derived from

rock weathering. The solubility of Fe(III) and Mn(IV) is greatly enhanced when complexed by ligands (Lovley and Woodward, 1996; Luther *et al.*, 1996; Stone, 1997). Contrary to the assumption that most dissolved iron is Fe(II), pore-water concentrations of Fe(III) can be comparable to Fe(II) (Ratering and Schnell, 2000), presumably due to organic complexation (Luther *et al.*, 1996; Tallefert *et al.*, 2000). Thus, iron cycling is partially mediated by the production and degradation of organic chelators.

Iron and manganese solubility increases dramatically upon reduction, yet most of the Fe(II) and Mn(II) in sediments at any given moment is not in a soluble form (Heron and Christensen, 1995). Dissolved forms of both metals sorb strongly onto cation exchange sites. Fe(II) precipitates as a variety of secondary minerals, the most common of which are iron monosulfide (FeS), pyrite (FeS_2), siderite ($FeCO_3$), vivianite ($Fe_3(PO_4)_2$), and magnetite ($Fe^{II}Fe^{III}_2O_4$), a magnetic, mixed valance mineral. Sulfide minerals dominate estuarine and marine sediments (Section 8.08.7), and are stable in neutral to weakly acidic habitats in the absence of O_2. Siderite formation is favored in water with carbonate alkalinity >1 mM (King, 1998), and it is the most abundant form of Fe(II) in sediments dominated by Fe(III) reduction or methanogenesis (Coleman *et al.*, 1993). These minerals are also produced by Fe(III)-reducing bacteria during ferrihydrite reduction (Zachara *et al.*, 2002). In contrast to Fe(II), Mn(II) is quite stable in the presence of O_2 at circumneutral pH (<8) and therefore tends to accumulate in oxic environments. Mn(II) may precipitate as $MnCO_3$ (van Cappellen *et al.*, 1998), but most is either adsorbed, dissolved or organically complexed (Burdige, 1993).

About 35% of the iron and 75% of the manganese in soils and sediments is in the form of free oxides (Canfield, 1997; Cornell and Schwertmann, 1996; Thamdrup, 2000). The remainder occurs as a minor constituent of silicate minerals. The lattice structure of Fe(III) oxide minerals varies widely. Freshly oxidized Fe(III) precipitates rapidly as ferrihydrite ($Fe(OH)_3$), a reddish-brown, amorphous, poorly crystalline mineral. Ferrihydrite is the dominant product of Fe(II) oxidation whether it occurs by abiotic oxidation, aerobic microbial oxidation, or anaerobic microbial oxidation (Straub *et al.*, 1998). Over a period of weeks to months, amorphous ferrihydrite crystals undergo diagenesis to yield well-ordered, strongly crystalline, stable minerals such as hematite(α-Fe_2O_3) and goethite (α-FeOOH) (Cornell and Schwertmann, 1996).

Fe(III)-reducing bacteria growing on ferrihydrite can produce extracellular fine-grained magnetite (Fe_3O_4) (Lovley *et al.*, 1987; Fredrickson *et al.*, 1998). Recently, it was discovered that *Shewanella oneidensis* (formerly *S. putrefaciens*) produced and deposited an unidentified iron mineral intracellularly (Glasauer *et al.*, 2002). Intracellular deposits of magnetite were previously known only from magnetotactic bacteria and a few higher organisms (Bazylinski and Moskowitz, 1997).

8.08.6.3 Microbial Reduction of Iron and Manganese

Microorganisms that reduce extracellular Fe(III) or Mn(III, IV) to support metabolism or growth (i.e., dissimilatory Fe(III)- or Mn (IV)-reducing bacteria) can be classified into two broad physiological categories. The most important of these for anaerobic carbon metabolism are the organisms that use metals as their primary terminal electron acceptor for the partial or complete oxidation of organic compounds or H_2, thereby conserving energy via Fe(III) or Mn(III, IV) respiration. The second group are fermenting bacteria that channel a small portion (<5%) of their organic-carbon-derived electron transport to Fe(III) or Mn(IV) reduction, thereby using metals as nonrespiratory electron sinks (Lovley, 1987, 1997). Most work on microbial metal reduction has focused on iron because it is abundant and interacts strongly with other elements, whether in an oxidized or reduced state. However, it is recognized that metal-reducing microorganisms are often capable of using several alternative elements as terminal electron acceptors, and likewise, a given element can be reduced by a wide variety of microorganisms.

8.08.6.3.1 Metabolic diversity in Fe(III)- and Mn(IV)-reducing organisms

The capacity for dissimilatory Fe(III) and Mn(IV) reduction is widely distributed among the subdivisions of the *Bacteria*, including all subclasses of the *Proteobacteria* (Coates *et al.*, 2001) and some hyperthermophiles. Most of the known species of Fe(III)-respiring bacteria are in the delta subclass of the *Proteobacteria*. This subclass includes the *Geobacteraceae* family, which is populated entirely by Fe(III)-reducing bacteria, including the well-studied species *Geobacter metallireducens* (Lovley *et al.*, 1993a). Metal reduction has also been reported in hyperthermophiles in the *Archea* (Vargas *et al.*, 1998). More than 40 isolates that couple anaerobic growth to dissimilatory Fe(III) respiration have been characterized (Coates *et al.*, 2001).

The energy sources used by Fe(III)- and Mn(IV)-respiring organisms include H_2 and acetate, which are the same primary substrates

that support methanogenesis and SO_4^{2-} reduction. Unlike the methanogens, some metal-respiring microorganisms also oxidize multicarbon organic compounds such as lactate, pyruvate, long-chain fatty acids, aromatic compounds, amino acids and glucose (Küsel *et al.*, 1999; Lovley, 2000b). These compounds can be completely oxidized to CO_2, or they can be fermented to acetate. Some fermentative organisms cannot grow solely on Fe(III), but reduce small quantities of Fe(III) as a nonrespiratory electron sink (Lovley, 1987, 2000b).

Complete oxidation of organic carbon to CO_2 is found in three of the four genera in the *Geobacteraceae* family (Lovley, 2000c), and phylogenetically distinct genera such as *Geovibrio* and *Deferribacter* (Greene *et al.*, 1997). The gamma and epsilon subclasses of the *Proteobacteria* tend to oxidize a more limited range of organic acids, and often carbon oxidation is incomplete (Lovley, 2000b). A well-studied member of this group is *Shewanella oneidensis* (formerly *S. putrefaciens*), one of the first described nonfermentative Fe(III)-reducers (Myers and Nealson, 1988; Nealson and Myers, 1992), and notable because it is also a facultative anaerobe (Kieft *et al.*, 1999). The ability to use H_2 for an energy source is common among incompletely oxidizing Fe(III)-reducers, and hyperthermophilic Fe(III)-reducers in the *Archea* and *Bacteria* (Slobodkin *et al.*, 2001; Vargas *et al.*, 1998). Relatively few Fe(III)-reducing bacteria outside these groups are known to oxidize H_2 (Coates *et al.*, 1999). However, some species of *Geobacter* can use both H_2 and acetate (Caccavo *et al.*, 1994, 1996).

Most Fe(III)-reducing organisms have the capacity to reduce Mn(IV) and at least one other common oxidant such as O_2, NO_3^-, SO_4^{2-}, elemental sulfur (S^0), or humic substances (Thamdrup, 2000; Senko and Stolz, 2001). In contrast to the small penalty in free energy that occurs when denitrifying bacteria switch from O_2 to NO_3^--respiration, growth on Fe(III) by the facultative anaerobe *S. oneidensis* is far inferior to that on O_2 (Kostka *et al.*, 2002a). Humic substances may prove to be the most common alternative electron acceptor for metal-reducing bacteria, particularly in freshwater environments where S^0 is not abundant (Lovley *et al.*, 1996a, 1998). There are several SO_4^{2-}-reducing bacteria that can also reduce Fe(III) and U(VI), although they generally cannot support growth on transition metals alone (Lovley *et al.*, 1993b). In fact, *Desulfovibrio desulfuricans* will simultaneously reduce SO_4^{2-} and Fe(III), or SO_4^{2-} and U(VI), if H_2 is available (Lovley and Phillips, 1992b; Coleman *et al.*, 1993).

Relatively little is known about the abundance of Fe(III)-reducing species *in situ*. Attempts to quantify dissimilatory Fe(III)-reducing bacteria in aquifer sediments suggest that they are dominated by the genera *Geobacter* or *Geothrix* (Anderson *et al.*, 1998; Rooney-Varga *et al.*, 1999; Snoeyenbos-West *et al.*, 2000). More ecosystems will need to be surveyed before generalizations are made about the dominant groups elsewhere (e.g., Rosselló-Mora *et al.*, 1995).

8.08.6.3.2 *Physical mechanisms for accessing oxides*

A fundamental difference between Fe(III) and Mn(IV) reduction and the other major pathways of anaerobic metabolism is that the oxidants are sparingly soluble solids. In order to access the terminal electron acceptor a microorganism must either: (i) make physical contact with an oxide surface, (ii) reduce an intermediate compound that can shuttle electrons from the cell to a distant oxide surface, or (iii) use the metal in a dissolved or chelated form. All three adaptations are observed among Fe(III)- and Mn(IV)-respiring organisms. Metal-respiring organisms do not necessarily require direct physical contact with oxides as some early studies suggested (Lovley and Phillips, 1986; Tugel *et al.*, 1986), but such contact may be common. Evidence of this is the observation that Fe(III) reductase activity tends to be localized in the outer membrane of both *G. sulfurreducens* and *S. oneidensis* (Beliaev and Saffarini, 1998; Gaspard *et al.*, 1998; C. R. Myers and J. M. Myers, 1992). Using atomic force microscopy, Lower *et al.* (2001) measured the adhesion strength of *S. oneidensis* cells to mineral surfaces and found that it increased rapidly upon exposure to goethite under anaerobic conditions. There was some evidence for a mobile iron reductase in the outer cell membrane that facilitated electron transport from the cell to the oxide surface. Grantham *et al.* (1997) reported that sites where *S. oneidensis* was observed adhering to Fe(III) oxide surfaces corresponded to pits caused by Fe(III) dissolution. The shape of the pits suggested that the organisms were more mobile under anaerobic conditions than aerobic conditions, an adaptation that may enhance their access to fresh Fe(III) oxide surfaces. A similar adaptation was observed in *G. metallireducens* which expressed flagella and was chemotaxic toward Fe(II) and Mn(II) when grown on insoluble oxides, but not when grown on soluble Fe(III) or Mn(IV) (Childers *et al.*, 2002). This adaptation had gone unnoticed because most culture work had previously been based on complexed, soluble Fe(III) media, highlighting the importance of culturing metal-reducing organisms on substrates that are common *in situ* such as ferrihydrite or goethite. More species must be tested to determine if chemotaxis is unique to

Fe(III)-reducing organisms that do not produce soluble electron shuttling compounds.

8.08.6.4 Factors that Regulate Fe(III) Reduction

Because Fe(III) respiration is the most recent major pathway of anaerobic metabolism to be investigated, there has been relatively little work on the processes that regulate it *in situ*. Rather, most advances have concerned the mechanisms by which microorganisms access Fe(III) oxides and interact with the physicochemical processes that govern Fe(III) oxide dissolution.

8.08.6.4.1 Electron shuttling compounds

Humic substances are effective electron shuttling compounds that are ubiquitous in soils and sediments (Stevenson, 1994). The redox-active moieties in these compounds are primarily quinones and related aromatic reductants (Lovley and Blunt-Harris, 1999; Scott *et al.*, 1998; Stone *et al.*, 1994). Because purified humic acids are expensive and it is difficult to evaluate their redox state, the synthetic quinone 2,6-anthraquinone disulphonate (AQDS) has been used as a model compound for studying the potential for humic acids to serve as terminal electron acceptors (Lovley *et al.*, 1996a). AQDS may not be an appropriate substitute for native humic acids in studies of reaction kinetics (Nurmi and Tratnyek, 2002), but it has proved to be useful for isolating humic-reducing microorganisms from a variety of ecosystems including lakes, wetlands and estuaries (Coates *et al.*, 1998). It appears that most, if not all, Fe(III)-reducing bacteria can obtain energy by the reduction of AQDS or humic substances (Lovley *et al.*, 2000). The strains tested to date include members of the *Geobacteraceae* family and hyperthermophiles. The metabolism is present in fermentative and nonfermentative organisms, and may be particularly widespread among thermophilic organisms (Lovley, 2000c).

There are several reasons why the ability to reduce humic substances could be an advantage to metal-respiring microorganisms. Higher rates of reduction should be possible with humic acids and other soluble oxidants than with solid-phase oxidants that require the organism to continually establish physical contact in order to access fresh oxide surfaces. Humic substances are ubiquitous and accumulate to levels that could make them significant alternative electron acceptors in some ecosystems. As with other anaerobic electron acceptors, the capacity to support respiration is enhanced by processes that regenerate humic substances in an oxidized form. Reduced humics and hydroquinones (i.e., reduced quinones) are rapidly oxidized by Fe(III) and Mn(IV), making them available again for microbial respiration. Some humic substances may be more efficient than microbial cells at accessing physical locations on metal oxides because they are relatively small (Zachara *et al.*, 1998). Finally, because enzymatically reduced humics may pass electrons to Fe(III), Mn(IV), U(VI), or other oxidants (Stone, 1987), an organism can indirectly access several potential "secondary" electron acceptors simultaneously rather than just one (Figure 18). The ability to shuttle electrons between microorganisms and inorganic oxidants explains why even low concentrations of AQDS (5 μmol kg^{-1}) and humics can stimulate Fe(III) reduction in sediments (Nevin and Lovley, 2000).

An actual contribution of humic substances to metal oxide reduction in natural systems has not been demonstrated, and there are processes such as adsorption or decomposition that could limit their effectiveness. Kostka *et al.* (2002a) observed that AQDS additions elicited a larger increase in Fe(III) reduction by *S. oneidensis* growing on ferrihydrite than smectite clay minerals. This suggests that the influence of humic substances may depend on soil or sediment mineralogy. Nevertheless, there is ample evidence to suggest that a portion of the anaerobic metabolism that was previously attributed to direct enzymatic Fe(III) and Mn(IV) reduction was actually nonenzymatic reduction by microbially reduced humic substances.

Variability in the effectiveness of humic substances as electron shuttling compounds is expected due to differences in their chemical structure. For example, the electron-accepting capacity of humic substances from three distinct sources varied nearly 700-fold in the following order: soils > sediments > dissolved river-borne (Scott *et al.*, 1998; Figure 19). Microorganisms are a source of quinone moities and other electron shuttling compounds. For example, *S. oneidensis*

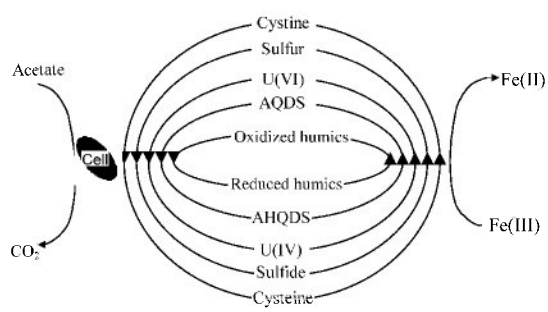

Figure 18 The variety of electron shuttles that promote Fe(III) reduction. (after Nevin and Lovley, 2000).

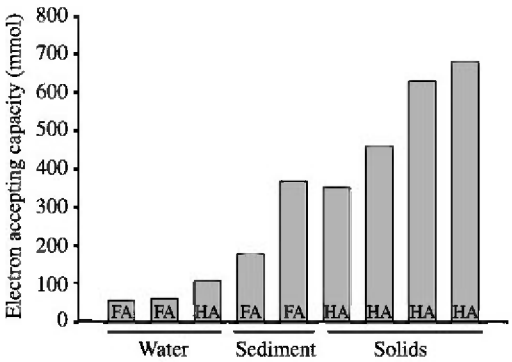

Figure 19 Ecosystem-level differences in the ability of humic substances to accept electrons. Samples were taken from the water column of freshwater lakes and rivers, aquatic sediments and soils (FA = fulvic acid, HA = humic acid) (after Scott *et al.*, 1998).

excretes an unidentified quinone-based compound that can reduce ferrihydrite and MnO_2 (Newman and Kolter, 2000), and *G. sulfurreducens* excretes a *c*-type cytochrome with similar capability (Seeliger *et al.*, 1998). These studies raise the prospect that some organisms can synthesize their own electron shuttle compounds if the supply of oxidants is limiting.

8.08.6.4.2 Fe(III) chelators

There are a variety of humic and nonhumic organic chelating agents that enhance the dissolution and solubility of metals (Stone, 1997; Luther *et al.*, 1992). Some forms of chelated Fe(III) are more rapidly reduced by bacteria than insoluble Fe(III) oxides (Lovley, 1991). Amending aquifer sediments with the synthetic chelator nitrilotriacetic acid (NTA) increased dissolved Fe(III) and stimulated the enzymatic reduction of Fe(III)-oxides (Lovley and Woodward, 1996). The increase in Fe(III) reduction was attributed to enhanced availability of dissolved Fe(III) rather than a stimulation of growth caused by trace elements or metabolizable organic carbon. However, the effect could also be explained by NTA complexation of Fe(II), which would prevent Fe(II)-poisoning of the mineral surface (see next paragraph). Other complexing agents such as hydroxamate have no effect on Fe(III) dissolution rates (Holmén *et al.*, 1999). As with humic acids, there is very little evidence that chelators substantially influence microbial iron metabolism *in situ*, and there are reasons that model chelators such as NTA are not good surrogates for those in natural systems (Straub *et al.*, 2001). The stability of ligand-Fe(III) complexes influence Fe(III) reduction rates because the cell must be able to out compete the chelator for Fe(III). In a study with *S. oneidensis*, the stability of soluble ligand-Fe(III) complexes was inversely related to Fe(III) reduction rates (Haas and DiChristina, 2002). Of the chelators tested, the highest Fe(III) reduction rates were produced by citrate, which is a weakly complexing ligand similar to most naturally occurring organic chelators.

Organic ligands also form complexes with Fe(II) (Luther *et al.*, 1996) and may enhance Fe(III) dissolution by removing the Fe(II) that coats and passively blocks access to fresh Fe(III) oxides surfaces. Urrutia *et al.* (1999) considered this mechanism and concluded that it was more important than Fe(III) complexation. Bacterial cells adsorbed to mineral surfaces also inhibit Fe(III) reduction rates (Urrutia *et al.*, 1999).

8.08.6.4.3 Mineral reactivity

Metal oxides in soils and sediments are mixtures of poorly crystalline, strongly crystalline and silicate-bound minerals. Differences in the intrinsic reactivity of these broad categories is sufficient to explain much of the variation in Fe(II) and Mn(II) reduction rates observed in nature, which spans several orders of magnitude. Mixtures of Fe(III) oxides are typically separated by exposing them to a sequence of chemical extractions involving increasingly strong solutions of acids, ligands, and reductants. For example, the poorly crystalline fraction can be extracted with a combination of aerobic and anaerobic oxalate solutions (Thamdrup and Canfield, 1996) or a dilute solution of HCl (0.5 M), while dithionate–citrate–bicarbonate is used to extract the strongly crystalline fraction. A limitation of such techniques is that the fractions are operationally defined and not mineral specific. Efforts have been made to calibrate wet chemical extraction protocols against minerals of known composition (Canfield, 1989; Haese *et al.*, 1997; Kostka and Luther, 1994). Nevertheless, no extraction technique is entirely discriminating for a specific metal phase or mineral (Zachara *et al.*, 2002). Recognizing the wide range of reactivities represented in a single operationally defined Fe(III) oxide pool, Postma (1993) proposed characterizing reactivity with a single continuous extraction. The "reactivity continuum" method allows straightforward comparisons of the bulk reactivity of pure and mixed iron (hydr)oxide pools (Figure 20).

The susceptibility of metal oxides to reduction and dissolution depends on mineralogy, crystallinity, surface area, the effectiveness of reducing and chelating agents, and microbial activity. Early culture studies with Fe(III)-respiring bacteria demonstrated that Fe(III) reduction rates vary with mineral form or crystallinity

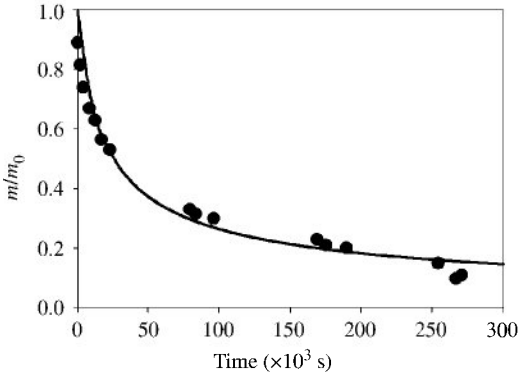

Figure 20 Application of the reactivity continuum method to Fe(III) oxide pools in oxidized aquifer sediments. The ratio m/m_0 is the fraction of undissolved Fe-oxide. The slope of the curve decreases with time because reactive minerals are increasingly depleted leaving behind relatively more recalcitrant forms (source Postma, 1993).

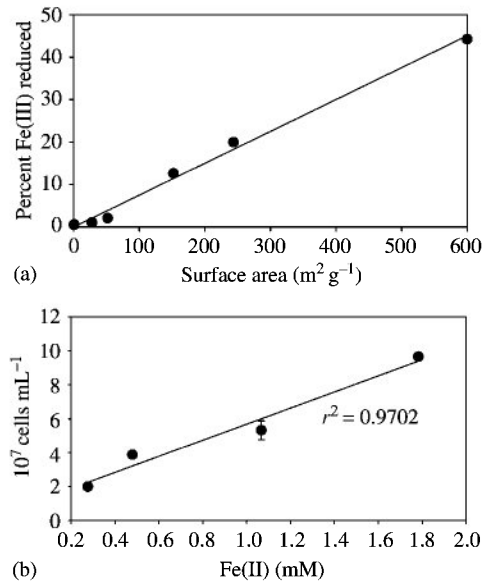

Figure 21 Relationship of Fe(III)-reducing bacteria activity and growth to oxide surface area. (a) Percent Fe(III) reduced as a function of oxide surface area. Surface area corresponded to different mineral types and included hematite, goethite, and ferrihydrite. (b) The density of *Shewanella oneidensis* cells as a function of the amount of structural Fe(III) reduction to Fe(II) in smectite clay, a strongly crystalline, high-surface-area Fe mineral. Differences in Fe(II) content reflect different amounts of clay particles inoculated into a minimal basal media (after Roden and Zachara, 1996 and Kostka *et al.*, 2002a, respectively).

(Lovley and Phillips, 1986). Minerals such as ferrihydrite and lepidocrocite (γ-FeOOH) are generally reduced more rapidly than relatively stable minerals such as goethite and hematite (Postma, 1993). Amorphous manganese oxides such as vernadite are more easily reduced than strongly crystalline forms such as pyrolusite, but the overall influence of crystallinity on reduction kinetics appears to be weaker for manganese and iron oxides (Burdige *et al.*, 1992).

It has now become evident that the primary reason for mineral-related differences in reactivity is not thermodynamic stability, but the fact that amorphous minerals have a far higher surface area than crystalline minerals (Figure 21). Kostka *et al.* (2002a) reported that Fe(III)-reducers grew nearly as well on smectite clay as on ferrihydrite, even though smectite clay is considered a crystalline mineral. Although smectite clay is more highly crystalline than ferrihydrite, the two minerals have a comparable surface area (~700 $m^2\ g^{-1}$) (Schwertmann and Cornell, 1991). As a result, Fe(III)-reducing bacteria were able to remove 20–50% of the total clay-bound iron and thereby alter the physical and chemical characteristics of these ubiquitous minerals (Kostka *et al.*, 1999a,c). Although Fe(III) reduction rates are reduced by one to two orders of magnitude in the presence of minerals with low- versus high surface area, there is ample evidence demonstrating that dissimilatory Fe(III)-reducing bacteria can gain energy for growth using goethite, hematite, and magnetite (Kostka and Nealson, 1995; Roden and Zachara, 1996), in some cases consuming up to 30% of the oxide-bound Fe(III). Fe(III)-rich clays and other highly crystalline forms account for much of the Fe(III) mass in soils and sediments (Stucki *et al.*, 1996), and may therefore support a substantial portion of the planet's anaerobic microbial carbon metabolism.

The reactivity of Fe(III) and Mn(IV) minerals is often highest immediately after they precipitate following Fe(II) or Mn(IV) oxidation. Because metal reduction is usually limited by electron acceptor availability (or co-limited with organic carbon), ecosystem processes that favor Fe(II) and Mn(II) oxidation also promote Fe(III) and Mn(IV) reduction. Regeneration by O_2 occurs slowly in rivers, lakes, and oceans because O_2 diffusion in water is exceedingly slow and sediments have a high biological oxygen demand. Oxidation is much faster when the sediments are mixed by currents or bioturbation (Aller, 1990). Periodic drawdown of the water table in intertidal sediments and wetlands rapidly introduces O_2 and accelerates metal oxidation. Wetland plants enhance metal oxidation by introducing O_2 directly in the soil from porous root systems (Kostka and Luther, 1995), a process known as radial oxygen loss, and by removing soil water by transpiration (Dacey and Howes, 1984). Metal oxides are also regenerated anaerobically through a variety of enzymatic and nonenzymatic mechanisms coupled to redox cycles of sulfur, iron, and

manganese. Because all of these processes can occur in wetland soils, a larger proportion of the total iron pool may be reactive in wetlands than nonwetland ecosystems (Weiss, 2002). Thus, wetlands may be "hot spots" of iron cycling.

8.08.6.4.4 Abiotic versus biotic reduction

Iron and manganese differ from other common terminal electron acceptors such as O_2, NO_3^-, SO_4^{2-}, and HCO_3^- in that they are subject to rapid nonenzymatic reduction. For example, Fe(II) reduces Mn(IV) according the generalized reaction (Lovley and Phillips, 1988; Postma, 1985):

$$2Fe(II) + Mn(IV) \rightarrow 2Fe(III) + Mn(II) \quad (23)$$

This reaction makes manganese the ultimate electron sink for a portion of the organic carbon consumed by dissimilatory Fe(III)-reducing bacteria, and it reduces diffusive losses of iron from sediments. Nonenzymatic Mn(IV) reduction by Fe(II) can be significant in high-Mn(IV) sediments (Aller, 1990), but its global significance is ultimately limited by the low content of manganese in the Earth's crust compared to iron.

Nonenzymatic reduction by sulfides is widely considered to be the primary mechanism for Fe(III) and Mn(IV) reduction in systems where SO_4^{2-} is abundant and sulfate reduction dominates anaerobic metabolism (Aller and Rude, 1988; Burdige and Nealson, 1986; Jacobson, 1994; Kostka and Luther, 1995; Postma, 1985; Pyzik and Sommer, 1981). Yet, Fe(III)-reducing bacteria are abundant in marine soils and sediments (Lowe *et al.*, 2000; Kostka *et al.*, 2002c), and several workers have noted that Fe(II) continues to accumulate when H_2S production is blocked by molybdate, apparently because of enzymatic Fe(III) reduction (Canfield, 1989; Canfield *et al.*, 1993b; Hines *et al.*, 1997; Jacobson, 1994; Joye *et al.*, 1996; Kostka *et al.*, 2002c; Lovley and Phillips, 1987; Sørensen, 1982).

Organic compounds have the potential to abiotically reduce Fe(III) and Mn(IV) (Luther *et al.*, 1992; Stone, 1987). Phenols and a variety of other aromatic compounds reduce Fe(III) rapidly at acidic pH, but slowly at circum neutral pH (LaKind and Stone, 1989). Humics can reduce Fe(III) effectively at circumneutral pH and they are abundant in soils and sediments. Because humics and other organic compounds often serve as electron shuttles between metal-reducing bacteria and metal oxides (Lovley *et al.*, 1996a), it may be difficult to separate microbial and nonmicrobial sources of electrons. Finally, aerobic photoreduction of Fe(III) has been observed in freshwater and marine environments (Barbeau *et al.*, 2001; Emmenegger *et al.*, 2001), but it is unknown to what degree this process contributes to nonenzymatic Fe(III) reduction in water columns or sediments.

The relative contributions of enzymatic and nonenzymatic pathways to Fe(III) reduction in marine environments is difficult to measure and highly variable. Because nonenzymatic reduction is rapid enough to compete with microorganisms for Fe(III)-oxide substrates (Thamdrup, 2000), nonenzymatic reduction should be favored over enzymatic reduction in environments where sulfides are abundant and reactive Fe(III) oxides are scarce. In such sediments, biological reduction may be restricted to microsites where sulfide levels are low and poorly-crystalline Fe(III) is abundant (Canfield, 1989). However, H_2S does not accumulate in the porewater of many marine environments (i.e. they are not *sulfidic*) because it reacts with metal oxides. In sediments where sulfide is produced but does not accumulate, enzymatic Fe(III) reduction rates are often substantial. Stoichiometric considerations suggest that enzymatic reduction should be a large part of the overall Fe(III) reduction in such sediments (Thamdrup, personal communication). For example, the oxidation of one mole of organic carbon to CO_2 can support the reduction of either 4 moles of Fe(III) to Fe(II) or 0.5 moles of SO_4^{2-} to S^{2-}. Assuming that SO_4^{2-} reduction produces H_2S, the H_2S could oxidize as little as 1/3 moles of Fe(III) (i.e. $3H_2S + 2FeOOH \rightarrow 2FeS + S$). In this case, the relative contributions of enzymatic and nonenzymatic processes to Fe(III) reduction would be comparable even if Fe(III) reduction contributed just 10% of total organic carbon oxidation. It is necessary to quantify the proportion of total Fe(III) reduction contributed by Fe(III)-reducing bacteria in order to understand the ecology of these ubiquitous organisms. However, from the perspective of overall microbial metabolism, it should be noted that Fe(III) reduction is ultimately a result of microbial respiration regardless of the mechanism. That is, Fe(III) serves indirectly as a terminal electron acceptor for SO_4^{2-}-reducing bacteria when Fe(III) undergoes non-enzymatic reduction by H_2S.

8.08.6.4.5 Separating enzymatic and nonenzymatic Fe(III) reduction

A common approach to estimating the contribution of microorganisms to Fe(III) and Mn(IV) reduction based on carbon mass balance. Total anaerobic microbial respiration is estimated by measuring the carbon mineralization rate (i.e., $\sum CO_2 + CH_4$ production). The carbon that was respired by SO_4^{2-}-reducing bacteria and methanogens is subtracted from the total, and the difference is assumed to be the contribution of Fe(III)- and Mn(IV)-reducing bacteria (Canfield *et al.*, 1993b; Thamdrup, 2000). Typically, the contribution of aerobic metabolism to carbon

mineralization is excluded by design, denitrification is assumed to be negligible because of limited NO_3^- availability, and it is assumed that nonmethanogenic fermentation does not completely oxidize organic carbon to CO_2. The approach is attractive in marine systems because methanogenesis is negligible, which simplifies the calculation, and SO_4^{2-} reduction can be measured accurately using a ^{35}S radioisotope technique (Jørgensen, 1978a,b,c). Sulfate reduction measurements in salt marsh soils are more problematic because severed roots can introduce fermentable organic carbon compounds, and spatial variability introduces error into the difference calculation.

A second method for separating enzymatic and nonenzymatic Fe(III) reduction by H_2S is to block SO_4^{2-} reduction with molybdate (MoO_4^{2-}). The technique has been used effectively to demonstrate the importance of enzymatic reduction in marine and freshwater sediments (Section 8.08.6.4.4). As with all inhibitor techniques, there is the possibility that molybdate additions directly or indirectly affect processes other than SO_4^{2-} reduction. For example, it could overestimate biotic Fe(III) reduction if the enzymatic process was stimulated by a cessation of competition with H_2S for Fe(III) substrates, or underestimate if SO_4^{2-} reduction was not completely blocked. Despite these *potential* limitations, the molybdate method produces patterns that are consistent with other types of geochemical data, and it is therefore widely used.

Perhaps the most elegant method for separating enzymatic and nonenzymatic Fe(III) reduction would exploit a difference in natural Fe-isotope fractionation. However, such a method has proved to be elusive. Experiments with the Fe(III)-reducing bacterium *Shewanella algae* yielded a 1.3‰ fractionation between the ferrihydrite substrate and soluble Fe(II), suggesting that variations in the natural abundance $\delta^{56}Fe$ could be interpreted biologically (Beard *et al.*, 1999). Unfortunately, even larger iron fractionations (up to 3.6‰) are possible by nonbiological mechanisms (Anbar *et al.*, 2000). The $^{16}O/^{18}O$ ratio of siderite ($FeCO_3$) was similar in the presence and absence of Fe(III)-reducing bacteria, suggesting that oxygen isotopes will not be a useful signature of enzymatic Fe(III) reduction (2001). However, the isotopic composition of biogenic iron minerals could prove to be useful as paleothermometers. Temperature-dependent fractionation of $\delta^{18}O$ in biogenic siderite occurred in cultures of Fe(III)-reducing bacteria (Zhang *et al.*, 1997; Zhang *et al.*, 2001), and in the intracellular magnetite produced by magnetotactic bacteria (Mandernack *et al.*, 1999). Radiolabeling the iron oxide pool to more precisely measure Fe(III) reduction rates proved unsuccessful because of rapid isotope exchange between the Fe(III) and Fe(II) pools (Roden and Lovley, 1993).

8.08.6.4.6 Fe(III) reduction in ecosystems

Field studies of enzymatic Fe(III) reduction are scarce, but they generally indicate that the availability of reactive Fe(III) and organic carbon govern rates. Thamdrup (2000) reported a positive relationship between the content of poorly crystalline Fe(III) in marine sediments and the fraction of carbon oxidation mediated by Fe(III)-reducing bacteria (Figure 22). The range of values indicates that Fe(III) reduction can account for up to 90% of the carbon oxidation in some sediments. Roden and Wetzel (2002) found a similar relationship in a freshwater marsh, also with a contribution from Fe(III) reduction of between 20% to 90% to the overall anaerobic carbon metabolism. It is noteworthy that Fe(III) reduction was never Fe(III)-saturated in either study, suggesting that Fe(III) availability may limit Fe(III) reduction within the range of labile Fe(III) concentrations that are generally found in soils and aquatic sediments. By analogy to denitrification, SO_4^{2-} reduction and methanogenesis, organic carbon availability is expected to also limit Fe(III) reduction. Roden and Wetzel (2002) reported a strong linear relationship ($r^2 = 0.99$) between microbial respiration (a measure of carbon availability) and initial Fe(III) reduction rate, and proposed that overall Fe(III) reduction was regulated by both carbon and Fe(III) availability according to the equation:

$$R_{Fe(III)} = \alpha b R_{oc} Fe(III)_{reac} \qquad (24)$$

where $R_{Fe(III)}$ and R_{oc} are rates of Fe(III) reduction and organic carbon decomposition, respectively. $Fe(III)_{reac}$ is the concentration of reactive Fe(III), α is the stoichiometric ratio of Fe(III) atoms reduced per carbon atoms oxidized (4 : 1 in this case), and b is a rate constant. Thus, for any given initial amount of reactive Fe(III), organic carbon

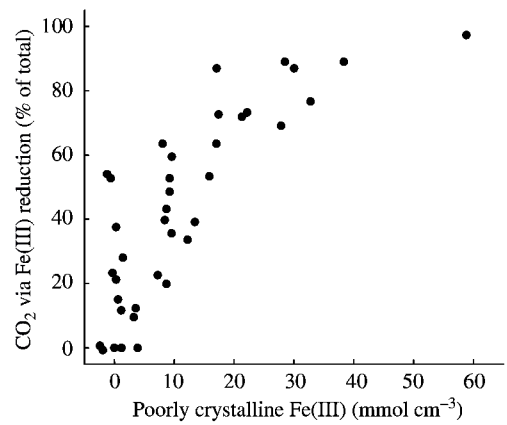

Figure 22 The fraction of carbon metabolism due to Fe(III) reduction in marine sediments as a function of the poorly crystalline Fe(III) content (after Thamdrup, 2000).

availability regulates Fe(III) reduction rates until the reactive Fe(III) pool has been exhausted.

8.08.6.5 Microbial Oxidation of Iron and Manganese

Fe(II) and Mn(II) oxidation regenerate high-quality (i.e., poorly crystalline) substrates for Fe(III) and Mn(IV)-reducing microorganisms. In contrast to other terminal electron acceptors, these elements rapidly precipitate upon oxidation and settle so that they are efficiently recycled in the ecosystem. Oxidation occurs under both aerobic and anaerobic conditions, and it can be biologically mediated or autocatalytic in either case. Mechanisms for chemical oxidation of Fe(II) under anaerobic conditions include reaction with Mn(IV) (Postma, 1985) and NO_2^- (Hansen *et al.*, 1994; Weber *et al.*, 2001). Organisms that oxidize Fe(II) and Mn(II) to support growth must compete with chemical oxidation for substrates.

8.08.6.5.1 Energetics of Fe(II) and Mn(II) oxidation

Microbial growth based on Fe(II) oxidation proceeds readily at acidic pH (<4) because Fe^{2+} (i.e., aqueous Fe(II)) is stable (Patrick and Henderson, 1981). In contrast, biological Fe(II) oxidation at circumneutral pH has been assumed to be unimportant because autocatalytic oxidation is rapid, and the reaction produces relatively little free energy (Straub *et al.*, 2001). However, neutralophilic Fe(II)-oxidizing bacteria growing on Fe(II) may actually be able to generate more free energy than acidophilic bacteria when one considers the forms of Fe(II) and Fe(III) in natural ecosystems. At a pH of 6 or 7, Fe(II) will often be in the form of $FeCO_3$, and Fe(III) will be in the form of an insoluble amorphous hydroxide, such as $Fe(OH)_3$ or FeOOH, which removes the product from the solution (Emerson, 2000). Under these conditions, Fe(II) oxidation coupled to O_2 respiration can generate substantial free energy.

Because Mn(II) is stable at pH <8, significant contributions from microorganisms to Mn(II) oxidation has been relatively easy to demonstrate, and a number of Mn(II) oxidizing bacteria have been isolated. Microbial oxidation is considered the primary mechanism of Mn(II) oxidation in circumneutral freshwater (Ghiorse, 1984; Nealson *et al.*, 1988). The sheathed bacterium *Leptothrix discophora* is perhaps the most-studied species of Mn(II)-oxidizing bacteria, and rate laws have been developed to describe Mn(II) oxidation as a function of pH, temperature, dissolved O_2, and Cu concentration (Zhang *et al.*, 2002). *Bacillus* sp. strain SG-1 has also been well studied. This species and related strains have been shown to oxidize Mn(II) even while in a spore stage (Francis and Tebo, 2002). All of the known organisms that perform Mn(II) oxidation are heterotrophic, and the process has not yet been linked to chemolithoautotrophy. Further details on Mn(II) oxidation are provided by Tebo *et al.* (1997) and Emerson (2000).

8.08.6.5.2 Anaerobic Fe(II) oxidation

Anaerobic Fe(II) oxidation is one of the most recently recognized categories of anaerobic metabolism. The first report of such metabolism described anaerobic, phototrophic organisms that required only Fe(II), CO_2, and light for growth (Widdel *et al.*, 1993):

$$4Fe^{2+} + CO_2 + 11H_2O \rightarrow 4Fe(OH)_3 + (CH_2O) + 8H^+ \quad (25)$$

Since then, several such bacteria have been isolated from freshwater and marine environments (Ehrenreich and Widdel, 1994; Heising and Schink, 1998; Heising *et al.*, 1999; Straub *et al.*, 1999). One implication of this metabolism is that microorganisms may have contributed to the banded iron formations, which resulted from widespread Fe(III)-oxide deposition in oceans at a time when there was very little atmospheric O_2 (Kump, 1993). Little is known about the physiology or ecology of these organisms (Straub *et al.*, 2001), but most strains can couple Fe(II) oxidation to NO_3^- reduction.

Even more recent was the first report of anaerobic Fe(II) oxidation coupled to NO_3^- reduction (Straub *et al.*, 1996; Hafenbradl *et al.*, 1996):

$$10FeCO_3 + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 10HCO_3^- + 8H^+ \quad (26)$$

Although NO_3^- reduction to NH_4^+ is thermodynamically feasible, all known strains produce primarily N_2 (Straub *et al.*, 2001), making this a novel metabolic pathway for N_2 production (Section 8.08.5.5.3). Cultures from a variety of environments grow solely on aqueous or solid-phase Fe(II) and CO_2 (i.e., they are chemolithoautotrophs), while others require Fe(II), CO_2 and an organic co-substrate such as acetate (i.e., they are putative mixotrophs). Organisms capable of anaerobic Fe(II) oxidation accounted for up to 58% of the total cultivatable denitrifying community in the profundal sediments of a lake (Hauck *et al.*, 2001), but not more than 0.8% of denitrifiers in other aquatic sediments (Straub and Buchholz-Cleven, 1998). Freshwater organisms can couple NO_3^- reduction to the oxidation of microbially

reduced goethite and other solid-phase Fe(II) (Weber *et al.*, 2001), and enrichment cultures from marine sediments oxidize FeS, but not FeS_2 (Schippers and Jørgensen, 2002). The contribution of these organisms to N_2 production and Fe(II) oxidation activity in ecosystems has not been evaluated. It has been suggested that their role in Fe(II) oxidation is ultimately limited by NO_3^- availability, which is typically low due to NO_3^- assimilation by plants and microorganisms. However, many of these organisms are facultative anaerobes and can use O_2 as an alternative electron acceptor for heterotropic growth. The capacity to switch between O_2 and NO_3^- as terminal electron acceptors may be a common trait among chemoautotrophic bacteria that link Fe(II) oxidation to N_2 production, just as it is among strictly heterotrophic denitrifying bacteria.

8.08.6.5.3 Aerobic Fe(II) oxidation

Iron oxidation occurs at the interface of aerobic and anaerobic environments according to the following generalized reactions:

$$Fe^{2+} + \tfrac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2}H_2O \quad (27)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3(s) + 3H^+ \quad (28)$$

The relative contributions of enzymatic versus nonenzymatic oxidation to overall Fe(II) oxidation rates is influenced by a variety of factors such as pH, concentrations of O_2 and Fe(II), and the rate of Fe(II) delivery (Neubauer *et al.*, 2002). At pH <4, the nonenzymatic oxidation of Fe(II) by O_2 is very slow, and microbial activity can increase oxidation rates by a factor $>10^6$ (Singer and Stumm, 1970). The practical implications of this observation for the abatement of acid mine drainage has motivated a great deal of research on chemolithotrophic, acidophilic prokaryotes such as *Acidothiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) and *Leptospirillum ferrooxidans* (Nordstrom and Southam, 1997). The influence of such organisms on the pH of mine drainage may vary, depending on the niche they occupy in an acid mine waste stream. *L. ferrooxidans* tolerates extremely low pH (<1.0) conditions typical of solutions in contact with pyrite ores, and it may be responsible for initiating a series of reactions that ultimately enhance pyrite dissolution and generates acidity (Schrenk *et al.*, 1998). Archea in the order *Thermoplasmales* are also abundant at such sites and presumably contribute to Fe(II) oxidation (Edwards *et al.*, 2000b). Contrary to its presumed role of enhancing pyrite dissolution, it has been shown that *A. ferriooxidans* actually occurs in somewhat less acidic environments where it would have little influence on pyrite dissolution (Schrenk *et al.*, 1998). In fact, *A. ferriooxidans* may serve a beneficial role by reducing aqueous loads of Fe(II) and other metals. As these studies illustrate, there is much insight to be gained from investigating the ecology of microorganisms *in situ*.

The only neutrophilic Fe(II)-oxidizer that had been cultured in the laboratory previous to the 1990s was *Gallionella ferruginea*, a microaerobe that forms a helical stalk (Emerson, 2000). *G. ferruginea* is a member of the beta-Proteobacteria, and is capable of chemolithoautotrophic or mixotrophic growth (Hallbeck and Pederson, 1991; Hallbeck *et al.*, 1993). A number of novel strains of chemolithoautotrophic Fe(II)-oxidizing bacteria have been isolated from groundwater (Emerson and Moyer, 1997) and hydrothermal vents (Emerson and Moyer, 2002). These strains are unicellular and do not form extracellular stalks. They are members of the gamma-*Proteobacteria* subclass in the family *Xanthomonaceae*. Another recent isolate of lithotrophic Fe(II)-oxidizing bacteria, strain TW-2, was recovered from a freshwater wetland and determined to be in the beta-*Proteobacteria* (Sobolev and Roden, 2003).

It is often assumed that Fe(II) oxidation is a nonenzymatic process at the circumneutral pH conditions that prevail in most ecosystems because autocatalytic oxidation is quite rapid. This may be the case when O_2 levels are high due to physical mixing, bioturbation, or rapid changes in water table depth. But when the position of the oxic–anoxic interface is stable, opposing diffusion gradients of Fe(II) and O_2 intersect to form microaerobic zones ($[O_2] < 0.5\%$), the ideal niche for organisms that require both reduced inorganic elements and O_2 for respiration. The half-life of nonenzymatic Fe^{2+} (i.e., dissolved Fe(II)) oxidation at low O_2 concentration is up to 300-fold longer than at high O_2 concentration (Roden *et al.*, in press). A long Fe^{2+} half-life should favor microbial oxidation. Indeed, chemolithoautotrophic bacteria competed successfully with nonenzymatic processes for O_2 and Fe(II) when grown in pure culture at circumneutral pH (Emerson and Revsbech, 1994).

Pure culture studies have alluded to some of the factors that may regulate enzymatic Fe(II) oxidation in complex natural environments such as sediments, soils, and the rhizosphere. Neubauer *et al.* (2002), used microcosms fed with environmentally relevant concentrations of O_2 and Fe(II) to investigate the metabolism of an Fe(II)-oxidizing strain isolated from the wetland rhizosphere. They found that both biotic and abiotic Fe(II) oxidation increased linearly ($r^2 \geq 0.90$) with the rate of Fe(II) addition (Figure 23). Since the experimental Fe(II) addition rate approximated *in situ* Fe(III)-diffusion rates in freshwater

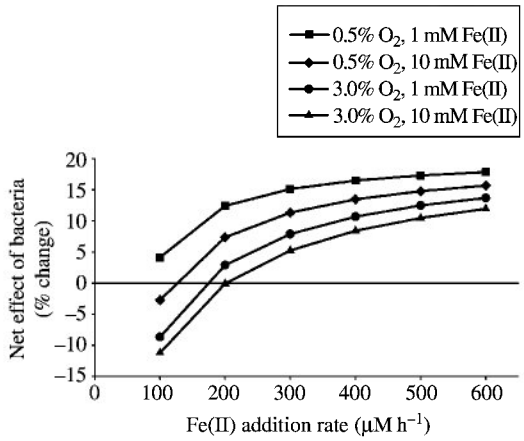

Figure 23 The influence of a chemolithoautotrophic bacterium on Fe(II) oxidation rates as a function of Fe(II) addition rate (*x*-axis) as determined in stirred microcosms. The effects varied depending on the O_2 concentration and the total Fe(II) oxide content in the microcosm. The bacterium was isolated from the rhizosphere of wetland plants (reproduced from Neubauer *et al.*, 2002).

wetland soils, these data suggested that microbial Fe(II) oxidation in wetlands is Fe(II)-limited. The bacteria fared best in competition with nonenzymatic oxidation when the total iron that had accumulated in the chambers was low, suggesting that competition is mediated in part by factors such as particle size distribution, texture, and mineralogy. Each of these factors influences the abundance and nature of surfaces that can adsorb Fe(II) and coordinate oxidation reactions. Finally, the presence of Fe(II)-oxidizing bacteria actually slowed the rate of overall Fe(II) oxidation in some circumstances. This presumably was caused by the inhibition of nonenzymatic Fe(II) oxidation via chelation and stabilization of aqueous Fe(II), perhaps by exopolymers or other organic extracellular molecules that are produced by Fe(II)-oxidizing bacteria (Emerson and Moyer, 1997). Similar compounds have been proposed to chelate Fe(III), allowing it to diffuse into anaerobic zones after microbial oxidation (Sobolev and Roden, 2001).

There is currently no satisfactory way to separate enzymatic from nonenzymatic Fe(II) oxidation *in situ*. However, pure culture studies have shown that bacteria mediated up to 90% of Fe(II) oxidation (Emerson and Revsbech, 1994; Neubauer *et al.*, 2002; Sobolev and Roden, 2001). In contrast to these studies, van Bodegom *et al.* (2001) reported that microbial Fe(II) oxidation was insignificant compared to nonenzymatic oxidation in a model rice system. However, their experiment included periods of vigorous sample agitation that may have artificially favored abiotic Fe(II) oxidation (Neubauer *et al.*, 2002). Indirect evidence that aerobic, chemolithotrophic Fe(II)-oxidizing bacteria contribute to Fe(III) deposition in circumneutral environments is the observation that they are ubiquitous and can account for 1% or more of the total microbial population (Weiss *et al.*, 2003).

8.08.6.6 Iron Cycling

Nealson (1983) articulated the first detailed description of iron cycling in which microbial metabolism was a central theme. Research since then has confirmed the more speculative elements of a microbial iron cycle, and contributed new insights on the processes that link Fe(III) reduction and Fe(II) oxidation in a "ferrous wheel," particularly in wetland soils. Microbial iron cycling promotes organic carbon oxidation via Fe(III) reduction, and it suppresses the metabolism of sulfate reducers and methanogens that do not compete effectively with Fe(III)-reducing bacteria for carbon substrates (Section 8.08.1). Many of the new developments in this area have come from the work of Roden and colleagues, who investigated a freshwater wetland where nonenzymatic Fe(III) reduction by sulfides was relatively unimportant (see synthesis by Roden *et al.*, in press). They proposed a model in which Fe(II)-oxidizing and Fe(III)-reducing bacteria are spatially organized into aggregates reminiscent of those that mediate anaerobic methane oxidation (Figure 10; Section 8.08.4.5). The aggregates have an Fe(III)-coated particle at the center, surrounded by a mass of Fe(III)-reducing bacteria, and then an outermost layer of Fe(II)-oxidizing bacteria (Sobolev and Roden, 2002). The Fe(II)-oxidizing bacteria enhance Fe(III) reduction by two mechanisms: (i) they generate a steep O_2 gradient that maintains anaerobic conditions inside the aggregate, and (ii) they produce compounds that chelate Fe(III) so that it can diffuse back into the anoxic center of the aggregate where it will be re-reduced to Fe(II). The Fe(II) produced by Fe(III)-reducing bacteria diffuses toward the periphery of the aggregate, completing the iron cycle. The end result would be the oxidation of organic carbon without the net consumption or depletion of Fe(III) oxides. Certain details of this model are supported by experimental evidence (Roden *et al.*, in press), but this model should be considered speculative. It is interesting to further speculate that these aggregates might also include bacteria that are capable of linking anaerobic Fe(II) oxidation to denitrification, with the NO_3^- provided by nitrifying bacteria at the periphery.

Iron cycling may also be favored by processes that operate at larger spatial and temporal scales than those proposed by Roden *et al.* (in press).

In wetland soils, iron cycling is promoted by several factors related to the presence of plants (Kostka and Luther, 1995; Roden and Wetzel, 1996; Frenzel *et al.*, 1999). Roots introduce O_2 into anaerobic soils (Schütz *et al.*, 1991; Hines, 1991; Holmer *et al.*, 2002), which supports biotic and abiotic Fe(II) oxidation (Neubauer *et al.*, 2002), and the deposition of poorly-crystalline Fe(III) oxides (Mendelssohn *et al.*, 1995). Due to repeated annual cycles of root growth and senescence, non-rhizosphere soils (i.e. mm or more from a root surface) in wetlands are enriched in poorly-crystalline Fe(III) oxides compared to sediments that lack plants altogether (Weiss, 2002). The combination of abundant poorly-crystalline Fe(III) minerals and labile organic carbon from roots favors rapid Fe(III) reduction during periods of anoxia. Because variations in plant development and photosynthetic activity can cause the rate of O_2 release by roots to vary hourly, daily and seasonally (Risgaard-Petersen and Jensen, 1997), parts of the root system may support net deposition of poorly-crystalline Fe(III) oxides at times when O_2 is released, then switch to net Fe(III)-reduction when O_2 is absent. Indeed, Fe(III)-reducing and Fe(II)-oxidizing bacteria occur on the same 1-cm lengths of plant roots (Weiss *et al.*, 2003). Burrowing animals influence sediments in much the same manner as roots (Kostka *et al.*, 2002c; Gribsholt *et al.*, 2003), and water table fluctuations can cause sediments to shift rapidly between reducing and oxidizing conditions. Although the processes discussed here have been demonstrated, their individual contributions to regulating Fe(III) cycling at an ecosystem level is largely unknown.

8.08.7 SULFUR

Sulfur (S) is an important element biochemically and geochemically. It is the fourteenth most abundant element in the Earth's crust and is ~1% of the dry mass of organisms where it serves many structural and enzymatic functions. Sulfur acts as a significant electron donor and acceptor in many bacterial metabolisms (Jørgensen, 1988). Microbial sulfur transformations are closely linked with the carbon cycle; sulfur reduction coupled to organic carbon oxidation is a major mineralization pathway in anoxic habitats, and autotrophic sulfur oxidation can occur aerobically and anaerobically. Sulfur compounds are often highly reactive, which results in a tight coupling of the oxidative and reductive portions of the biological sulfur cycle, particularly at the redoxcline where sulfur cycling can be extremely rapid (Figure 24). Although sulfur cycling occurs in terrestrial and freshwater aquatic environments, it is most prominent in marine ecosystems due to

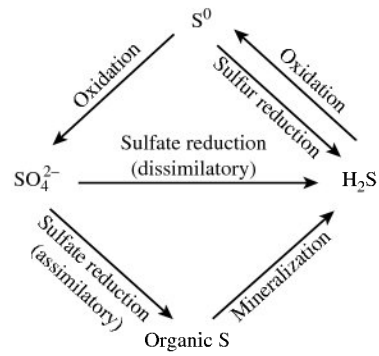

Figure 24 A simplified biological redox cycle for sulfur.

the abundance of sulfate (SO_4^{2-}) in seawater. Many specialized, small-scale environments, including hypersaline and geothermal (hot spring and hydrothermal vent) ecosystems also thrive on microbial sulfur transformations (e.g., Baas Becking, 1934; Ehrlich, 2002). In marine environments, a large fraction (up to 80%) of the organic carbon is respired through SO_4^{2-} reduction (Canfield *et al.*, 1993a, b), so copious amounts of sulfide are available for geochemical and microbial transformations.

8.08.7.1 Sulfur Geochemistry

The average crustal abundance of sulfur is 260 $\mu g\ g^{-1}$ and most on Earth is present as metal sulfide, gypsum, anhydrite, and dissolved sulfate. Sulfur can be found in a range of valence states from the highly reduced sulfide (−2) to the most oxidized form in SO_4^{2-} (+6). There are several intermediate valence forms of sulfur that can serve as both electron donors and acceptors for bacteria depending on environmental conditions, the most important being elemental sulfur and thiosulfate (Odom and Singleton, 1993). Sulfate is highly soluble and is the second most abundant anion in freshwater (after bicarbonate) and in seawater (after chloride) with the ocean acting as a major reservoir of SO_4^{2-}. Sulfate is also found in evaporite deposits, primarily as gypsum ($CaSO_4{\cdot}2H_2O$). Gypsum dissolution is a source of SO_4^{2-} for microorganisms (Machel, 2001). Sulfate-containing minerals are common oxidation products of sulfide minerals and can include anhydrous sulfates such as barite ($BaSO_4$) and hydroxylated sulfates such as alunite ($KAl_3(SO_4)_2(OH)_6$) and jarosite ($KFe_3(SO_4)_2(OH)_6$) (Deer *et al.*, 1992). Elemental sulfur (S^0) is formed hydrothermally and as an oxidation product of sulfide weathering. However, S^0 is also a common product of sulfide oxidation by bacteria and can serve as an electron acceptor for bacterial respiration (Jørgensen, 1982a).

Reduced S(II), or *sulfide*, is present in a variety of forms, most of which are solids. Dissolved sulfide exists as bisulfide ion (HS^-) at neutral pH, sulfide ion (S^{2-}) at alkaline pH, and hydrogen sulfide (H_2S) at low pH. H_2S is the only form of sulfide that is volatile and it imparts a characteristic "rotten egg" smell to sediments at low tide. Dissolve sulfides react strongly with base and transition metal ions to form insoluble sulfide minerals, the most prominent of which are iron sulfides that make anoxic sediments black. Freshly precipitated iron sulfides are amorphous, but tend to crystallize rather quickly into other acid-soluble authigenic minerals such as Mackinawite (tetragonal FeS) and Greigite (cubic Fe_3S_4). Many metals form insoluble sulfide minerals, which result in economically important deposits, and as many as 95 sulfide minerals appear in standard lists including the monosulfides galena (PbS), covellite (CuS), and cinnabar (HgS). Economically important metal sulfide deposits may be precipitated from hydrothermal solutions in vein-type or replacement deposits, but some of the largest zinc, copper, and lead deposits were formed from diagenetic reactions in sedimentary basins from biogenic sulfide. The acid-soluble forms of iron-containing sulfides convert to disulfide pyrite (cubic FeS_2) by the addition of elemental sulfur. Pyrite is not acid soluble and accumulates as the major end product of sulfur diagenesis in reducing sediments (Goldhaber and Kaplan, 1975), a process that occurs slowly over several years. Pyrite can also be formed rapidly when iron monosulfide levels are undersaturated, as is usually the case in salt marsh sediments (Howarth, 1979; Howarth and Merkel, 1984). Disulfides can also incorporate other elements to form minerals such as arsenopyrite (FeAsS) and molybdenite (MoS_2).

8.08.7.2 Microbial Reduction of Sulfate

8.08.7.2.1 Overview of sulfate reduction

Biological SO_4^{2-} reduction is an ancient process and evolved earlier than ~3.5 Gyr ago (Shen *et al.*, 2001). Sulfate-reducing bacteria are anaerobes that are situated at the terminus of the anaerobic food web where they act as an important cog in the sulfur and carbon cycles. Sulfate-reducing bacteria liberate CO_2 and S(II) as the primary end products of organic matter mineralization. They gain energy by coupling the oxidation of organic compounds or H_2 to SO_4^{2-} reduction (Figure 25). The process is also termed *dissimilatory* SO_4^{2-} *reduction* to differentiate it from *assimilatory* SO_4^{2-} *reduction*, which produces reduced sulfur for biosynthesis. Assimilatory SO_4^{2-} reduction is common among organisms and does not lead to the excretion of sulfide. The presence of H_2S is

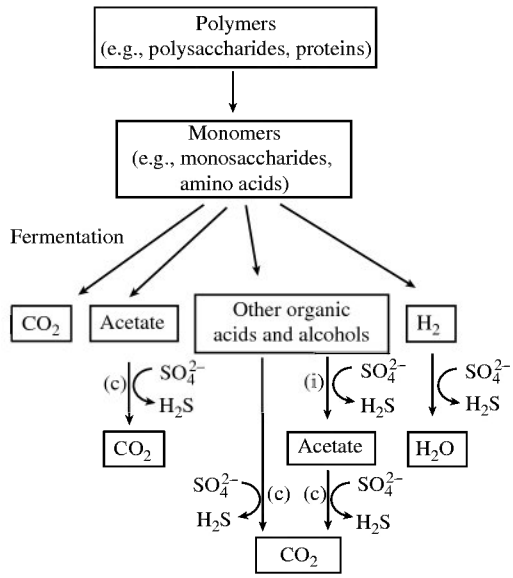

Figure 25 Anaerobic decomposition with sulfate reduction as the terminal step. Fermentation leads to several possible products including low molecular weight organic acids and alcohols and hydrogen and carbon dioxide. Incomplete oxidizers (i) produce acetate as an end product, whereas complete oxidizers (c) mineralize organic compounds, including acetate, to carbon dioxide.

noted by its characteristic foul smell, black ferrous sulfide precipitates, or white patches of S^0 as an oxidation product. Dissimilatory SO_4^{2-} reduction can account for half or more of the total organic carbon mineralization in many environments (Canfield *et al.*, 1993a; Jørgensen, 1982b). It has been estimated that $\sim 5 \times 10^{12}$ kg yr^{-1} of SO_4^{2-}-S is reduced by bacteria globally, with greater than 95% the activity occurring in the ocean (Skyring, 1987). Most of the SO_4^{2-} reduction in marine ecosystems occurs in coastal regions that receive high inputs of organic material. However, SO_4^{2-} reduction in freshwater environments can account for a significant portion of anaerobic mineralization processes, and in some instances can be the dominant pathway (Bak and Pfennig, 1991; Holmer and Storkholm, 2001; Lovley and Klug, 1983a; Urban *et al.*, 1994).

Besides its importance in the degradation of organic compounds, the reduced sulfur produced during SO_4^{2-} reduction is important geochemically since it is highly reactive and involved in the precipitation of highly insoluble metal sulfides and the accumulation of dissolved sulfide. The reduced products can be readily reoxidized under oxidizing conditions and can act as substrates for autotrophic bacteria including phototrophs, thus completing a dynamic sulfur cycle. The dissolved and solid-phase products of SO_4^{2-} reduction are responsible for consuming a significant portion of

the O_2 that diffuses into sediments, and this O_2 sink can be equal to or greater than the amount of O_2 consumed by aerobic bacterial respiration (Jørgensen, 1977). In typical coastal marine sediments, it is possible that 90% of the reduced sulfur produced during sulfate reduction each year is recycled back to sulfate. The remaining sulfide is buried as a metal sulfide and eventually transformed into FeS_2 (Jørgensen, 1982a).

The primary role of SO_4^{2-} reducers in the carbon cycle is the mineralization of relatively small organic substrates to CO_2. Although the group as a whole contains a diverse array of metabolic capabilities, SO_4^{2-} reducers do not generally degrade polymers. An exception is the thermophilic Archeal species *Archeoglobus* (Stetter, 1988; Stetter *et al.*, 1987), and few other species seem to be capable of degrading compounds more complicated than simple monomers or fermentation products. The energy gain from dissimilatory SO_4^{2-} reduction is relatively low. The free-energy yield ($\Delta G'$) of the complete oxidation of acetate or lactate to CO_2 is -48 kJ or -128 kJ, respectively, whereas acetate or lactate oxidation with O_2 yields -844 kJ or -1323 kJ, respectively. However, the sulfide produced by these reactions can act as an energy source for other bacteria, especially photoautotrophic and chemoautotrophic bacteria in aerobic surface sediments (Jørgensen, 1988).

8.08.7.2.2 Metabolic diversity

(i) *Complete and incomplete organic carbon oxidation*. Physiologically, SO_4^{2-} reduction is divided between complete and incomplete oxidation processes. Incomplete oxidizers utilize a variety of substrates, many of which are incompletely degraded to acetate. For example:

$$2\,\text{lactate} + SO_4^{2-} \rightarrow 2\,\text{acetate} + 2CO_2 + 2H_2O + S^{2-} \quad (29)$$

or

$$4\,\text{propionate} + 3SO_4^{2-} \rightarrow 4\,\text{acetate} + 4CO_2 + 2H_2O + 3S^{2-} \quad (30)$$

Some incomplete oxidizers are also capable of fermenting substrates without SO_4^{2-} respiration, for example:

$$3\,\text{lactate} \rightarrow 2\,\text{propionate} + \text{acetate} + CO_2 \quad (31)$$

However, some strains of SO_4^{2-} reducers are capable of fermentations that yield H_2 as an end product that must be removed quickly by a bacterial partner. It was noted early that strains produced acetate as an end product, so SO_4^{2-} reducers were regarded as fermenting bacteria for decades. It was not until the 1950s that the respiratory nature of SO_4^{2-} reduction was elucidated (Ishimoto *et al.*, 1954; Postgate, 1954). All SO_4^{2-} reducers isolated prior to about 1977 were incomplete oxidizers, including most members of the genus *Desulfovibrio*, the first of which was isolated over 100 yr ago (Voordouw, 1995). Although no acetate-consuming isolates existed prior to the late 1970s, it was postulated for many years that SO_4^{2-}-reducing bacteria were capable of utilizing acetate as an electron donor (Widdel, 1988).

The first acetate utilizer isolated was a gram-positive, sporulating bacterium of the genus *Desulfotomaculum* (Widdel and Pfennig, 1977). Since then, several species of acetate-utilizing gram-negative bacteria have been recovered representing a suite of metabolic capabilities and representing new genera. Complete oxidation entails the production of CO_2 as a major end product and includes the use of acetate, for example:

$$\text{acetate} + SO_4^{2-} \rightarrow 2CO_2 + H_2O + S^{2-} \quad (32)$$

Although physiologically distinct in many ways, the incomplete and complete oxidizing SO_4^{2-}-reducing bacteria can coexist, with the former supplying acetate to the latter in some instances.

Sulfate-reducing bacteria can engage in interspecies H_2 transfer, in which H_2 equivalents are transferred to other bacteria in lieu of SO_4^{2-} respiration. For example:

Desulfovibrio

$$2\,\text{lactate} + 4H_2O \rightarrow 2\,\text{acetate} + 2CO_2 + 4H_2 \quad (33)$$

Hydrogenotrophic methanogen

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (34)$$

In essence, SO_4^{2-} reducers in this case are fermenting bacteria that depend on H_2-consuming methanogens to maintain a low H_2 partial pressure, thereby making fermentation thermodynamically feasible. In addition, methanogenic bacteria can consume the acetate produced by the SO_4^{2-} reducers.

(ii) *Electron donors*. Energy sources used by SO_4^{2-}-reducing bacteria are similar to those utilized by metal-reducing and methanogenic bacteria. It is generally accepted that SO_4^{2-}-reducing bacteria oxidize fermentation products-such as fatty acids, alcohols, and H_2 (Christensen, 1984; Parkes *et al.*, 1989; Sørensen *et al.*, 1981), but this list is incomplete and is continually growing as new metabolisms are discovered. Sulfate reducers can also metabolize chemolithotrophically (autotrophically) when utilizing H_2 as an electron donor. Since most of the substrates used by SO_4^{2-} reducers are provided by a variety

of other bacteria, these compounds represent rapidly recycled intermediates that accumulate quickly when SO_4^{2-} reduction is inhibited by the use of SO_4^{2-} analogues such as the group IV oxyanions molybdate and selenate (Oremland and Capone, 1988; Smith and Klug, 1981; Taylor and Oremland, 1979).

Besides the common fermentation products listed above, SO_4^{2-}-reducing bacteria have been shown to consume a variety of other substrates including xenobiotics and other aromatic compounds (Bolliger *et al.*, 2001; Elshahed and McInerney, 2001b; Kniemeyer *et al.*, 2003; Kuever *et al.*, 2001; Lovley *et al.*, 1995), carbohydrates such as fructose and sucrose (Sass *et al.*, 2002), amino acids (Burdige, 1989; Coleman, 1960), alkanes and alkenes up to C_{20} (Aeckersberg *et al.*, 1991, 1998), phosphite (Schink *et al.*, 2002), aldehydes (Tasaki *et al.*, 1992), dicarboxylic acids (Postgate, 1984), glycolate (Friedrich and Schink, 1995), methylated nitrogen and sulfur compounds (Finster *et al.*, 1997; Heijthuijsen and Hansen, 1989; Kiene, 1988; van der Maarel *et al.*, 1996a), acetone (Platen *et al.*, 1990), and sulfonates such as taurine and cysteate (Visscher *et al.*, 1999).

Sulfate-reducing bacteria are able to use a variety of organic compounds as both electron acceptors and electron donors. For example, *Desulfovibrio* species can ferment fumarate or malate, but will reduce these species to succinate if an additional electron donor is available (Miller and Wakerley, 1966).

(iii) *Electron acceptors.* Although the reduction of SO_4^{2-} is considered to be the classic role of SO_4^{2-}-reducing bacteria, these organisms are capable of utilizing a wide variety of inorganic sulfur compounds as electron acceptors including sulfite, bisulfite, metabisulfite, dithionite, tetrathionate, thiosulfate, dimethylsulfoxide, sulfur dioxide, and elemental sulfur (S^0) (Fitz and Cypionka, 1990). Although some species can grow with S^0 as an electron acceptor, the growth of many species is inhibited in the presence of S^0 (Bak and Widdel, 1986; Widdel and Pfennig, 1982), presumably due to increases in redox potential (Rabus *et al.*, 2000).

Nitrate and NO_2^- reduction are rather widespread in SO_4^{2-} reducers (Keith and Herbert, 1983; McCready *et al.*, 1983; Mitchell *et al.*, 1986; Moura *et al.*, 1997; Seitz and Cypionka, 1986), and in some cases NO_3^- is preferred over SO_4^{2-} (Seitz and Cypionka, 1986). The bisulfite reductase enzyme in SO_4^{2-} reducers displays some activity toward NO_2^-, which partially explains the ubiquity of NO_2^- reduction. However, it appears that specific NO_2^- reductases are also present (Liu and Peck Jr., 1981). Unlike denitrifying bacteria that reduce NO_3^- to N_2, the end product of NO_3^- reduction in SO_4^{2-} reducers is NH_4^+ (Widdel and Pfennig, 1982). Thus, SO_4^{2-} reducers contribute to DNRA (Section 8.08.5.4).

Iron reduction has been observed in SO_4^{2-}-reducing bacteria (Bale *et al.*, 1997; Knoblauch *et al.*, 1999b; Lovley *et al.*, 1993b), but this ability is more ubiquitous in the non-SO_4^{2-}-reducing members of the δ Proteobacteria (Kostka *et al.*, 2002a; Nielsen *et al.*, 2002), and cell growth has not been observed. Sulfate reducers are capable of reducing a variety of other metals such as uranium, chromium, technetium, and gold, but like iron, no growth occurs (Lovley and Phillips, 1992b, 1994b; Lovley *et al.*, 1993b). The reduction of arsenate to arsenite supports growth of SO_4^{2-}-reducing bacteria and, in some cases, arsenate is the preferred electron acceptor (Macy *et al.*, 2000; Newman *et al.*, 1997a,b; Stolz and Oremland, 1999). Sulfate reducers can reduce selenate (Stolz and Oremland, 1999) and Mn(IV) (Tebo and Obraztsova, 1998).

Other entries on the list nonsulfur inorganic electron acceptors used by SO_4^{2-} reducers are carbonate, which is reduced to acetate (Klemps *et al.*, 1985), and O_2 (Dilling and Cypionka, 1990). Oxygen reduction is a relatively common feature of SO_4^{2-}-reducing bacteria (Cypionka, 2000; Dannenberg *et al.*, 1992). However, aerobic growth in pure cultures is poor or absent and it appears that O_2 reduction, despite being enzymatic, is primarily an O_2 removal mechanism (Cypionka, 2000). Many of the SO_4^{2-}-reducing bacteria isolated from oxic environments belong to the genus *Desulfovibrio*, although other genera dominate in some instances (Krekeler *et al.*, 1997; Sass *et al.*, 1997; Wieringa *et al.*, 2000). Organic compounds that can serve as electron acceptors for SO_4^{2-} reduction include malate, aspartate, cysteine, sulfonates, pyruvate, acrylate, and oxidized glutathione (Rabus *et al.*, 2000).

Sulfate-reducing bacteria couple the reductive dehalogenation of aromatic compounds to growth (DeWeerd *et al.*, 1990; Dolfing and Tiedje, 1987), and both chlorinated benzoates and bromophenols are used as electron acceptors (Boyle *et al.*, 1999; Mohn and Tiedje, 1990). Separate populations of SO_4^{2-} reducers can be either sources (indirectly) or sinks for acrylate. Acrylate is formed during the breakdown of the marine osmolyte dimethylsulfoniopropionate, a process that is catalyzed by SO_4^{2-} reducers in sediments, and then reduced by other SO_4^{2-}-reducing species (van der Maarel *et al.*, 1998, 1996d).

8.08.7.3 Taxonomic Considerations

Sulfate-reducing bacteria are a complex physiologic group and classifying them has traditionally required the consideration of several properties, the most important of which are motility, cell

shape, the guanine plus cytosine content of DNA, the presence of desulfoviridin and cytochromes, growth temperature, use of various electron donors, and the ability to conduct complete or incomplete oxidation. New analysis of ribosomal RNA (rRNA) sequences has allowed for a more thorough organization of the SO_4^{2-}-reducing bacteria into four major groups: gram-negative mesophilic, gram-positive spore forming, thermophilic bacterial, and thermophilic Archeal (Castro *et al.*, 2000). The gram-negative mesophilic group of SO_4^{2-} reducers is placed within the delta (δ) subdivision of the *Proteobacteria* and includes two major families, the *Desulfovibrionaceae* and *Desulfobacteriaceae* although many genera fall outside of these families. The *Desulfovibrionaceae* includes the genera *Desulfovibrio* and *Desulfomicrobium*, and this family appears to be rather phylogenetically distinct (Devereux *et al.*, 1990). The *Desulfobacteriaceae* family is much less distinct and includes several genera (perhaps >20), including many of the complete oxidizing species (Castro *et al.*, 2000; Widdel and Bak, 1992). The gram-positive spore-forming SO_4^{2-} reducers constitute primarily members of the genus *Desulfotomaculum*.

Prior to the late 1970s, only two genera of SO_4^{2-}-reducing bacteria were known, *Desulfovibrio* and *Desulfotomaculum* (Widdel, 1988; Widdel and Bak, 1992). The desulfovibrios have received the most attention because they are relatively easily isolated from the environment and are not difficult to maintain in laboratory culture. They were originally described as gram-negative bacteria that are curved rods, do not produce spores, and utilize primarily H_2 and lactate as electron donors (Postgate and Campbell, 1966). However, the group has been expanded to include members that are capable of using several other electron donors and electron acceptors, the latter including NO_3^-, O_2, and metal oxides (Barton *et al.*, 1983; Cypionka, 2000; Lovley *et al.*, 1993b; Moura *et al.*, 1997). They are the classic examples of SO_4^{2-} reducers that conduct incomplete metabolism with acetate as an important end product. Phylogenetic analyses have shown the *desulfovibrios* to be sufficiently diverse and distinct to warrant placement within their own family (Devereux *et al.*, 1990). *Desulfovibrio* species are routinely isolated from marine sediments and probably are an important component of SO_4^{2-} reduction in the sea. The *desulfovibrios* are the only group observed to enter into syntrophic relationships with methanogenic bacteria (Fiebig and Gottschalk, 1983; Pankhania *et al.*, 1988).

Desulfotomaculum species are gram-positive, rod shaped, spore-forming SO_4^{2-}-reducing bacteria that as a group display wide phylogenetic diversity (Rabus *et al.*, 2000). They are also quite phylogenetically distinct from all other SO_4^{2-}-reducing bacteria. The genus exhibits a great nutritional versatility comparable to that of non-spore-forming sulfate reducers, including the use of H_2, alcohols, fatty acids, other aliphatic monocarboxylic or dicarboxylic acids, alanine, hexoses, or phenyl-substituted organic acids as electron donors for dissimilatory SO_4^{2-} reduction (Widdel and Pfennig, 1999). *Desulfotomaculum* species are not considered to be particularly important in marine sediments compared to the non-spore-forming SO_4^{2-} reducers. However, they are easily isolated from organic-rich sediments and they possess metabolic capabilities that are known to be important in sediments. Their ability to form spores gives them a competitive advantage in some habitats and they are found to dominate environments like rice paddy sediments that undergo wetting–drying cycles that could harm nonsporing cells (Rabus *et al.*, 2000). *Dtm. acetoxidans* was the first SO_4^{2-} reducer isolated that was capable of acetate oxidation (Widdel and Pfennig, 1977).

Since the early 1980s, many new lineages of SO_4^{2-} reducers have been isolated and described. The phylogeny of SO_4^{2-} reducers has expanded to include the family *Desulfobacteriaceae* (Widdel and Bak, 1992), which includes many new genera that are capable of complete and/or incomplete oxidation; the genera *Desulfobacter*, *Desulfobacterium*, and *Desulfococcus* to name a few. Members of the filamentous, gliding SO_4^{2-}-reducing genus *Desulfonema* also appear to be common inhabitants of organic-rich sediments, especially within sharp redox gradients (Fukui *et al.*, 1999). Sulfate reducers as a whole are phylogenetically distinct from other bacteria, which has led to the discovery of signature DNA sequences of ribosomal subunit genes that have been used as oligonucleotide probes and polymerase chain reaction primers for the detection, determination of relative abundance, and microscopic visualization of members of the group (Amann *et al.*, 1990a,b; Daly *et al.*, 2000; Devereux *et al.*, 1992; Devereux and Stahl, 1993). Probes specific for SO_4^{2-}-reducing bacteria have been applied to marine water columns (Ramsing *et al.*, 1996; Teske *et al.*, 1996), biofilms and bacterial mats (Fukui *et al.*, 1999; Minz *et al.*, 1999a; Ramsing *et al.*, 1993; Santegoeds *et al.*, 1999), and sediments (Hines *et al.*, 1999; LlobetBrossa *et al.*, 1998; Rooney-Varga *et al.*, 1997; Sahm *et al.*, 1999b; Sass *et al.*, 1998). In addition, reverse sample genome probing (Voordouw *et al.*, 1991), hydrogenase (Wawer *et al.*, 1997) and dissimilatory sulfite-reductase genes (Minz *et al.*, 1999b; Wagner *et al.*, 1998), and denaturing gradient gel electrophoresis (Okabe *et al.*, 2002) have been used to study SO_4^{2-} reducers in nature.

8.08.7.4 Sulfate-reducing Populations

The population composition of SO_4^{2-}-reducing bacteria has been investigated using culturing, biochemical, and genetic methods. In general, estimates of abundance using viable counting methods such as colony counts on solid media or growth in liquid media after serial dilutions (most probable number (MPN) methods), are 10^2–10^5 ml^{-1}, which appear low when considered in terms of the rate of *in situ* SO_4^{2-} reduction and culture estimates of rates per cell. This discrepancy is undoubtedly due to the inability of viable counting techniques to recover the majority of bacteria present. However, MPN techniques yield more realistic estimates of SO_4^{2-} reducer abundance (10^6–10^8 ml^{-1}) when applied to organic-rich habitats such as marine microbial mats (Visscher *et al.*, 1992; Ramsing *et al.*, 1993) and salt marsh sediments (Hines *et al.*, 1999).

Due to the complex nature of the anaerobic bacterial food web, it is generally held that SO_4^{2-} reducers account for only ~5% of the total bacteria present despite their important role at the end of the food web (Devereux *et al.*, 1996; Li *et al.*, 1999; LlobetBrossa *et al.*, 1998). *In situ* hybridization techniques that use fluorescent oligonucleotide probes to visualize individual cells of specific bacteria groups have yielded SO_4^{2-}-reducing bacteria counts in marine sediments as high as 3×10^7 ml^{-1}, which represented up to 6% of the total *Bacteria* (LlobetBrossa *et al.*, 1998). Hybridizations of bulk sedimentary RNA with probes also demonstrated that ~1–6% of the total bacteria present in sediments were SO_4^{2-}-reducing (Devereux *et al.*, 1996), although this technique demonstrated that 20% of the prokaryotes in a subtidal sediment were likely SO_4^{2-}-reducing (Sahm *et al.*, 1999b). Sulfate reducers in salt marsh sediments can account for >30% of the total bacteria (Hines *et al.*, 1999). This high percentage is likely due to the fact that marsh grasses exude organic substrates from roots that can be used directly by SO_4^{2-} reducers, thus circumventing the need for fermenting bacteria.

A wide variety of SO_4^{2-}-reducing bacteria are found within sediments. Enumerations of bacteria using MPN methods supplemented with specific substrates for SO_4^{2-}-reducing groups have shown the presence of bacteria able to use many substrates including lactate, ethanol, acetate, malate, and propionate (Laanbroek and Pfennig, 1981). Lactate and acetate utilizers often outnumber other groups illustrating the importance of both incomplete and complete oxidizing SO_4^{2-} reducers in sediments. Sulfate-reducing marine sediments display an abundance of even chain bacterial phospholipid fatty acids indicative of the presence of acetate-utilizing bacteria of the genus *Desulfobacter* (Parkes *et al.*, 1993). Using MPN methods, acetate-utilizing sulfate reducers were most abundant in a marine microbial mat (Visscher *et al.*, 1992; Teske *et al.*, 1998), while ethanol utilizers greatly outnumbered acetate utilizers in a salt marsh sediment (Hines *et al.*, 1999).

Molecular analyses have furthered the description of SO_4^{2-}-reducing groups in depositional environments. Both whole-cell *in situ* hybridizations and hybridizations using RNA extracted from sediments have been employed to investigate the diversity of SO_4^{2-} reducers (Ramsing *et al.*, 1993; Rooney-Varga *et al.*, 1997). Incomplete oxidizing groups of sulfate reducers seem to dominate in marine sediments, primarily members of the *Desulfovibrionaceae*, but also *Desulfobulbus* species are abundant. However, the distribution of groups varies with depth and among habitats. For example, members of the *Desulfobacteriaceae* and *Desulfovibrionaceae* were equally abundant in the upper 2.0 cm of sediments, but the incomplete oxidizers (*Desulfovibrionaceae* species) dominated at greater depths (Devereux *et al.*, 1996). Complete oxidizers were essentially absent from Arctic Ocean sediments (Sahm *et al.*, 1999a), yet they dominated sediments inhabited by salt marsh grasses (Hines *et al.*, 1999). Complete oxidizers, i.e., *Desulfobacteriaceae* species accounted for over 20% of the total recovered RNA in some instances in the marsh, while *Desulfovibrionaceae* species were a small fraction in all cases (Hines *et al.*, 1999; Rooney-Varga *et al.*, 1997). Members of the *Desulfobacteriaceae* are metabolically diverse and may be well suited for environments that undergo widely changing seasonal redox changes like those encountered in salt marsh sediments and at the sediment surface. The presence of high numbers of *Desulfobulbus* species in marsh sediments (Hines *et al.*, 1999) may reflect the ability of members of the genus to conduct sulfur disproportionation reactions, which would be stimulated by plant-mediated redox cycling within the rhizosphere and the production of intermediate redox states of sulfur.

8.08.7.5 Factors Regulating Sulfate Reduction Activity

8.08.7.5.1 Sulfate-reducing activity

Knowledge of the role of SO_4^{2-} reduction in sediments has increased greatly since the introduction of the use of ^{35}S as a tracer for determining sulfate reduction rates (Jørgensen and Fenchel, 1974; Sorokin, 1962). At first, rates were estimated from the incorporation of ^{35}S into dissolved sulfide (H_2S) and acid volatile sulfides (iron monosulfides, FeS), which were thought to be the only significant products (Jørgensen, 1978a). However, it was determined that in some habitats, such as salt

marsh sediments and near the oxic/anoxic boundary, a significant fraction of the reduced sulfur produced during SO_4^{2-} reduction is rapidly converted to pyrite (FeS_2), a compound that was previously thought to be produced at rates on the order of several years (Howarth, 1979). Methods now routinely include a reduction step using reduced chromium under acidic conditions to incorporate all major reduced inorganic sulfur species that may form during SO_4^{2-} reduction (Westrich, 1983; Fossing and Jørgensen, 1989; Meier *et al.*, 2000). In highly organic soils and sediments, it is usually necessary to recover the carbon-bonded ^{35}S label (Wieder and Lang, 1988). Sulfate reduction rates can also be determined from losses of SO_4^{2-} in incubated sediments and from mathematical models that describe the loss of SO_4^{2-} with sediment depth in terms of sedimentation and diffusion (Jørgensen, 1978a,b,c). Depth increases in reduced sulfur compounds and the abundance of SO_4^{2-}-reducing bacteria are useful as comparative indicators of the SO_4^{2-} reduction process, but they are poor indicators of actual rates of activity.

The rate of organic matter input (i.e., sedimentation) and the availability of SO_4^{2-} control the rate of SO_4^{2-} reduction in sediments. Sulfate is rarely limiting in marine systems except in brackish estuarine waters and with depth in sediments where SO_4^{2-} has been depleted. Sulfate reduction rates in sediments can span several orders of magnitude, but typical near-shore rates in the upper 5–10 cm of marine sediments are often 50–500 nmol cm^{-3} d^{-1} (Skyring, 1987). Rates in sediment with unusually rapid sedimentation rates can be reach 2,000 nmol ml^{-1} d^{-1} (Crill and Martens, 1987). Sulfate reduction in salt marshes and microbial mats can reach rates as high as 4,000 nmol ml^{-1} d^{-1} and 14,000 nmol ml^{-1} d^{-1}, respectively (Canfield and Des Marais, 1991; Hines *et al.*, 1999). Anaerobic CH_4 oxidation supports a large fraction of the SO_4^{2-} reduction in some marine sediments (Table 6).

8.08.7.5.2 Temperature

Sulfate reduction occurs over a wide range (0–110 °C) of temperatures (Castro *et al.*, 2000; Elsgaard *et al.*, 1994; Jørgensen *et al.*, 1992; Knoblauch and Jorgensen, 1999; Knoblauch *et al.*, 1999a; Kostka *et al.*, 1999b; Sievert and Kuever, 2000; Stetter *et al.*, 1993). Above ~115 °C, SO_4^{2-} reduction is believed to occur only by thermochemical reactions (Machel, 2001). Like other biological processes, biological SO_4^{2-} reduction is affected strongly by temperature. Seasonal changes in SO_4^{2-} reduction activity often follow temperature well, except for lags in activity due to the time required to remove oxygen and other competing electron acceptors (Crill and Martens, 1987; Hines *et al.*, 1982; Jørgensen, 1977). In general, rates of activity can vary by factors of <5 to >30 between winter and summer, and these changes have profound influence on redox conditions and the accumulation of reduced species (Jørgensen, 1977).

8.08.7.5.3 Carbon

Sulfate reduction is controlled strongly by the quantity and quality of organic matter present. In marine sediments, there is a strong relationship between organic sedimentation rate and SO_4^{2-} reduction rate (Goldhaber and Kaplan, 1975) and it is generally held that SO_4^{2-} reduction is limited by organic matter availability except when SO_4^{2-} concentration is quite low (Boudreau and Westrich, 1984; Dornblaser *et al.*, 1994; Westrich and Berner, 1984). Although SO_4^{2-} reducers usually consume a relatively narrow suite of organic compounds, the tight coupling of SO_4^{2-} reduction with degradative processes prior to SO_4^{2-} reduction results in a stoichiometric relationship between the degradation of complex organic matter and the reduction of SO_4^{2-} (Richards, 1965). In general, there is a 2 : 1 molar relationship between labile carbon deposited into the SO_4^{2-} reduction zone and SO_4^{2-} reduced (Thamdrup and Canfield, 1996). The quantity of nitrogen and phosphorus mineralized during SO_4^{2-} reduction can be predicted using C/N/P ratios of organic matter and SO_4^{2-} reduction stoichiometry (Hines and Lyons, 1982; Martens *et al.*, 1978). Sulfate reduction activity responds rapidly to increased inputs of organic material with the seasonal deposition of spring phytoplankton blooms resulting in significant increases in activity in lake (Hadas and Pinkas, 1995) and ocean sediments (Boetius *et al.*, 2000a). Although it is clear that low-molecular-weight fatty acids are important substrates for SO_4^{2-}-reducing bacteria, different groups of SO_4^{2-} reducers may consume different acids (Boschker *et al.*, 2001).

Some electron donors are more readily metabolized by methanogens than SO_4^{2-} reducers and these "noncompetitive" substrates can allow methanogenesis to occur in the presence of active SO_4^{2-} reduction (Oremland *et al.*, 1982). Examples of these types of substrates include C_1 compounds like methylated amines, methylated sulfur compounds (e.g., dimethylsulfide and methane thiol), and methanol. Methylated nitrogen and sulfur compounds are common degradation products of osmoregulating compounds found in certain marine algae and salt marsh grasses, and their use by methanogens partially explains the occurrence of significant concentrations of CH_4 in SO_4^{2-}-containing estuarine and

salt marsh sediments (Dacey *et al.*, 1987; Kiene, 1996b). In fact, the degradation of the osmoregulant glycine betaine in marine sediments produces acetate and trimethylamine, the former of which is consumed by sulfate-reducing bacteria, while the latter is consumed by methanogens (King, 1984). Methanol is a degradation product of plant structural components such as pectin.

8.08.7.5.4 Sulfate and molecular oxygen concentrations

The SO_4^{2-} concentration in sediments affects SO_4^{2-} reduction only when concentrations are quite low. The reduction of SO_4^{2-} in marine sediments appears to be zero-order with respect to SO_4^{2-} to concentrations of ~2 mM (Boudreau and Westrich, 1984; Goldhaber and Kaplan, 1974). In freshwaters, SO_4^{2-} concentrations must be much lower before they limit SO_4^{2-} reduction (Bak and Pfennig, 1991; Lovley and Klug, 1983b; Sinke *et al.*, 1992). Because freshwater contains very little SO_4^{2-} compared to seawater, the importance of SO_4^{2-} reduction in sediments increases in an estuary as the salinity increases (Capone and Kiene, 1988). Therefore, the vertical extent of the SO_4^{2-} reduction zone increases substantially as more SO_4^{2-} becomes available, while the methanogenic zone is "pushed" deeper into the sediment and its contribution to carbon mineralization decreases in importance.

Sulfate-reducing bacteria are generally anaerobic, but recent studies have shown that some varieties are capable of O_2 use, are able to withstand several hours of full aeration, and are common inhabitants of oxic regions of microbial mats (Cypionka, 2000; Krekeler *et al.*, 1997). Sulfate reducers withstand O_2 stress better in the presence of a co-metabolizing bacterium that presumably consumes O_2 (Gottschalk and Szwezyk, 1985). However, studies of co-cultures of a SO_4^{2-} reducer and a facultative anaerobe suggested the occurrence of O_2-dependent growth by the SO_4^{2-} reducer in the absence of SO_4^{2-} (Sigalevich *et al.*, 2000a,b; Sigalevich and Cohen, 2000).

Sulfate-reducing populations within active sediments, like microbial mats, can exhibit a bimodal distribution with two distinct maxima of differing populations, one within surficial oxic layers and another in deeper anoxic sediments (Minz *et al.*, 1999a; Ramsing *et al.*, 1993; Risatti *et al.*, 1994; Sass *et al.*, 1997). Sulfate reducers within or near oxic regions can benefit from labile organic compounds released by photosynthetic bacteria (Canfield and Des Marais, 1991; Teske *et al.*, 1998; Visscher *et al.*, 1992) or vascular plants (Scheid and Stubner, 2001; Blaabjerg and Finster, 1998; Hines *et al.*, 1999). Indeed, rates of SO_4^{2-} reduction measured in oxic regions of microbial mats can equal or exceed those noted in deeper anoxic lamina (Canfield and Des Marais, 1991; Frund and Cohen, 1992; Visscher *et al.*, 1992). The complete oxidizing and gliding SO_4^{2-} reducer, *Desulfonema*, is a common inhabitant of the oxic–anoxic interface in microbial mats (Fukui *et al.*, 1999; Risatti *et al.*, 1994; Teske *et al.*, 1998), and is also abundant within the partially oxygenated rhizosphere of salt marsh plants (Rooney-Varga *et al.*, 1997). Filamentous morphology, aggregate formation, and diurnal migration are all adaptations that allow this species to thrive in rapidly changing conditions.

8.08.7.6 Microbial Reduction of Sulfur

The microbial reduction of elemental sulfur (S^0) was not discovered as early as the reduction of SO_4^{2-}. The first report of the sole use of S^0 as a terminal electron acceptor for growth was reported well into the twentieth century (Pelsh, 1936), and the first pure culture (*Desulfuromonas acetoxidans*) was isolated four decades later (Pfennig and Biebl, 1976). *D. acetoxidans* was discovered as a partner living syntrophically with an anaerobic phototroph that oxidized sulfide to S^0 and provided organic electrons donors. Hence, a complete anaerobic sulfur cycle was maintained. Several other S^0 reducers were isolated soon thereafter, which couple the reduction of S^0 with the oxidation of compounds like acetate, formate, lactate, fumarate, aspartate, dimethylsulfoxide, and H_2, (Widdel, 1988). Some SO_4^{2-}-reducing bacteria possess the ability to reduce S^0 (Biebl and Pfennig, 1977; He *et al.*, 1986), but this is relatively rare. Certain Fe(III)-reducing bacteria also possess the ability to reduce S^0, and species have been isolated that are able to use a variety of other electrons acceptors such as NO_3^-, Mn(IV), thiosulfate, and O_2 (Caccavo *et al.*, 1994; Myers and Nealson, 1988; Roden and Lovley, 1993). Dissimilatory S^0 reduction also occurs in bacteria that are known to grow aerobically at normal O_2 tension (Balashova, 1985; Lovley *et al.*, 1989; Myers and Nealson, 1988). The ability to use organic disulfide molecules, such as cysteine or glutathione, as terminal electron acceptors appears to be restricted to certain members of the S^0-reducing bacteria (Pfennig and Biebl, 1976). Among the S^0-reducing bacteria, several species are able to generate ATP during the reduction of S^0 (Hedderich *et al.*, 1999). However, many members of the Archea are strictly fermentative S^0 reducers that use S^0 reduction as an electron sink in lieu of respiratory S^0 reduction (Stetter, 1996). Since S^0 is highly insoluble, it is likely that many S^0 reducers utilize polysulfide as an electron

acceptor, or at least as an intermediate in S^0 reduction (Hedderich *et al.*, 1999). In general, the habitat of sulfur reducers is similar to that of SO_4^{2-} reducers, so ecologically they coexist. However, in most instances SO_4^{2-} reducers produce more sulfide.

Several thermophilic microorganisms are capable of sulfur reduction (Bonch-Osmolovskaya *et al.*, 1990; Huber *et al.*, 1992; L'Haridon *et al.*, 1998) and this capability was employed in the early isolation of thermophilic Archea (Stetter, 1996), some of which are capable of aerobic growth using sulfur as an electron donor and anaerobic growth using sulfur as an electron acceptor (Segerer *et al.*, 1985). In fact, most of the sulfur-reducing bacteria known belong to the Archeal domain (Hedderich *et al.*, 1999). In the presence of sulfur, methanogenic Archea, especially thermophilic strains, reduce sulfur to H_2S, while CH_4 formation is highly curtailed (Stetter and Gaag, 1983). Sulfur reduction is common in microorganisms situated within deep branches of the phylogenetic tree suggesting that sulfur reduction is a very ancient process (Stetter, 1997; Woese, 1987).

A *Desulfuromonas* sp. was the first eubacterium to be classified using rRNA techniques and it was found to affiliate with the δ-subclass of Proteobacteria and closely with the completely oxidizing SO_4^{2-}-reducing bacteria (Fowler *et al.*, 1986; Liesack and Finster, 1994). However, sulfur reducing eubacteria are quite diverse and are found within other subclasses of the Proteobacteria, many of which are not related phylogenetically to SO_4^{2-} reducers (Lau *et al.*, 1987; Schleifer and Ludwig, 1989). In general, the sulfur reducers as a group still require proper classification (Widdel and Pfennig, 1999).

8.08.7.7 Disproportionation

It has been demonstrated that SO_4^{2-}-reducing bacteria have the ability to conduct inorganic fermentations of sulfur compounds, or *sulfur disproportionation* (Bak and Cypionka, 1987). The ability to disproportionate thiosulfate, sulfite and elemental sulfur (S^0) has been found in many SO_4^{2-}-reducing genera (Kramer and Cypionka, 1989), and it occurs as follows:

Thiosulfate

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (35)$$

Elemental sulfur

$$4S^0 + 4H_2O \rightarrow SO_4^{2-} + 3HS^- + 5H^+ \quad (36)$$

Sulfite

$$4SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^- \quad (37)$$

Pure cultures have been isolated that are capable of all these reactions including an isolate that is an obligate disproportionator and does not reduce SO_4^{2-} (Finster *et al.*, 1998).

Disproportionation reactions are important in sediments because they provide a mechanism for anaerobic sulfur cycling (Jørgensen, 1990a), and SO_4^{2-} reducers capable of this type of metabolism are numerically abundant (Bak and Pfennig, 1987; Jørgensen and Bak, 1991). Thiosulfate appears to be a key component of the sulfur cycle in sediments because it can be oxidized, reduced, and disproportionated. Sulfate-reducing bacteria are key components of this cycling since they are capable of reducing SO_4^{2-} and thiosulfate, and they are able to disproportionate thiosulfate. A "thiosulfate shunt" seems to exist in sediments where thiosulfate couples both reductive and oxidative pathways of the sulfur cycle (Jørgensen, 1990b) (Figure 26). Thiosulfate can be the main product of sulfide oxidation in reducing sediments, and much of this can be disproportionated with the remainder either oxidized to SO_4^{2-} or reduced to sulfide. Hence, most of the sulfide produced during SO_4^{2-} reduction may be ultimately oxidized to SO_4^{2-} after passing through a thiosulfate intermediate.

Although S^0 disproportionation is important in the sulfur cycle, it is not an energetically favorable reaction under standard conditions (Bak and Cypionka, 1987) and some have argued that it is not a major mode of metabolism in terms of bacterial energetics or growth (Jørgensen and Bak, 1991). However, bacteria are able to grow via this process when metal oxides are present to rapidly remove sulfide (Lovley and Phillips, 1994a; Thamdrup *et al.*, 1993). Such sulfide scavenging by sedimentary metal oxides may allow the

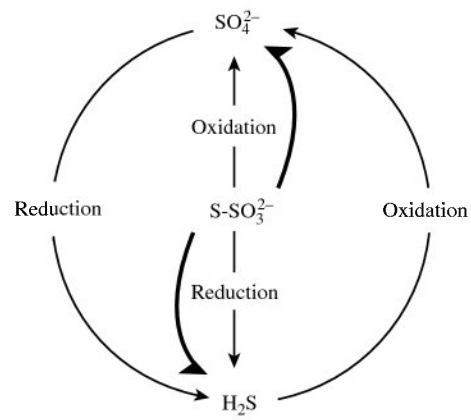

Figure 26 Thiosulfate disproportionation (heavy lines). Typical oxidation (aerobic) and reduction (anaerobic) reactions are included (light lines). A combination of these reactions leads to a thiosulfate shunt that allows for the anaerobic production of intermediate redox states of S in anoxic sediments.

process to act as a common metabolic transformation (Finster *et al.*, 1998).

8.08.7.8 Sulfur Gases

Gaseous sulfur compounds are produced and consumed by microorganisms and they play a significant role in the global sulfur cycle because they connect the terrestrial, freshwater, and marine environments via the atmosphere, and they affect atmospheric chemistry and physics. The most important volatile sulfur compounds are H_2S, dimethylsulfide (DMS), methane thiol (MeSH), carbonyl sulfide (OCS), carbon disulfide (CS_2), and dimethyl disulfide (DMDS) (Bates *et al.*, 1992; Hines, 1996; Kiene, 1996a; Lomans *et al.*, 2002b). Reduced sulfur compounds are subject to chemical and photochemical oxidation in the atmosphere, which yield acidic products that contribute to acid precipitation, and aerosol particles that directly attenuate incoming solar radiation or lead to cloud condensation nuclei, both of which affect the global radiative balance (Charlson *et al.*, 1987; Kelly and Baker, 1990; Panter and Penzhorn, 1980). Early work predicted that H_2S produced by dissimilatory SO_4^{2-} reduction was the primary biogenic sulfur gas emitted into the atmosphere (Bremner and Steele, 1978; Natusch and Slatt, 1978). However, although the biogenic production of organosulfur gases has been known since the 1930s (Haas, 1935), it was only since the 1970s that the importance of these gases has been recognized (Graedel, 1979; Lovelock *et al.*, 1972; Rodhe and Isaksen, 1980). It is now clear that DMS emissions constitute the bulk of the sulfur that enters the atmosphere each year (~75%, primarily from oceanic sources). See Chapter 8.14 of this volume for details on the role of sulfur gases in the global sulfur cycle.

8.08.7.8.1 Hydrogen sulfide

The liberation of H_2S is controlled not only by the rate of its production by SO_4^{2-}-reducing bacteria, but also by its pH-dependent speciation, its tendency to rapidly precipitate as metal sulfides, and its rapid chemical and biological oxidation. Only the protonated species (H_2S) is volatile, and at neutral pH, most inorganic sulfide is present as bisulfide ion (HS^-), whereas sulfide (S^{2-}) dominates under alkaline conditions. These three species are known collectively as ΣH_2S. Hence, the escape of sulfide should be enhanced at low pH. Sulfate reduction is most dominant in marine sediments and this is where the highest emissions of gaseous H_2S occur (Hines, 1996). However, DeLaune *et al.* (2002) reported higher emissions of H_2S from brackish marshes than from true salt marshes. In most cases, despite the fact that dissolved sulfide concentrations can be very high (mM) (Hines *et al.*, 1989; King *et al.*, 1982), only a small portion of the gross production of sulfide escapes to the atmosphere (Jørgensen and Okholm-Hansen, 1985; Kristensen *et al.*, 2000). Bodenbender *et al.* (1999) reported H_2S fluxes from intertidal marine sediments that were up to 2.6×10^4 times less than the rate of SO_4^{2-} reduction. Release of H_2S is usually highest at night, because during the day, photosynthetic microorganisms at the sediment surface either directly consume sulfide or increase the penetration of the O_2 into sediments that enhances chemical oxidation (Bodenbender *et al.*, 1999; Castro and Dierberg, 1987; Hansen *et al.*, 1978; Jørgensen and Okholm-Hansen, 1985). Sulfide emissions from intertidal sediments can increase during flooding due to tidal pumping (Jørgensen and Okholm-Hansen, 1985). Vegetated sediments tend to release less H_2S due to the oxidation of sulfides by O_2 released from roots (Hines, 1996). In general, sulfureta-like sediments, i.e., highly reducing, unvegetated, and sulfide-rich environments, allow for a significant loss of H_2S to the atmosphere since O_2 penetration is minimal and metals capable of precipitating sulfide are already removed. However, these environments are the exception rather than the rule.

8.08.7.8.2 Methylsulfides

Numerous studies of DMS formation and consumption have appeared since the recognition of the importance of this compound in marine biogeochemistry and atmospheric chemistry. Indeed, DMS accounts for ~90% of the biogenic sulfur emissions from the marine environment (Andreae and Crutzen, 1997). A link has been proposed among DMS production and flux, the atmospheric oxidation of DMS, and the subsequent formation of SO_4^{2-} aerosols and cloud condensation nuclei (Charlson *et al.*, 1987). In this model, increasing global temperatures lead to enhanced production of DMS, which attenuates further warming by radiative backscatter from aerosols and reflection of radiation from increased cloud cover. Although most work to date has been conducted on the cycling of DMS in seawater, considerable effort has also been invested in understanding the biogeochemistry of DMS in wetlands and sediments (Kiene, 1996b).

Several compounds can serve as precursors of DMS including dimethylsulfonium compounds such as dimethylsulfoniopropionate (DMSP), S-containing amino acids, MeSH, dimethylsulfoxide (DMSO), and methoxylated aromatic compounds, which are capable of methylating

inorganic sulfide and MeSH (Bak *et al.*, 1992; Finster *et al.*, 1990; Kadota and Ishida, 1972; Kiene and Taylor, 1988a,b; Kreft and Schink, 1993; Lomans *et al.*, 2002b). Methionine is ubiquitous in organisms and has been shown to be a precursor of several sulfur gases including DMS (Bremner and Steele, 1978; Kiene and Capone, 1988; Kiene and Visscher, 1987b; Zhang *et al.*, 2000; Zinder and Brock, 1978c). Methionine degradation produces MeSH, which can be subsequently methylated to DMS (Kiene and Capone, 1988; Kiene and Visscher, 1987b). S-methyl-cysteine can also be degraded to MeSH, whereas S-methyl-methionine degrades primarily to DMS (Kiene and Capone, 1988). DMSO reduction by bacteria also produces DMS (Griebler, 1997; Zinder and Brock, 1978a; Zinder and Brock, 1978b) and several SO_4^{2-}-reducing bacteria with this ability have been isolated (Bale *et al.*, 1997; Jonkers *et al.*, 1996; van der Maarel *et al.*, 1998).

DMSP is a major source of DMS in marine systems (Kiene, 1996a). DMSP is produced by marine micro- and macroalgae and halophytic plants where it acts as an osmoregulant or as part of an antioxidant system (Sunda *et al.*, 2002; Yoch, 2002). Although many phytoplankton contain a DMSP lyase enzyme, bacteria are thought to be the primary degraders of DMSP in seawater (Kiene, 1992). Fungi associated with decaying salt marsh plants also possess a DMSP lyase (Bacic *et al.*, 1998). Cleavage of DMSP can lead to the production of DMS and acrylate, but DMSP can also be demethylated to 3-methylmercaptopropionate, which can be demethiolated to MeSH or demethylated to 3-mercaptopropionate (Kiene and Taylor, 1988a,b; van der Maarel *et al.*, 1996c; Visscher and Taylor, 1994). Members of the *Proteobacteria*, especially the α-group, appear to be important DMS producers in the sea (Gonzalez *et al.*, 1999). However, DMS producers belonging to the β-, δ-, and γ-subdivisions have been identified in seawater (de Souza and Yoch, 1995; Jonkers *et al.*, 1998; Ledyard *et al.*, 1993; van der Maarel *et al.*, 1996b) and members of the γ-subdivision dominated DMS-producing isolates from a salt marsh (Ansede *et al.*, 2001). It has been hypothesized for aerobic seawater that the pathway used for DMSP degradation is controlled by DMSP concentrations and the demand for sulfur, and that DMS formation is usually favored at high DMSP concentrations (Kiene *et al.*, 2000).

Both direct cleavage of DMSP to DMS/acrylate and DMSP demethylation pathways have been observed in anoxic sediments and oceanic waters (Kiene and Taylor, 1988b; Yoch, 2002). However, unlike oxic environments, the demethylation of DMSP in anoxic sediments appears to require two organisms, one to demethylate DMSP to 3-methiolpropionate, followed by another to form MeSH (van der Maarel *et al.*, 1995, 1993). Sulfate-reducing bacteria have been isolated that are capable of cleaving DMSP to DMS and acrylate (van der Maarel *et al.*, 1996d) as well as demethylating DMSP (van der Maarel *et al.*, 1996c). A SO_4^{2-}-reducing bacterium has been isolated that cleaves DMSP and then reduces the liberated acrylic acid (van der Maarel *et al.*, 1996d). Acrylate and SO_4^{2-} reduction occurred simultaneously, but SO_4^{2-} reduction yielded more growth.

DMS (and MeSH) concentrations in freshwater sediments are considerably lower than in marine habitats, primarily due to the low concentrations of DMSP in freshwater plants (Bechard and Rayburn, 1979). However, some small ponds and wetlands exhibit DMS levels similar to those in seawater (3–25 nM) (Kiene and Hines, 1995; Nriagu *et al.*, 1987), and DMS emissions from freshwater habitats can be significant (Hines, 1996; Kelly and Smith, 1990). It has been known for sometime that freshwater bacteria have the ability to form DMS from DMSP, and an obligate anaerobe with this ability was isolated early (Wagner and Stadtman, 1962). Freshwater sediments are capable of producing DMS from DMSP amendments even though these sediments may be nearly DMS-free (Yoch *et al.*, 2001). Enrichments of these sediments with DMSP led to the isolation of a suite of gram-positive DMS-producing bacteria related to *Rhodococcus* spp.

Most studies indicate that the primary path of DMS formation in freshwater sediments is via the methylation of inorganic sulfide to MeSH, which is subsequently methylated to DMS (Bak *et al.*, 1992; Drotar *et al.*, 1987a; Finster *et al.*, 1990; Kiene and Hines, 1995; Lomans *et al.*, 2001a, 1997). Thiol methyltransferase enzymes that catalyze this methylation are relatively widespread in microorganisms including bacteria and protozoa (Drotar *et al.*, 1987a,b; Drotar and Fall, 1985). Methoxylated aromatic compounds, which are degradation products of lignin, have been shown to be important as methyl donors for the methylation of sulfide and MeSH (Finster *et al.*, 1990; Kiene and Hines, 1995), and several anaerobic bacteria have been isolated that can perform this sulfide-mediated *O*-demethylation reaction (Bak *et al.*, 1992; Lomans *et al.*, 2001a; Mechichi *et al.*, 1999). The hydroxylated aromatic residue that remains after demethylation is degraded to acetate or acetate and butyrate (Kreft and Schink, 1993). This differs from the more common pathway for acetate production in which homoacetogenic bacteria transfer methyl groups from methoxylated aromatic compounds to CO to produce acetate (Bache and Pfennig, 1981).

DMS and other volatile organo-sulfur compounds rarely accumulate in sediments, because they are consumed by microorganisms as rapidly

as they are produced (Lomans *et al.*, 2002a,b). Several bacteria are able to degrade DMS and MeSH aerobically, but most of those isolated are *Thiobacillus*, *Methylophaga* or *Hyphomicrobium* spp. (Cho *et al.*, 1991; de Bont *et al.*, 1981; de Zwart *et al.*, 1996; Sivelä and Sundmann, 1975; Smith and Kelly, 1988a; Visscher *et al.*, 1991). Many bacteria are capable of oxidizing DMS to DMSO in the presence of an additional carbon source (Zhang *et al.*, 1991).

Zinder and Brock (1978d) were the first to report the anaerobic conversion of DMS and MeSH to CO_2 and CH_4 in freshwater lake sediments and sewage sludge. Since then, this process has been reported to occur in many anoxic environments including salt marsh and mangrove sediments, hypersaline lakes, and a variety of freshwater sediments (Jonkers *et al.*, 2000; Kiene, 1988; Kiene *et al.*, 1986; Lomans *et al.*, 1999a, 2001b; Lyimo *et al.*, 2000, 2002). Degradation of DMS and MeSH in anaerobic environments is catalyzed primarily by methanogenic, SO_4^{2-}-reducing, NO_3^--reducing, and phototrophic bacteria (Kiene, 1988, 1991a; Kiene *et al.*, 1986; Liu *et al.*, 1990; Lomans *et al.*, 2002b; Lyimo *et al.*, 2000; Oremland *et al.*, 1989; Tanimoto and Bak, 1994; Visscher *et al.*, 1995; Zeyer *et al.*, 1987). Despite the ubiquity of CH_4 formation from DMS and MeSH degradation, SO_4^{2-}-reducing bacteria appear to dominate degradation in marine sediments (Kiene, 1996a) and SO_4^{2-}-reducing bacteria are known to degrade DMS in freshwater environments as well. Methanogens and SO_4^{2-}-reducing bacteria appear to compete for DMS, but methanogens out-compete SO_4^{2-} reducers at high DMS concentrations even when SO_4^{2-} is abundant (Lomans *et al.*, 2002b). The role of SO_4^{2-} reduction in DMS degradation is based primarily on the observed decrease in DMS consumption when SO_4^{2-} reduction is inhibited by molybdate or tungstate (Kiene and Visscher, 1987a). However, a pure culture of a DMS-utilizing SO_4^{2-} reducer has been isolated from a thermophilic digester (Tanimoto and Bak, 1994). The energetic contribution of transformation of methylated sulfur compounds to anaerobic bacteria remains unclear, but the thermodynamics of the use these compounds by methanogens and SO_4^{2-} reducers has recently been evaluated (Scholten *et al.*, 2003). A pure culture of a DMS-degrading denitrifying bacterium has also been isolated (Visscher and Taylor, 1993b).

Several methanogens capable of using DMS have been isolated from marine and hypersaline environments (Finster *et al.*, 1992; Kiene *et al.*, 1986; Oremland *et al.*, 1989; Rajagopal and Daniels, 1986), and a freshwater methanogen with this capability has also been isolated and represents a new genus (Lomans *et al.*, 1999b). The degradation of DMS and MeSH appears to occur similarly to other C_1 compounds such as methanol and methylated amines (Ferry, 1999), but the consumption of methylated sulfur compounds appears to be due to separate inducible enzymes (Ni and Boone, 1993). The methyltransferase responsible for the use of DMS in *Methanosarcina barkeri* is distinct from those used for acetate, methanol, or methylated amines (Tallant and Krzycki, 1997; Tallant *et al.*, 2001). The methanogenic degradation of two moles of DMS yields three moles of CH_4 and one of CO_2. One mole of methyl groups is oxidized to CO_2, which generates reducing equivalents for the reduction of three moles of methyl groups to CH_4 (Finster *et al.*, 1992; Kiene *et al.*, 1986). Complete degradation of methylated sulfides to CO_2 by methanogens may occur when H_2 is removed by hydrogenotrophic bacteria such as SO_4^{2-} or NO_3^- reducers (interspecies H_2 transfer) (Lomans *et al.*, 1999c). Methanogens in oligotrophic peat in northern bogs do not consume DMS, which accumulates during incubations (Kiene and Hines, 1995). It was shown that acetate accumulates in these peats as well (Hines *et al.*, 2001).

8.08.7.8.3 Carbonyl sulfide and carbon disulfide

Bacteria are also involved in the formation and degradation of other volatile sulfur compounds such as carbonyl sulfide (OCS) and carbon disulfide (CS_2). Much less is known about the bacterially mediated transformations of these compounds compared to the methylated sulfur species, especially with respect to the role of anaerobic bacteria. They both constitute a rather minor portion of the global flux of biogenic sulfur gases to the atmosphere (Bates *et al.*, 1992; Hines, 1996). Carbonyl sulfide is a long-lived species in the atmosphere and is the most abundant gaseous form of S, whereas CS_2 has a low atmospheric abundance. Both species appear to form photochemically in aquatic systems, but both also can be formed biologically from a variety of organosulfur precursors including S-containing amino acids (Banwart and Bremner, 1975; Bremner and Steele, 1978) and more unusual sulfur compounds like djenkolic acid ($CH_2[SCH_2CH(CH_2)COOH]_2$) and lanthionine ($S[CH_2CH(NH_2)COOH]_2$) (Piluk *et al.*, 1998). In general, these gases are formed anaerobically in organic-rich environments, but the mechanisms of formation are unclear in most instances (Bremner and Steele, 1978; Kiene, 1996a; Wakeham *et al.*, 1984). Both OCS and CS_2 are toxic to certain bacteria (Borjesson, 2001; Bremner and Bundy, 1874; Seefeldt *et al.*, 1995) and their release from roots has been implicated as a plant defense mechanism (Kanda and Tsuruta, 1995). Both OCS and CS_2 are consumed

aerobically, primarily by bacteria involved in oxidation of inorganic sulfur compounds (Kelly and Baker, 1990; Kelly *et al.*, 1994). They are also consumed in anaerobic sediments (Zinder and Brock, 1978a), but the mechanism for this consumption is unresolved. CS_2 can be oxidized to OCS and H_2S anaerobically (Smith and Kelly, 1988b) and denitrifying bacteria have been isolated that are capable of degrading CS_2 anaerobically (Jordan *et al.*, 1997).

8.08.7.9 Microbial Oxidation of Sulfur

Sulfide and a wide variety of additional organic and inorganic reduced sulfur compounds can be used as electron donors for microbial redox reactions. In addition to its importance in "natural" depositional and hot spring environments, sulfur oxidation plays a significant role in sewage sludge, paper- and wood-mill effluents, coal desulfurization, and leaching of ores and minerals. Sulfide-oxidizing microbes remove noxious sulfides (organic and inorganic) and interact with minerals through oxidation of metal sulfides (e.g., FeS, FeS_2), deposition of elemental sulfur (S^0), precipitation of gypsum and dissolution of calcium carbonate (Baas Becking, 1934; Bos and Kuenen, 1990; Edwards *et al.*, 2000a; Ehrlich, 2002; Lens and Kuenen, 2001; Visscher and Van Gemerden, 1993).

The Gibbs free energy yield of carbon oxidation with SO_4^{2-} as a terminal electron acceptor is about 4–10 times lower than with oxygen. However, sulfide, the product of SO_4^{2-} reduction is an excellent electron donor; the reaction of which with oxygen:

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad (38)$$

has a $\Delta G^{0\prime}$ of $-798\ \text{kJ mol}^{-1}$. Alternatively, anaerobic oxidation using NO_3^-, which couples the sulfur and nitrogen cycles, provides ample energy as well:

$$5HS^- + 8NO_3^- \rightarrow 5SO_4^{2-} + 4N_2 + 3OH^- + H_2O \qquad (39)$$

with a $\Delta G^{0\prime}$ of $-744\ \text{kJ mol}^{-1}$ H_2S. Chemolithotrophic microbes that derive their energy in this fashion are collectively referred to as colorless sulfur bacteria (CSB) in contrast to a physiologically distinct group of pigmented organisms (e.g., purple-sulfur bacteria (PSB)). The latter couple the oxidation of sulfur to photoautotrophic CO_2 fixation (Van Niel, 1931, 1941):

$$HS^- + 2CO_2 + 2H_2O \rightarrow SO_4^{2-} + 2[CH_2O] + H^+$$

HS^- and certain other reduced sulfur-compounds can serve as electron donors for photosystem (PS) I in anoxygenic photolithotrophs (purple and green bacteria) and a few cyanobacteria species. The electrons of HS^- are donated to PS I (Section 8.08.2.1) and utilized to generate reducing biochemical equivalents (NAD(P)H) and ATP. In contrast to oxygenic photolithoautotrophs, the oxidation product of photosynthesis is SO_4^{2-} instead of O_2. In both CSB and phototrophic sulfur bacteria, much of the energy generated from sulfur oxidation is used to assimilate CO_2 into cell material.

Other reduced sulfur compounds that play a significant role in oxidation–reduction reactions in the environment include inorganic compounds such as FeS, FeS_2, $S_2O_3^{2-}$, S_x^{2-}(polysulfides), $S_yO_6^{2-}$ (polythionates), and S^0; organic compounds include methylsulfides (CH_3SH, $(CH_3)_2S$). Clearly, in sulfidic environments, reduced sulfur compounds represent an important source of energy for chemo- and photolithoautotrophic sulfur oxidizers. However, sulfur oxidizers must compete with a variety of biotic and abiotic reactions for terminal electron acceptors.

8.08.7.9.1 Colorless sulfur bacteria

CSB gain energy through chemolithotrophic sulfur-oxidation using O_2 or NO_3^- as terminal electron acceptors. CSB include a wide variety of morphologically and phylogenetically distinct groups (e.g., filamentous *Beggiatoa* and *Thioploca* spp. and single-cell *Thiobacillus* and *Thiomicrospira* spp.). The filamentous CSB have a conspicuous morphology, with cell sizes that allow observation with the naked eye. For example, *Thiomargarita namibiensis* has a cell diameter of up to 0.75 mm (Schulz *et al.*, 1999; Schulz and Jorgensen, 2001) and filaments of certain *Thioploca* species may be as long 70 mm (Jørgensen and Gallardo, 1999). Not surprisingly, Winogradsky (1887) made his early observations of "chemosynthetic" sulfur oxidation in *Beggiatoa* spp. It has been claimed that the *Thioploca-Beggiatoa*-dominated mats in the upwelling area off the coast of Chile embody the largest microbial ecosystem, estimated $10^4\ \text{km}^2$ (Jørgensen and Gallardo, 1999), although it is possible that other such systems exist (Gallardo *et al.*, 1998; Namsaraev *et al.*, 1994). However, the single-celled CSB are abundant in most sulfur-containing ecosystems and are metabolically diverse (Kelly, 1982, 1988; Kelly *et al.*, 1997; Kuenen and Beudeker, 1982). Thus, they can be considered to be at least equally important from a global perspective.

8.08.7.9.2 Single-cell colorless sulfur bacteria

Single-celled, rod-shaped microbes that derive energy from oxidation of reduced

sulfur-compounds were traditionally classified as *Thiobacillus* spp. (Vishniac and Santer, 1957), whereas smaller spirillum-shaped species were *Thiomicrospira* (Timmer-Ten Hoor, 1975). Based on molecular phylogeny, the validity of early classification has recently been challenged and the phylogeny of single-celled CSB is currently under revision. Furthermore, physiological studies revealed the need for a careful description of the products of sulfide-oxidation (Gottschall and Kuenen, 1980; Tuttle and Jannasch, 1977; Vishniac and Santer, 1957). Many heterotrophic microorganisms that seem capable of oxidizing HS^- or $S_2O_3^{2-}$ produce polythionates ($S_yO_6^{2-}$) rather than SO_4^{2-} as reaction products (Tuttle and Jannasch, 1977, 1979). Some heterotrophs have been found to oxidize SO_3^{2-} and other reduced sulfur compounds to SO_4^{2-} (Sorokin and Lysenko, 1993), but since thiosulfate utilization is generally not tested as a metabolic trait, it is possible that many more heterotrophs possess the capacity to oxidize sulfur-compounds. Although these heterotrophs are not considered to be CSB, their role in HS^- oxidation in the environment may be significant (Sorokin and Lysenko, 1993; Tuttle and Jannasch, 1979).

Single-celled CSB oxidize a wide variety of inorganic and some organic sulfur-compounds, mixtures of which are typically present in the environment (Luther and Church, 1988; Luther *et al.*, 1999; Visscher and Van Gemerden, 1991; Zopfi *et al.*, 2001). In mixtures of sulfur-compounds, CSB have a strong preference for HS^- (Kuenen and Beudeker, 1982), and under oxygen-limitation, S^0, polythionates and polysulfides are the oxidation products in species that do not utilize NO_3^- (Van den Ende and Van Gemerden, 1993). S^0 deposited outside the cell, consists primarily of long-chained polythionates that form micelle-like structures (Steudel *et al.*, 1987). This "zero-valent" sulfur contains hydrophilic, charged sulfonate groups on the outside, which explains how growth on highly insoluble "elemental" sulfur is possible. The enzymes involved in the sulfide oxidation pathway, which are well characterized (Kelly, 1982; Kelly *et al.*, 1997), include two that oxidize sulfite to sulfate: sulfite oxidase and reverse adenine phosphosulfate reductase (Kappler *et al.*, 2001). The latter is highly efficient in energy conservation and couples oxidation of sulfite directly to ATP generation. This could in part explain the observation that two groups of thiobacilli can be distinguished based on the amount of energy required for autotrophic growth (Kelly, 1982, 1988). The first group has a low growth yield and oxidizes ~4 mol HS^- per mole of CO_2 fixed into structural cell material. Members include *Thiobacillus neapolitanus*, *T. thiooxidans*, *T. versutus*. The second group has a high growth yield and requires ~2 mol of HS^- oxidized per mole CO_2 fixed. Members of the second group include *T. thioparus*, *T. aquaesulis* and *T. denitrificans*. Autotrophic thiobacilli deploy the Calvin cycle for CO_2 fixation and some have cytoplasmic inclusions that contain crystalline Rubisco (Beudeker *et al.*, 1980). Many CSB are not obligate chemolithoautrophs, but are either facultative autotrophs (e.g., *T. pantotropha, T.*(or *Paracoccus*) *denitrificans*, *T.* (or *Paracoccus*) *versutus*) or so-called mixotrophs (chemolithoorganotrophs; e.g., *T. novellus*) that use sulfur-compounds as electron donors but use organic carbon for biomass.

Single-celled CSB are found in a variety of environments, both terrestrial and aquatic, ranging from freshwater to hypersaline, with pH values from <1 to >10, and displaying psychrophilic, mesophilic, and thermophilic temperature responses. Several species are symbionts in hydrothermal vent invertebrates (e.g., the tube worm *Riftia pachyptilia*, the bivalve *Calyptogena magnifica*, the mussel *Bathymodiolus puteoserpentis;* Cavanaugh, 1994; Cavanaugh *et al.*, 1981; Southward *et al.*, 2001) as well as in bivalves (e.g., *Thyasira* sp.) and oligochetes and clams in estuarine and other environments (Dubilier *et al.*, 1999; Krueger *et al.*, 1996; Wood and Kelly, 1989).

Thiobacilli are typically present in high densities, especially in marine sediments near the oxycline, where densities of 10^6–10^9 cells cm^{-3} have been reported (Gonzalez *et al.*, 1999; Sorokin, 1972; Teske *et al.*, 1996; Visscher *et al.*, 1991, 1992, 1995). Similar densities could be expected in sewage outfall and wastewater treatment facilities, where high rates of SR or of HS^- loading can be expected (Lens and Kuenen, 2001). Some CSB species thrive in extreme environments. For example, *T. ferrooxidans* is commonly found in acid mine drainage where it oxidizes Fe(II) to Fe(III), Cu(I) to Cu(II), and HS^- to SO_4^{2-}. This organism is capable of pyrite oxidation and plays an important role in leaching of ores (e.g., copper, nickel, and uranium) and desulfurization of coal (Bos and Kuenen, 1990). *T. thioparus* is versatile in organic carbon utilization and oxidizes MeSH and DMS (Kanagawa and Kelly, 1986; Smith and Kelly, 1988a; Visscher and Taylor, 1993b; Visscher and Van Gemerden, 1991). Another *Thiobacillus* sp., resembling *T. thioparus*, is able to degrade DMS with NO_3^- instead of O_2 and can also oxidize the thiol group of a range of alkylthiols to sulfate (Visscher and Taylor, 1993a).

The genus *Thiomicrospira* contains three known species, all of which are chemolithoautotrophs. These organisms thrive under microaerophilic conditions, and one strain can couple sulfide and thiosulfate oxidation to NO_3^-

reduction (Timmer-Ten Hoor, 1975). Another exceptional genus of sulfide-oxidizing Eubacteria is *Achromatium*, a facultative autotroph that stores S^0 and calcite intracellularly (Head *et al.*, 2000) and is found in freshwater, brackish, and marine environments. The three known species that belong to this genus all have mixotrophic growth capabilities. The calcite deposits make this organism one of the largest free-living single-celled prokaryotic cells known (estimated biovolume of up to 8×10^4 μm^3 cell^{-1}) (Schulz and Jorgensen, 2001). The role of the calcite is obscure; it could perhaps act as a buffer in the cytoplasm when SO_4^{2-} is produced, or provide CO_2 for autotrophic growth. The latter scenario is somewhat unlikely, since precipitation of $CaCO_3$ yields H^+, and CSB are actually believed to dissolve this mineral when growing autotrophically (Visscher *et al.*, 1998).

The Archeabacteria contain two genera of sulfur-oxidizing organisms, both of which are hyperthermophiles. *Sulfolobus* is an aerobic sulfur-oxidizer, which is also capable of using Fe^{2+} and organic carbon (facultative chemolithoautotroph that grows chemoorganoheterotrophically). *Acidianus* resembles *Sulfolobus*, but can grow both in the presence and absence of O_2. Under oxic conditions it oxidizes S^0 to SO_4^{2-}, while under anoxic conditions, S^0 is used as electron acceptor and sulfide is produced.

8.08.7.9.3 Filamentous colorless sulfur bacteria

The filamentous CSB include the genera *Thioploca*, *Thiothrix*, and *Beggiatoa* (Larkin and Strohl, 1983; Schulz *et al.*, 1999), which are relatively closely grouped phylogenetically. *Beggiatoa* spp. chemolithoautotrophs were the first to be described (Winogradsky, 1887), but later investigations demonstrated that they might have a chemolithoorganotrophic (or mixotrophic) lifestyle. These organisms are found in freshwater and marine habitats at the interface of O_2 and HS^- (Schulz and Jorgensen, 2001).

Beggiatoa stores "elemental" sulfur inside the cell, which gives the filaments a white appearance. Massive blooms of this organism in sulfidic hot springs, salt marshes, and marine sediments make this gliding organism conspicuous (Larkin and Strohl, 1983) when it forms veils or mats. Hydrothermal vents at the deep seafloor support sulfide-based ecosystems with unusually large *Beggiatoa* spp. (Jannasch *et al.*, 1989) in addition to unicellular CSB in trophosomes of *Riftia* spp. (Cavanaugh, 1994). In some *Beggiatoa* spp., the sulfide can be oxidized with O_2 or NO_3^- as an electron acceptor, and N_2 is the product of nitrate reduction (Sweerts *et al.*, 1990).

Thioploca spp. are sheathed filamentous CSB, that resemble *Beggiatoa* spp. in many characteristics. They thrive in the O_2-minimum zone in marine and freshwater mud and seem to accumulate NO_3^- in their vacuoles (as do some *Beggiatoa* spp.), which enables these organisms to thrive under conditions where HS^- and O_2 or NO_3^- are not present simultaneously. The NO_3^- concentration in the vacuole can reach 0.5 M, or more than 100 times that outside the cell (Jørgensen and Gallardo, 1999). However, under anoxic conditions, NO_3^- is reduced to NH_4^+ and not N_2 (Otte *et al.*, 1999). In addition to a positive tactic response to O_2, *Thioploca* spp. also move towards NO_3^- and low concentrations of sulfide; microaerophilic conditions are preferred. *Thioploca* sheaths may contain SO_4^{2-}-reducing bacteria (*Desulfonema* spp.; (Jørgensen and Gallardo, 1999)) that may provide sufficient HS^-. Filaments that have characteristic tapered ends can live outside the sheath, and S^0 is stored intracellularly. *Thioploca* spp. have a mixotrophic lifestyle and, based on cell diameter, four size categories can be distinguished: two small groups (2.5–5 and 12–20 μm), a larger group (30–43 μm), and a group with gigantic cells (125 μm). Although the mats off the Chilean and Peruvian coast are quite extensive (see above), *Thioploca* spp. have been found at other offshore locations including the Arabian Sea, the Benguela Current, and the Mediterranean Sea, as well as in lakes (Lake Erie, Lake Baikal, Lake Biwa) (Gallardo *et al.*, 1998; Jørgensen and Gallardo, 1999; Namsaraev *et al.*, 1994; Nishino *et al.*, 1998).

8.08.7.9.4 Anoxyphototrophic bacteria

Purple and green sulfur bacteria use reduced sulfur compounds (e.g., HS^-, S^0, S_x^{2-}, $S_2O_3^{2-}$) as electron donors for photosynthesis. Purple and green nonsulfur bacteria seem to have limited capabilities to use these donors as well. Interestingly, some PSB have the ability to oxidize sulfide chemolithotrophically in the presence of O_2 (De Wit and Van Gemerden, 1987). For example, although *Thiocapsa roseopersicina* can use HS^- phototrophically, in the presence of O_2, it requires anoxia for synthesis of photopigments (De Wit and Van Gemerden, 1987). If these conditions are not met, *T. roseopersicina* will ultimately be nonpigmented and resort to chemotrophic sulfide oxidation for energy requirements. Immediately following even a brief period of anoxia, the reverse shift from chemolithotrophy to photolithotrophy occurs.

A phylogenetic link between PSB and thiobacilli has been suggested, in which the PSB (and/or other phototrophic sulfur bacteria)

are considered to be the ancestors of certain thiobacilli. Blooms of phototrophic bacteria are observed in lakes (e.g., Guerrero *et al.*, 1985; Overmann, 1997)), microbial mats (Pierson *et al.*, 1987; Van Gemerden, 1993) and in salt marshes (Baas Becking, 1934). Purple nonsulfur bacteria (PNSB) can use a wide variety of organic carbon compounds (Madigan, 1988) and typically thrive in habitats where sulfur and organic carbon loading are high (e.g., sewage effluents). Some PSB and PNSB can use organic sulfur compounds, either as electron donors (Visscher and Taylor, 1993c; Visscher and Van Gemerden, 1991) or as electron donors and/or carbon sources (Chien *et al.*, 1999).

8.08.7.9.5 *Ecological aspects of sulfide oxidation*

As the phylogeny, physiology, and biochemistry of sulfide-oxidizing microbes is under revision, the ecological aspects and biogeochemical role of these organisms in carbon, sulfur, and nitrogen cycles is anticipated to change as well. For example, as outlined above, the capability to oxidize sulfide or thiosulfate is not one of the "standard tests" that is performed when characterizing organisms. This may well explain the scattered distribution of single-celled CSB in the phylogenetic trees (although the sequence of 16S rRNA and metabolic traits are not synonymous). In addition to the abundance of sulfur-oxidizers, it is important to acknowledge the environmental characteristics, especially those related to O_2 and HS^- (and other sulfur-compounds; Jørgensen *et al.*, 1979; Luther *et al.*, 1991 ; Visscher and Van den Ende, 1994; Visscher and Van Gemerden, 1993; Zopfi *et al.*, 2001).

The expanding role of cultured organisms that were not considered to be involved in sulfur transformations and some novel insights in associations of various sulfur bacteria were recently reviewed (Overmann and van Gemerden, 2000). One example of interactions among sulfide-oxidizers is that of CSB and PSB. When PSB (*Thiocapsa roseopersina*) and CSB (*Thiobacillus thioparus*) compete for sulfide under alternating oxic–anoxic conditions, PSB outcompete CSB despite a better affinity for sulfides in the latter group of organisms (Visscher *et al.*, 1992). The storage of S^0 intracellularly and the capability to use light provide a competitive advantage. Similarly, other investigators hinted that a combination of environmental factors, including light quality and quantity and sulfide and oxygen concentrations, need to be considered when evaluating the competitive position between CSB and PSB (Jørgensen and Des Marais, 1986). In further investigations, where the oxygen/sulfide ratios were varied, the PSB were only able to coexist with the CSB when the ratio was less than 1.6 (indicating O_2-limitation), in which case the PSB used partially oxidized sulfur compounds excreted by the CSB (Van den Ende *et al.*, 1996).

Although several physiological and ecological aspects of sulfur-oxidation have been outlined above, others exist, including the fermentation of sulfur compounds (Bak and Cypionka, 1987), the use of HS^- or $S_2O_3^{2-}$ as electron donor in certain cyanobacteria (e.g., De Wit and Van Gemerden, 1987; Pringault and GarciaPichel, 2000), and the coexistence of CSB and SRB under atmospheric O_2 concentrations (Van den Ende and Van Gemerden, 1993). The need for an improved understanding of ecophysiological functioning of microbes and their distribution as well as biogeochemical transformations in the sulfur cycle are still unambiguous despite 125 years of research.

8.08.8 COUPLED ANAEROBIC ELEMENT CYCLES

Although it is pragmatic to consider the element cycles independently, an understanding of how elements and organisms interact is required to interpret the relative contributions of the various pathways to anaerobic metabolism in nature. This concluding section elaborates on these interactions and briefly reviews the relative contribution of the major anaerobic pathways to carbon metabolism in ecosystems.

8.08.8.1 Evidence of Competitive Interactions

The outcome of competition between microorganisms for electron donors can be predicted from thermodynamic theory (Section 8.08.1.2; Table 1; Zehnder and Stumm, 1988), and these predictions are generally consistent with empirical data. Temporal succession of the microbial metabolic pathways that dominate respiration occurs upon the flooding of an oxidized soil or sediment (Figure 1). Not surprisingly, most examples of temporal succession in anaerobic respiration processes have come from wetland soils, which are subject to cycles of flooding and exposure (Turner and Patrick, 1968; Ponnamperuma, 1972; Achtnich *et al.*, 1995a; Yao *et al.*, 1999). However, the same pattern is observed in sediments and even upland soils (Peters and Conrad, 1996).

Yao *et al.* (1999) recognized three phases of reduction following the flooding of rice paddy soil. In the *reduction phase* (phase I), the inorganic electron acceptors were sequentially consumed and CO_2 emissions were highest; in phase II, CH_4 production became dominant and peaked, and in

phase III there was a steady-state ratio of CH_4 and CO_2. Peak CH_4 production during phase II was highly correlated ($r^2 = 0.79$) with the ratio of nitrogen to inorganic electron acceptors, reflecting the dual influence of carbon quality and competition on methanogenesis. Methane production is favored by a high nitrogen content, because it is indicative of a large labile carbon pool (Section 8.08.4.3.4), which is consumed preferentially by nonmethanogenic bacteria until the inorganic electron acceptor pools are exhausted. Thus, electron flow through methanogenesis is highest when the labile carbon pool is large and the inorganic electron acceptor pools are small. The length of phase I increases with the inorganic electron acceptor pool size, and decreases with a decrease in the labile pool size or an increase in temperature (van Hulzen *et al.*, 1999).

Spatial zonation develops due the progressive consumption of terminal electron acceptors from their source to points downgradient. Such zonation occurs as a function of depth in systems that are continually inundated such as aquatic sediments. It also occurs downstream of an organic carbon point source in rivers and aquifers (Lovley *et al.*, 1994a).

A final source of observations that indicate competition for electron donors comes from direct manipulation of soils or sediments. Addition of 30 g ferrihydrite per kg soil reduced CH_4 emission from a paddy soil by 84% (Jäckel and Schnell, 2000), and similar responses have been reported following a fall in the water table (Krüger *et al.*, 2001). Freeman *et al.* (1994) attributed a decline in CH_4 emissions following drought to inhibition by SO_4^{2-} reduction, which is consistent with the results of direct SO_4^{2-} addition studies (Gauci *et al.*, 2002).

8.08.8.2 Mechanisms of Competition

Molecular hydrogen (H_2) is the most abundant product of fermentation and the most common electron donor in terminal anaerobic metabolism. Extremely low (nanamolar level) H_2 concentrations in anaerobic sediments are evidence of keen competition for H_2. Metabolic pathways that yield relatively large amounts of free energy (i.e., those with more negative ΔG values) tend to be associated with low pore-water H_2 concentrations (Table 1; Figure 27). Thus, SO_4^{2-}-reducers inhibit H_2-dependent methanogens by reducing the H_2 concentration to a value below the threshold at which CH_4 production is thermodynamically feasible (Lovley *et al.*, 1982); Fe(III) reducers have the same influence on SO_4^{2-} reducers, and so forth according to the free-energy yield of the processes when operating near chemical equilibrium (Lovley and Goodwin, 1988; Postma and Jakobsen, 1996).

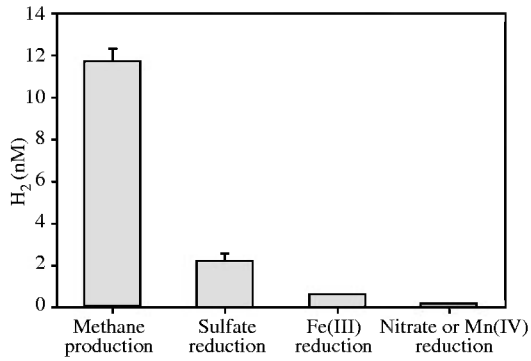

Figure 27 Steady-state H_2 concentrations in sediments with different dominant terminal electron accepting processes (Lovely and Goodwin, 1988) (reproduced by permission of Elsevier from *Geochim. Cosmochim. Acta.* **1988**, *52*, 2993–3003).

The link between bioenergetics and H_2 offers a nondestructive method of determining the dominant terminal electron accepting process *in situ* (Hoehler *et al.*, 1998). Such an approach is most effective when the influence of temperature, pH, mineral reactivity and other factors are taken into account by calculating the free energy of the system. Hoehler *et al.* (1998) observed that the ΔG value for a given terminal respiration process remained constant when temperature and pH were manipulated, and suggested that it reflected the need for microorganisms to operate at their energetic limit in order to successfully compete for H_2. The theoretical minimum energy yield that can support life (i.e., the *biological energy quantum*) is one-third ATP per round of metabolism or about -20 kJ mol^{-1} (Schink, 1997). This limit agrees at least roughly with *in situ* observations. The low energy yields of fermentation, methanogenesis, and most other forms of anaerobic metabolism suggest that anaerobic organisms function at a level of near-starvation (Valentine, 2001).

Thermodynamic considerations suggest that competition for acetate should also favor the metabolic pathway with the highest free-energy yield. However, acetate concentrations have been reported to change in response to a shift in the dominant metabolic pathway in some cases but not others (Achtnich *et al.*, 1995b; Chidthaisong and Conrad, 2000; Sigren *et al.*, 1997). Thermodynamic control of acetate concentrations may be superseded by kinetic effects due to its slow diffusion rate.

A more recent addition to the list of terminal electron acceptors is humic substances (Section 8.08.6.4.1). Cervantes *et al.* (2000) demonstrated that a humic acid analogue (AQDS) inhibited methanogenesis due to a combination of toxic and

competitive effects. The thermodynamic yield of AQDS reduction was estimated to be more favorable than SO_4^{2-} reduction or methanogenesis, but less favorable than Fe(III) reduction. Because humic substances can quickly transfer electrons to other terminal acceptors such as Fe(III), they are rapidly recycled and could be important in anaerobic metabolism despite typically low concentrations.

8.08.8.3 Exceptions

There are many instances when the succession of anaerobic microbial pathways or segregation in space varies from the classical pattern (Section 8.08.8.1). There is a tendency for overlap between pairs of terminal electron acceptors with similar free-energy yields (e.g., NO_3^- and Mn; Fe and SO_4^{2-}). These exceptions can often be explained by the absence of competition for electron donors (Oremland *et al.*, 1982; Crill and Martens, 1986; Holmer and Kristensen, 1994; Chidthaisong and Conrad, 2000; Mitterer *et al.*, 2001; McGuire *et al.*, 2002). Small amounts of CH_4 are produced in rice paddy soils immediately upon the onset of anoxia, despite the presence of NO_3^-, Fe(III) and SO_4^{2-}, and a high redox potential (Roy *et al.*, 1997;Yao and Conrad, 1999). The initial burst of methanogenic activity coincides with a peak in H_2 concentrations, suggesting that H_2 production from fermentation can exceed demand for a period of time (Yao and Conrad, 1999). Once Fe(III) reduction and SO_4^{2-} reduction draw down H_2 to a level below the threshold required for methanogenesis, CH_4 production ceases. A seemingly uncommon reason for the absence of competition between terminal electron accepting processes is that the organisms require different electron donor substrates (Section 8.08.7.5.3).

The order of competing terminal electron accepting processes can vary with any number of factors that influence the thermodynamics of the system. One factor that must be considered in ecosystems with mineral sediments or soils is the composition of Fe(III) and Mn(IV) minerals. The typical sequence of Fe(III) reduction before SO_4^{2-} reduction can be reversed with a change in the abundance of labile Fe(III) minerals such as ferrihydrite (Postma and Jakobsen, 1996). This is one explanation for the common observation that the zones of Fe(III) reduction and SO_4^{2-} reduction overlap in marine sediments (Boesen and Postma, 1988; Canfield, 1989; Canfield *et al.*, 1993b; Goldhaber *et al.*, 1977; Jakobsen and Postma, 1994). Postma and Jakobsen (1996) predicted that the overlap between Fe(III) reduction and SO_4^{2-} reduction should increase as Fe(III) oxide stability (or surface area) increases.

Spatial heterogeneity is a likely explanation for the coexistence of competing terminal electron accepting processes. It is well established that denitrifying bacteria are active in anaerobic microsites imbedded in upland soils (Section 8.08.5.3.2; Figure 14), and there is evidence of methanogenic activity, the most O_2 sensitive of anaerobic metabolisms, in upland soils as well (von Fischer and Hedin, 2002). Redox potentials are notoriously variable in anaerobic sediments, suggesting that redox microsites occur in the total absence of O_2. Spatial variability has been proposed to explain deviations from the expected suppression of SO_4^{2-} reduction by Fe(III) reduction (Hoehler *et al.*, 1998). It is now possible to observe such small-scale variability in microbial populations using molecular techniques (e.g., Boetius *et al.*, 2000b; Orphan *et al.*, 2001b) and element-specific microelectrodes, including a recently developed system for measuring iron speciation (e.g., Luther *et al.*, 2001).

8.08.8.4 Noncompetitive Interactions

In many cases, the segregation of terminal electron accepting processes is due to factors other than competition. The ability of NO_3^- to suppress Fe(III) reduction (DiChristina, 1992) and methanogenesis has been shown to be due in part to inhibition by denitrification intermediates (Klüber and Conrad, 1998, p. 331; Roy and Conrad, 1999). Concentrations of <100 μM NO_3^-, 1–2 μM NO and <1 mM N_2O have been reported to completely inhibit hydrogenotrophic methanogenesis (Balderston and Payne, 1976; Klüber and Conrad, 1998), while somewhat higher concentrations are necessary to inhibit acetogenic methanogenesis (Clarens *et al.*, 1998). Chidthaisong and Conrad (2000) reported that NO_3^- amendments inhibited glucose turnover in a paddy soil, which indicates that nitrogen oxide toxicity may have affected the methanogens indirectly by inhibiting fermentation.

8.08.8.5 Contributions to Carbon Metabolism

The factors that influence the flux of energy through aerobic–anaerobic interface ecosystems have been the focus of this review chapter. Here we consider the net effect of these influences on carbon metabolism in marine and freshwater ecosystems. Thamdrup (2000) recently compiled studies that reported the relative contributions of O_2 reduction, Fe(III) reduction, and SO_4^{2-} reduction to carbon metabolism in marine ecosystems ($n = 16$). On average, the dominant pathway was SO_4^{2-} reduction (62 ± 17%, $\bar{x}$ ± SD). Aerobic respiration and Fe(III) respiration contributed equally to carbon metabolism (18 ± 10% and 17 ± 15%, respectively). Compared to previous compilations, ~50% of the amount

attributed to O_2 reduction is now credited to Fe(III) reduction, while the contribution of SO_4^{2-} reduction is unchanged (Thamdrup, 2000). Fe(III) reduction can also be the dominant carbon oxidation pathway in salt marsh sediments (Kostka *et al.*, 2002c; Gribsholt *et al.*, 2003).

There have been remarkably few attempts to determine the contribution of Fe(III) reduction to anaerobic carbon metabolism in freshwater ecosystems where it may be the dominant pathway. The most extensive such study examined 16 rice paddy soils collected from China, Italy, and the Philippines (Yao *et al.*, 1999). Fe(III) reduction was 58–79% of carbon metabolism during the reduction phase (Section 8.08.8.1), with most of the remainder attributed to methanogenesis. Fe(III) reduction contributed ~70% of the anaerobic metabolism in a *Juncus effusus* marsh (Roden and Wetzel, 1996).

There is growing evidence that plant activity causes carbon metabolism to shift away from methanogenesis to Fe(III) reduction. Roden and Wetzel (1996) reported that the contribution of methanogenesis to anaerobic carbon metabolism shifted from 69% in the absence of plants to <30% in their presence. Similar observations have been reported for freshwater marshes dominated by *Scirpus lacustris* and *Phragmites australis* (van der Nat and Middelburg, 1998) and a rice paddy soil (Frenzel *et al.*, 1999). The mechanism for this effect is an abundance of poorly crystalline Fe(III) in the rhizosphere compared to the bulk soil (Kostka and Luther 1995; Weiss, 2002), and perhaps a larger labile organic carbon supply. Fe(III) and Mn(IV) respiration are subordinate terminal electron-accepting processes in saturated soils and sediments in the absence of a mechanism for their regeneration to oxides. Plants, bioturbation, or physical mixing have this effect. In salt marshes, bioturbation by crabs may be more important to Fe(III) regeneration than radial O_2 loss from roots in some cases (Kostka *et al.*, 2002c), but not in others (Gribsholt *et al.*, 2003). In marine sediments, there is very little Fe(III) or Mn(IV) reduction in the absence of mixing (Thamdrup, 2000).

8.08.8.6 Concluding Remarks

Research since 1990 has greatly expanded our understanding of anaerobic metabolism. Novel microorganisms have been discovered, such as those performing anaerobic ammonium oxidation to N_2 (anammox). These organisms were predicted to exist based on thermodynamic considerations, but have since been shown to contribute substantially to N_2 production in some marine sediments. Organisms that were known to exist for sometime have been shown to perform unexpected types of metabolism, such as nitrifying bacteria that are capable of denitrification and Fe(III)-reducing bacteria that produce CH_4. The reduction of Fe(III) was thought to be primarily an abiotic process, but it is now understood to account for much of the anaerobic carbon metabolism in many freshwater ecosystems. Microorganisms appear to play a larger role in Fe(II) oxidation at circumneutral pH that previously believed. Progress has been aided by the development of molecular and stable isotope techniques that yield detailed descriptions of microbial communities. Such techniques have recently provided strong support for the hypothesis that anaerobic CH_4 oxidation is achieved by a syntrophic relationship between *Archea* and *Bacteria*. The significance of these new microorganisms and metabolic pathways for element cycling *in situ* remains to be determined, but it is likely to lead to important revisions of the element budgets for carbon, nitrogen, manganese, iron, and sulfur, and the mechanisms that regulate their cycling. The recent downward revision of aerobic carbon mineralization rates in marine sediments that followed the discovery of respiratory Fe(III) reducing bacteria (Section 8.08.8.4) is a poignant example of how important the field of environmental microbiology has become for advancing our knowledge of biogeochemistry. Likewise, geochemistry has made significant contributions to environmental microbiology by discovering the activity of anaerobic CH_4 oxidizing bacteria and the anammox process. More exciting discoveries can be expected as these fields continue to become integrated.

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