Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems

Amy J Burgin[1*], Wendy H Yang[2], Stephen K Hamilton[3], and Whendee L Silver[2]

Microbial metabolism couples elemental reactions, driving biogeochemical cycles. Assimilatory coupling of elemental cycles, such as the carbon (C), nitrogen (N), and phosphorus cycles, occurs when these elements are incorporated into biomass or released through its decomposition. In addition, many microbes are capable of dissimilatory coupling, catalyzing energy-releasing reactions linked to transformations in the oxidation state of elements, and releasing the transformed elements to the environment. Different inorganic elements provide varying amounts of energy yield, and the interaction of these processes creates a microbial energy economy. Dissimilatory reactions involving C, N, iron, and sulfur provide particularly important examples where microbially mediated oxidation–reduction (redox) transformations affect nutrient availability for net primary production, greenhouse-gas emissions, levels of contaminants and natural toxic factors, and other ecosystem dynamics. Recent discoveries of previously unrecognized microbial dissimilatory processes are leading to re-evaluation of traditional perceptions of biogeochemical cycles.

Front Ecol Environ 2011; 9(1): 44–52, doi:10.1890/090227

All organisms have to make a living by harnessing energy, collecting nutrients, and expelling waste. The joining together, or coupling, of elemental cycles occurs when elements required for biosynthesis are assimilated into microbial, plant, and animal biomass, and again when that biomass is decomposed; in both cases, elements cycle in proportion to their abundance in the food consumed and the biomass produced. Carbon (C), nitrogen (N), and phosphorus (P) provide well-studied examples of this "assimilatory" coupling, which is the focus of a body of theory known as ecological stoichiometry (Sterner and Elser 2002). Prokaryotic microbes also assimilate these elements into biomass, but the microbial world includes diverse additional metabolic pathways, many of which strongly influence biogeochemical cycles (Falkowski *et al.* 2008). Organisms can be classified according to their sources of energy; the metabolic diversity of microbes far exceeds that of plants and animals (Figure 1).

In a nutshell:

- Oxidation–reduction (redox) reactions associated with microbial metabolism couple inorganic element transformations to the release of energy
- Direct couplings via dissimilatory pathways include microbially mediated oxidation and reduction of iron, manganese, sulfur, and nitrogen through metabolic processes that only microbes can perform
- Indirect coupling occurs when the products of dissimilatory transformations react in the environment to affect the cycling of other elements, such as when oxidized iron binds phosphorus in soils or sediments to control phosphorus availability
- Recently discovered microbial processes provide the foundation for previously unrecognized linkages in biogeochemical cycles

Biogeochemists are particularly interested in certain groups of microbes that couple elemental cycles via their energy metabolism, mediating oxidation–reduction (redox) reactions and releasing the transformed products into the environment in processes known as "dissimilatory transformations". Two distinct classes of dissimilatory transformations are (1) the anaerobic respiration of organic matter (a heterotrophic metabolism in which inorganic substances serve as electron acceptors), and (2) lithotrophic (rock eating) oxidation of inorganic substances to obtain energy (Figure 1). With a little insight into these metabolic pathways, biogeochemists can often observe the products of dissimilatory transformations in nature (Figure 2). Given the critical importance of microbes in coupling biogeochemical cycles, our goals are to (1) explain how redox chemistry related to microbial metabolism creates a microbial energy economy, (2) describe examples of the diverse ways in which microbial metabolic reactions couple biogeochemical cycles, both directly and indirectly, and (3) highlight areas of needed research on these topics across aquatic and terrestrial ecosystems.

The microbial energy economy

The microbial energy economy refers to the many metabolic pathways that microbes use to harness energy (Figure 1). Like any economy, the microbial energy economy is built

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[1] *Department of Earth and Environmental Sciences, Wright State University, Dayton, OH* [*] *(burginam@gmail.com);* [2] *Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, CA;* [3] *WK Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, MI*

from raw materials from which goods and services are produced. The raw materials – various dissolved chemicals, gases, and sunlight – are resources microbes can exploit. These resources are converted into biomolecules, such as enzymes and nucleic acids. Biomolecules can then be used for services, such as energy generation, growth, and reproduction. We will focus on how microbes combine different raw materials (resources) to mediate reactions that produce energy (services). The metaphor of an economy is apt because it suggests a diversity of interacting and potentially competing players (ie the microbial assemblage), and similar to the conventional sense of an economy, some metabolic pathways are energetically more valuable than others, which confers competitive advantages. In this energy economy, microbes couple elemental cycles directly through their metabolism, or indirectly through the reaction of the products of dissimilatory transformations, which can then affect other biogeochemical cycles. To understand these interactions, however, we must first elaborate on the concept of redox chemistry and how it relates to microbial metabolism.

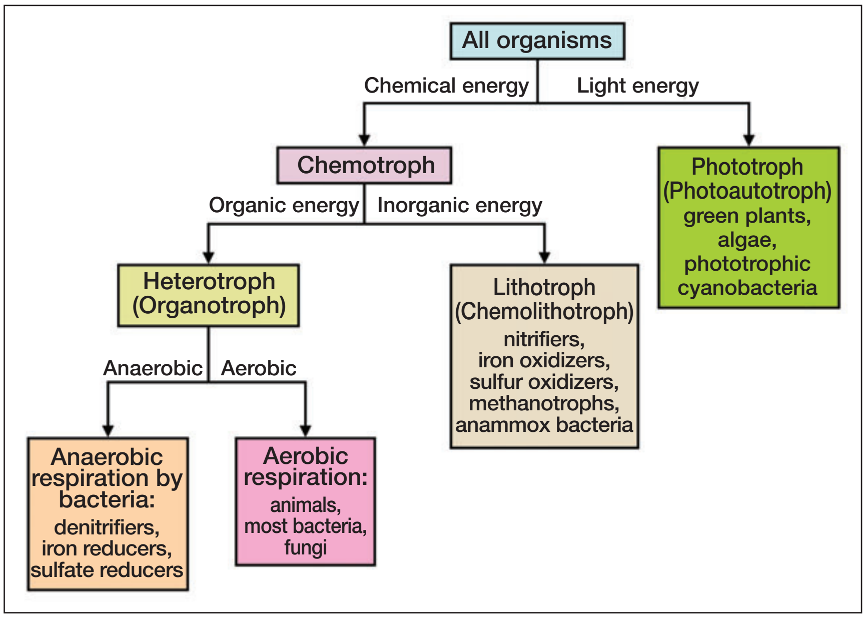

Figure 1. *Classification of organisms based on their primary source of energy reveals the metabolic diversity of microbes as compared with that of higher plants and animals. Other forms of anaerobic microbial metabolism (eg fermentation) exist but are not included here.*

Redox reactions are chemical reactions in which atoms change their oxidation state. In its simplest sense, oxidation occurs when a molecule, atom, or ion loses electrons, and reduction occurs when such units or particles gain electrons (Figure 3a). Because electrons cannot exist freely, every oxidation must be accompanied by a reduction, with the two parts of the overall reaction called "half reactions". Many redox reactions can occur abiotically, but they take place much faster when catalyzed by enzymes. Enzymes do not alter whether a reaction is thermodynamically favorable (which cannot be done), but they increase the speed of a reaction.

Microbes catalyze redox reactions to release energy. This energy is subsequently captured in biomolecules, including adenosine triphosphate (ATP), the energy currency of life. Electrons from the electron donor are shuttled onto electron transport chains contained within a cell's membrane (Figure 3b). As the electrons move through the chain, protons (H^+, originating from water) are pumped out of the cell, thereby creating an electrochemical gradient that effectively charges the cell like a battery. This charge provides the power to fuel ATP synthesis via the class of enzymes called adenosine triphosphatases (ATPases; White 2000). After completing the course through the membrane chain, the electrons are transferred to the terminal electron acceptor, which is then reduced (eg Fe[III] → Fe[II] in Figure 3b).

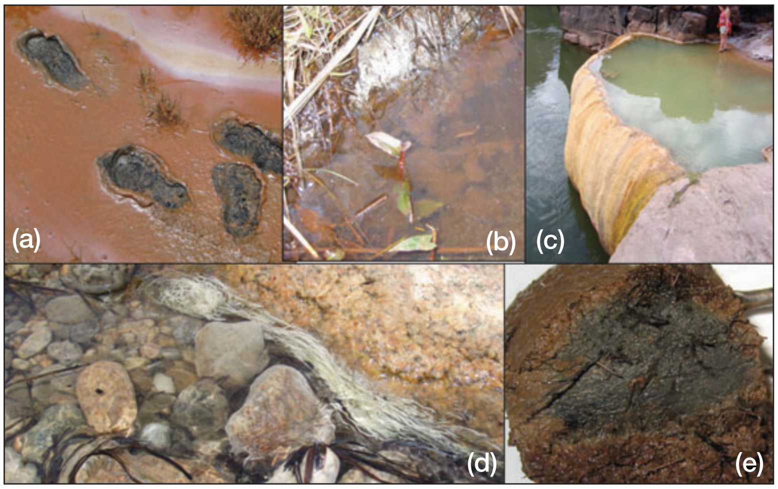

Figure 2. *Evidence of microbial biogeochemistry is ubiquitous: (a) footprints in an Australian salt marsh reveal a layer of black iron sulfide minerals below the oxidized iron upper layer, with a manganese sheen nearby; (b) iron oxides (rust-colored flakes, or floc, underwater) and sulfur-oxidizing bacteria (white material on vertical face) in an area of groundwater seepage out of a freshwater wetland in Brisbane, Australia; (c) a carbonate-depositing spring along the Colorado River in the Grand Canyon, Arizona, where groundwater brings solutes from deep magmatic sources, showing iron oxide staining; (d) filamentous sulfur bacteria growing in a stream in Massachusetts appear white because they deposit elemental sulfur intracellularly; and (e) a cross section of a wet soil core (from a high marsh soil in Tomales Bay, California) 5 cm in diameter exposed to ambient oxygen conditions shows a rust-colored outer layer, reflecting oxidized iron, and a distinct gleyed (grayish) center, reflecting reduced iron minerals where oxygen demand exceeds diffusive oxygen resupply.*

"Redox potential" (also known as "reduction potential"

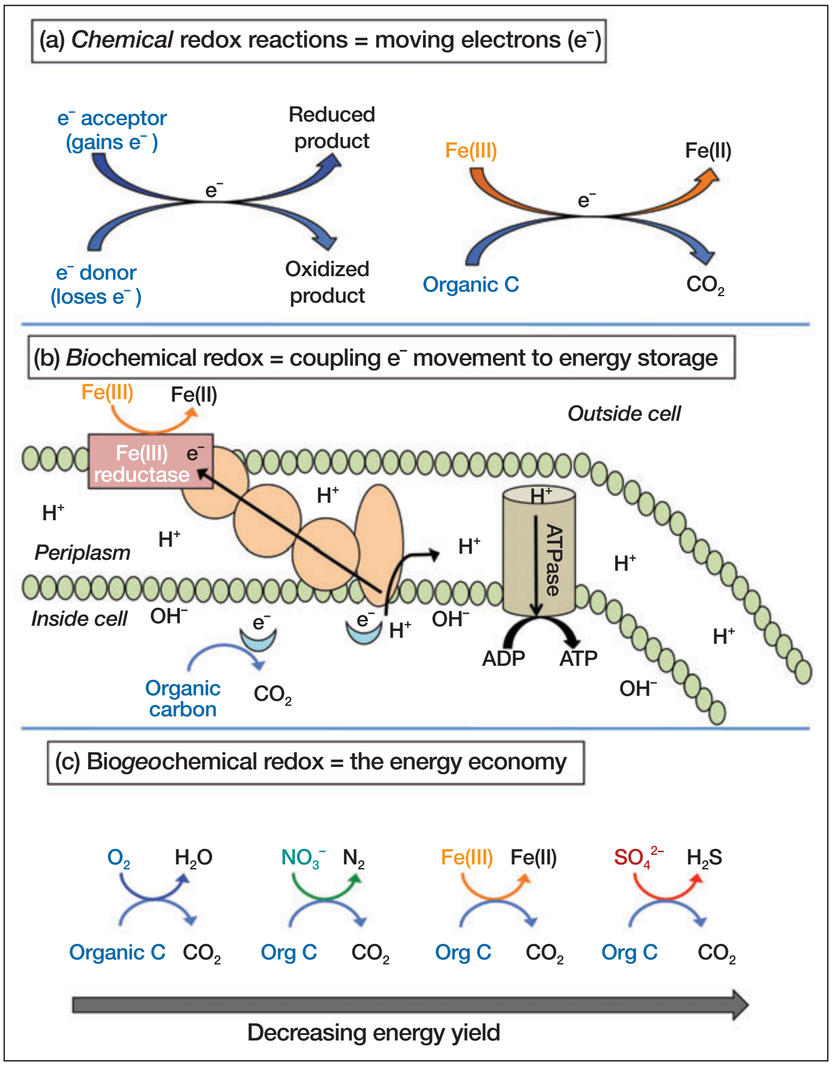

Figure 3. *Redox reactions are fundamental to biological energy metabolism: (a) schematic of coupled redox half-reactions and anaerobic respiration coupled to iron reduction as an example. (b) Biochemical coupling of proton (H^+) and electron transport from organic carbon across the cell membrane to yield reduced iron, in this example portraying* Thiobacillus ferrooxidans*. ADP = adenosine diphosphate, a precursor to adenosine triphosphate (ATP) (modified from Weber* et al. *2006a). (c) The sequentially decreasing energy yield of potential terminal-electron-accepting processes dictates the outcome of competition among microbes that conduct anaerobic respiration.*

or "oxidation–reduction potential") describes whether an environment is relatively oxidizing or reducing, and gives an indication of what thermodynamic reactions occur therein. Scientists often refer to certain environments as exhibiting "reducing" conditions, where oxygen (O_2) is absent and reduced substances are abundant (eg sediments in wetlands), and others as having "oxidizing" conditions (eg well-drained soils with O_2 in the pore spaces).

■ Anaerobic respiration as a source of reduced substances

Unlike most other forms of life, certain heterotrophic microbes are not limited to aerobic respiration but can conduct anaerobic respiration using a variety of chemicals as alternative electron acceptors for the oxidation of organic matter (White 2000; Megonigal *et al.* 2004; Madsen 2008; Figure 1). Electron acceptors yield differing amounts of energy in redox reactions (Figure 3c). The energy yield depends on environmental pH, which means that a given redox reaction can be thermodynamically favorable (ie likely to occur) in one environment but not in another. Typically, the most energy is generated from respiration supported by O_2 as the electron acceptor, followed by nitrate (NO_3^-), ferric iron (Fe[III]), and sulfate (SO_4^{2-}), with the latter two processes conducted by specialized microbial groups. Methanogenesis – a distinctive form of metabolism carried out only by certain members of the domain Archaea – becomes the predominant form of anaerobic heterotrophic metabolism when these alternative electron acceptors are depleted. Thus, putting Figure 3 together, we see that (Figure 3a) chemical redox reactions can be (Figure 3b) exploited by cells to capture energy; the efficiency of energy capture varies, based on which oxidants (electron acceptors) are available (Figure 3c). The efficiency of energy yield influences the outcome of competition among microbes, with the more energetically favorable processes tending to prevail as long as the supply of electron acceptors for those processes is not exhausted.

The availability of electron donors and acceptors to microbes is influenced by biotic and abiotic factors, including: (1) the geologic characteristics of the soil; (2) past and present primary productivity, which influences the amounts of organic C; and (3) pH, which is influenced by mineral weathering, organic and mineral acids, and dissolved carbon dioxide (CO_2). Along with myriad other processes, microbial metabolism both produces and consumes acids and hence affects pH. Gradients of electron-acceptor availability can be found in aquatic and terrestrial ecosystems, including lakes (Figure 4a), sediments (Figure 4b), and soil aggregates (Figure 4c), and these gradients occur over vastly different spatial scales (Fenchel *et al.* 1998). Microscale variability of electron donors and acceptors is probably more marked in unsaturated soils, where uniform water distribution is not as effective as a homogenizing factor and soil aggregation can create heterogeneity in redox zones; the potential importance of such variability for microbial redox reactions is not well understood (Sexstone *et al.* 1985).

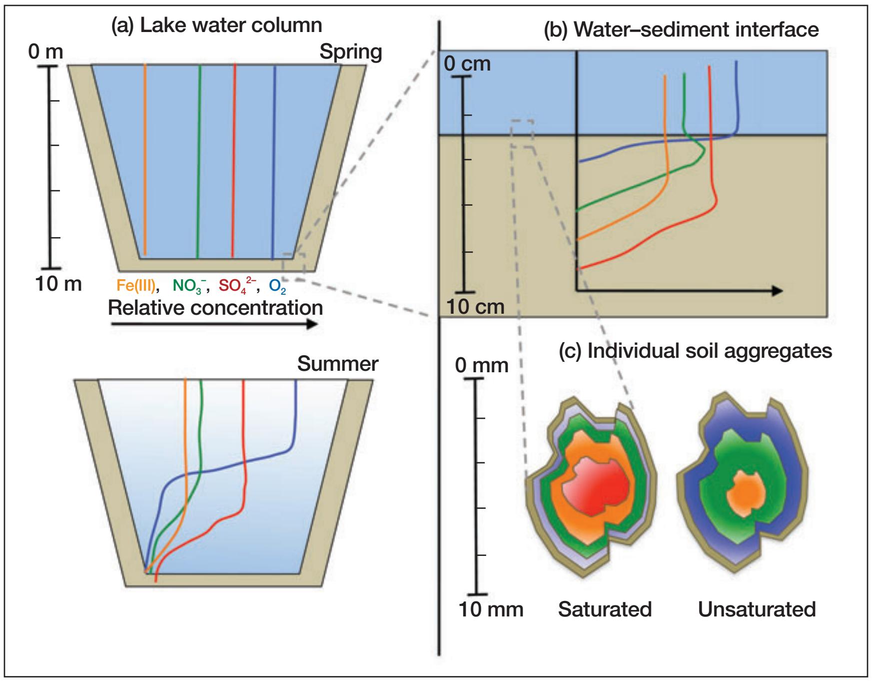

Figure 4. *Examples of how redox stratification can develop (a) over the course of the summer in a stratified water column of a lake, (b) in the first few millimeters of sediments below the sediment–water interface in oxic waters, and (c) within soil aggregates that have high water content or high biological oxygen demand, making them anoxic in the interior, despite an oxic soil atmosphere on the outside. The colored lines correspond to the molecule of the same color (eg green for NO_3^-) with concentration increasing along the arrow in (a) and (b), and increasing with color intensity (increasing trends toward the red-orange end of the spectrum) in (c).*

In the biosphere as a whole, most of the production and decomposition of organic matter occurs via aerobic processes and is conducted by photoautotrophs and chemoheterotrophs (Figure 1). Why then do biogeochemists devote so much attention to microbial processes that occur in the absence of O_2? One reason is that anaerobic decomposition is crucial in removing organic matter and regenerating available nutrients; without it, environments prone to anaerobic conditions, including lakes, oceans, and water-saturated soils, would fill in with incompletely decomposed biomass, and nutrients would be locked up in those organic pools. Over shorter time scales, the inorganic redox reactions associated with these dissimilatory transformations consume or produce highly reactive substances and thereby alter the environment in which they are produced. For example, anaerobic respiration based on NO_3^- reduction to dinitrogen (N_2; denitrification) is the most important sink (removal process) for reactive N, helping to remove excess NO_3^- resulting from human activities (Seitzinger *et al.* 2006). However, anaerobic respiration based on NO_3^- reduction also produces nitrous oxide (N_2O), a catalyst for stratospheric ozone depletion and a potent greenhouse gas (Conrad 1996). Iron reduction liberates elements associated with oxidized Fe minerals, including P (Chacon *et al.* 2006; Liptzin and Silver 2009) and trace metals such as arsenic, and produces highly mobile dissolved Fe(II) that can be transported by water and eventually oxidized in distant locations (Megonigal *et al.* 2004; Figure 2). Sulfate reduction yields sulfide (free sulfide occurs mainly as H_2S or HS^-, depending on pH, but is hereafter referred to as H_2S), a toxic substance that limits life in sediments and reacts readily with metals, including Fe (Wang and Chapman 1999).

The redox reactions that we describe here are prevalent in anoxic waters and sediments as well as at their interfaces with oxic (oxygenated) waters, but they are also surprisingly important in environments that are not considered depleted in O_2. Wetland soils have well-described patterns in redox potential, varying in time (seasonally, episodically, or with tidal regimes) and space (vertically with depth, and horizontally resulting from flooding patterns). Although upland soils are less likely to experience inundation, they also exhibit temporal and spatial variation in redox potential. In upland soils, redox fluctuations are driven by the interplay between biology and physics, with aerobic respiration by plants and microbes constrained by O_2 diffusion from the surface (Silver *et al.* 1999). Biological O_2 demand can exceed the rate of resupply via diffusion, particularly in finely textured or organic soils, resulting in anaerobic hotspots or events. The prevalence of anaerobic processes, such as denitrification and methanogenesis, in upland ecosystems is evidence of low redox microsites or events (Conrad 1996). Unlike that in wetlands and water columns, redox in upland soils often varies considerably over much smaller spatial scales (Figure 4) and fluctuates on short temporal scales of hours to days (Silver *et al.* 1999; Schuur and Matson 2001). These fluctuations can facilitate rapid redox cycling and consequent coupling of biogeochemical cycles. For example, in highly weathered humid tropical forest soils, episodic rainfall is associated with high biological activity that depletes O_2, reduces Fe, and releases CO_2 and P (Chacon *et al.* 2006; Liptzin and Silver 2009). In upland tropical forest soils, Fe reduction has been shown to account for over 50% of total soil respiration (Dubinsky *et al.* 2010). Iron can later be reoxidized under anaerobic conditions when coupled to nitrite (NO_2^-) or NO_3^- reduction (Weber *et al.* 2006b) or under aerobic conditions, regenerating oxidized Fe to sustain Fe reduction (see Panel 1).

So far we have emphasized dissimilatory transformations linked to heterotrophic metabolism in which organic C is the electron donor or energy source (Figure 1; see also Figure 3c). Dissimilatory transformations

Panel 1: The ferrous wheel

Iron is the most abundant redox-active metal on Earth, and Fe redox dynamics have the potential to catalyze many biogeochemical reactions in marine, freshwater, and soil environments. Iron often rapidly cycles between redox states (Fe[II] reduced; Fe[III] oxidized) because microorganisms use it as an electron acceptor or donor, and it also reacts abiotically with minerals and organic matter. Iron reduction can be facilitated by humic substances that act as "electron shuttles", kinetic intermediates that facilitate extracellular electron transport, which are particularly important in the reduction of insoluble iron oxides (Lovley *et al.* 1996). Reduced Fe can then be reoxidized in contact with O_2 or anaerobically via a range of coupled biogeochemical processes (Weber *et al.* 2006a). Thus, Fe cycling serves as a perpetual motion engine, a ferrous wheel (Figure 5).

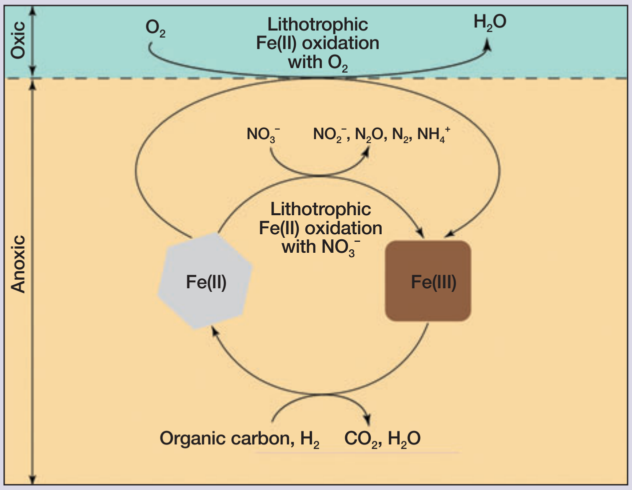

***Figure 5.** Depiction of the ferrous wheel.*

One hypothesized reaction involving Fe has fueled considerable recent controversy: the abiotic reduction of NO_3^- to dissolved organic nitrogen in soils. The debate arose from observed total N retention (Nadelhoffer *et al.* 1999) coupled with the rapid disappearance of NO_3^- (sensu Berntson and Aber 2000; Perakis and Hedin 2001; Dail *et al.* 2001; Huygens *et al.* 2008) in soils exposed to simulated N deposition. Davidson *et al.* (2003) hypothesized that Fe(II) oxidation catalyzes NO_3^- reduction in anaerobic microsites in soils, and that the resulting NO_2^- reacts abiotically with dissolved organic matter to form dissolved organic N. Colman *et al.* (2007) tested the hypothesis of Davidson *et al.* (2003) using 45 soil samples from a wide range of ecosystems. They found that apparent abiotic NO_3^- immobilization could be explained by an analytical artifact of Fe interference with the measurement of NO_3^- concentrations. Despite a fascinating debate that ensued in the literature (Colman *et al.* 2008; Davidson *et al.* 2008), including additional indirect evidence purporting abiotic NO_3^- removal, there remains little direct evidence of substantial abiotic NO_3^- conversion to dissolved organic N in soils (Schmidt and Matzner 2009).

resulting from chemolithotrophic (rock eating) metabolism also affect biogeochemical cycles. The remainder of this paper describes ecologically important examples in which microbial metabolism couples elemental cycles directly through chemolithotrophic metabolism and indirectly through the production of reactive substances of both oxidized and reduced states.

■ Chemolithotrophy: capturing the energy in reduced inorganic substances

Chemolithotrophic processes are of particular interest because they couple elemental cycles very differently from the traditionally considered C couplings dictated by heterotrophic reactions (Schlesinger *et al.* 2011). Chemolithotrophs oxidize inorganic substances to obtain and store energy (Figure 1); in doing so, often they function to re-oxidize the reduced substances generated by anaerobic respiration (they may also utilize reduced substances of geological origin, such as those in hot springs). Some of the best characterized chemolithotrophic pathways are aerobic reactions in which microbes use O_2 to oxidize reduced forms of N (ammonium, NH_4^+, and NO_2^-), Fe (Fe[II]), and S (H_2S and elemental sulfur, S^0), yielding NO_3^-, Fe(III), and SO_4^{2-}, respectively. These chemolithotrophic microbes typically grow at redox interfaces where anoxic water or soil aggregates containing reduced substances contact O_2 via water flow or molecular diffusion (eg groundwater springs, deep-sea hydrothermal vents, anoxic–oxic interfaces in water columns or soils, and sediment surfaces). Chemolithotrophy is not supported only by O_2, but can also be supported by alternative oxidizing agents (ie several forms of chemolithotrophic metabolism are anaerobic).

Sulfur-oxidizing bacteria can use NO_3^- rather than O_2 as an electron acceptor, effectively decoupling N cycling processes, such as denitrification, from organic C respiration and linking them instead to S cycling (Burgin and Hamilton 2007). A spectacular example is the discovery in the 1990s of vast mats of benthic, S-oxidizing bacteria in continental shelf sediments (Fossing *et al.* 1995). A distinguishing feature of these bacteria is their gliding motility, which enables them to capture and store NO_3^- in overlying water and subsequently use the stored NO_3^- to oxidize H_2S obtained at greater depth in the sediments, potentially subsisting for weeks on stored NO_3^- (Schulz and Jørgensen 2001). In this ecosystem, S is arguably more important than organic C as the proximate factor influencing N dynamics, although the reduced S commonly originates from organic matter degradation. Although this coupling of the S and N cycles has been recognized in marine systems (Brettar and Rheinheimer 1991; Brunet and Garcia-Gil 1996), S concentrations in freshwater systems are thought to be too low to drive a substantial fraction of N cycling. There is, however, increasing evidence of freshwater S-driven NO_3^- reduction; for example, we found that in Michigan wetlands and lakes (Payne *et al.* 2009), 10–40% of NO_3^- removal can be attributed to S oxidation (Burgin and Hamilton 2008). These recent studies show that S and N cycling can be coupled in freshwater and marine ecosystems, but more research is needed to verify its importance relative to other processes and controls (eg C). Furthermore, this link has scarcely been explored in terrestrial ecosystems.

Iron-oxidizing bacteria can also use NO_3^- as an electron acceptor. For example, dissimilatory NO_3^- reduction to NH_4^+ (DNRA) can be coupled to anaerobic Fe oxidation to influence ecosystem N cycling (Weber *et al.* 2006b). More importantly, this link between N and Fe cycling allows regeneration of oxidized Fe to perpetuate Fe cycling under prolonged anoxic conditions. This concept is elaborated further in Panel 1.

The discovery of anaerobic ammonium (NH_4^+) oxidation (anammox) coupled to NO_2^- reduction in the environment has transformed our concept of N cycling because, unlike nitrification–denitrification, anammox provides a pathway for reactive forms of N to be converted to the unreactive form of N_2 without the potential for groundwater pollution through NO_3^- leaching or the release of N_2O and nitric oxide. Ammonium oxidation was once thought to be a strictly aerobic chemolithotrophic process (known as nitrification). In 1977, anammox was calculated to be energetically favorable (Broda 1977; Kuenen 2008), but only in the 1990s did new tools, including molecular markers and stable isotope measurements, help confirm that it does occur in nature (Kuenen 2008). Anammox might account for 50–70% of the marine N_2 production previously attributed entirely to denitrification (Thamdrup and Dalsgaard 2002; Dalsgaard *et al.* 2005), but it is not always an important N_2 production pathway relative to denitrification (Ward *et al.* 2009). The past decade of anammox research has focused on quantifying anammox rates in a range of ecosystems to determine where the process occurs naturally. Now that anammox has been demonstrated in a wide range of aquatic ecosystems (Jetten *et al.* 2009), researchers can move toward understanding what factors control the relative importance of anammox and denitrification as N_2 production pathways in these ecosystems. However, in terrestrial ecosystems, conclusive detection of anammox activity has yet to be documented, despite molecular evidence for the presence of anammox bacteria in diverse soils (Humbert *et al.* 2010).

Theoretically, anaerobic NH_4^+ oxidation can also be coupled to manganese (Mn) and Fe reduction to produce NO_2^- or N_2 directly, but these pathways have not been quantified in nature (Luther *et al.* 1997; Hulth *et al.* 1999). The Mn-associated pathway is more energetically favorable than the Fe-associated pathway (Feammox), but the greater abundance of Fe in many soils suggests that Feammox has the potential to drive substantial terrestrial N cycling, particularly where soils (and aquatic sediments) experience periodic anoxia. A major challenge in detecting Feammox is distinguishing it from nitrification (in soils where O_2 can be relatively abundant) and anammox (in anoxic soils and sediments). Nitrite production has been observed under Fe-reducing conditions in wetland sediments and wastewater reactors (Clement *et al.* 2005; Sawayama 2006; Chen *et al.* 2008; Shrestha *et al.* 2009), suggesting that Feammox does occur. However, no evidence of N_2 production via Feammox has been reported. This is a very new topic in coupled biogeochemical cycles, so continued efforts to quantify Feammox may yield exciting new insights into aquatic and terrestrial N cycling.

Anaerobic oxidation of methane (CH_4) coupled to SO_4^{2-} reduction is poorly understood but currently thought to be a predominantly marine process and one that plays a critical role in global C cycling, potentially consuming 7–25% of globally produced CH_4 (Knittel and Boetius 2009). Methanotrophs are chemolithotrophic microbes that oxidize CH_4 to obtain energy, using either O_2 or SO_4^{2-} as electron acceptors. Anaerobic methane oxidation was originally assumed to be important mostly in the oceanic CH_4–SO_4^{2-} transition zone, where methane from lower anoxic zones encounters SO_4^{2-} from upper oxic zones (Reeburgh 2007). However, recent studies have revealed anaerobic CH_4 oxidation coupled to NO_2^- reduction (Raghoebarsing *et al.* 2006) and Mn and Fe reduction (Beal *et al.* 2009). These findings imply that anaerobic CH_4 oxidation could be important outside of the oceanic CH_4–SO_4^{2-} transition zone and could couple CH_4 oxidation with many different biogeochemical cycles. In addition, this suggests that the importance of anaerobic CH_4 oxidation should be explored in terrestrial ecosystems as well.

Chemolithotrophic microbes can exist in mutualistic relationships with more complex organisms, where the energy harnessed in chemical oxidation reactions benefits both partners. For example, in submarine hydrothermal vents, efflux of H_2S- and CH_4-rich water allows S-oxidizers and methanotrophs to flourish. These microbes occur both as free-living organisms and in mutualisms, where they reduce inorganic C to organic forms that support heterotrophic macrofauna like tube worms, mussels, and clams (Martin *et al.* 2008). Hydrothermal vents support entire food webs, which thrive in the absence of sunlight as an energy source. Work on vent field symbioses has led to an increased recognition of the role of chemolithotrophic symbioses in more widespread environments (Dubilier *et al.* 2008) and has also provided clues to the metabolic processes of Earth's earliest life forms that preceded the development of an oxidized atmosphere (Martin *et al.* 2008).

Indirect coupling of elemental cycles

In considering direct coupling of biogeochemical cycles, we focused on examples of transformations catalyzed by one specific microbe through its chemolithotrophic

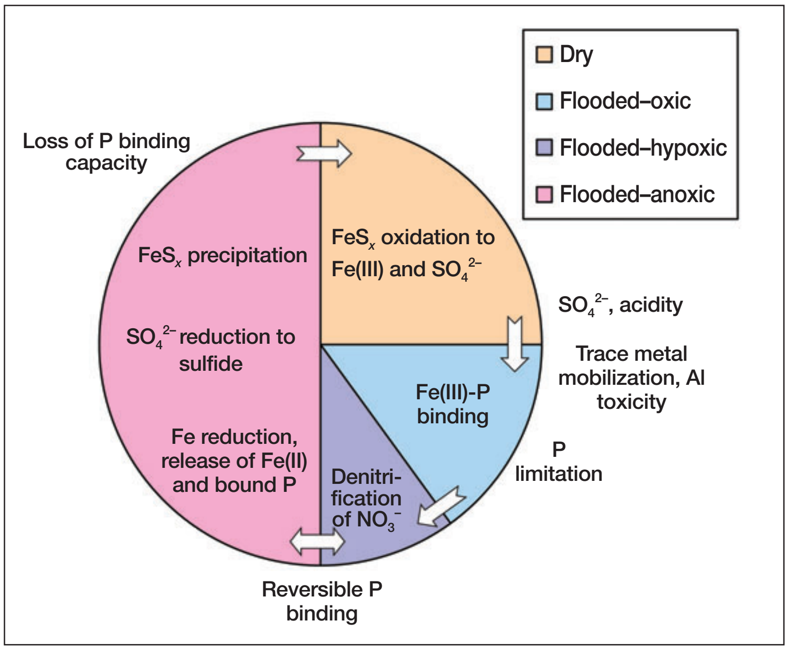

Figure 6. *Hypothetical cycle of microbial Fe and S transformations and their implications for P availability in an aquatic environment subject to fluctuations in water levels and periodic drying of sediments. Under dry or oxic flooded conditions, Fe(II) is oxidized to yield iron oxyhydroxides (FeOOH, a form of Fe[III]), which strongly bind P. During inundation, oxygen (O_2) and then nitrate (NO_3^-) eventually become depleted (via denitrification with organic C, or another electron donor), and Fe(III) reduction by anaerobic respiration produces Fe(II), releasing bound P to the sediment pore water and overlying water column. After oxidized Fe is depleted, sulfate (SO_4^{2-}) reduction commences (driven by organic C, or another electron donor), and the resultant H_2S precipitates with Fe(II) in the sediments. The Fe-S minerals (FeS and FeS_2: here called FeS_x) tend to be stable until the sediments dry out, upon which oxidation can occur, mediated by chemolithotrophs when nitrate is available. Resultant production of SO_4^{2-} and increased acidity can mobilize trace metals and, if pH decreases enough, generate toxic forms of aluminum (Al).*

metabolism. However, microbes live in close proximity, often under variable conditions, and the metabolic capability of a particular microbe can depend on other organisms with different metabolic capabilities. Microbial ecologists use the term "syntrophy" to describe interdependent microbial metabolism (Madigan and Martinko 2006; Knittel and Boetius 2009). In syntrophic interactions, one species produces a product needed for the metabolic processes of another species. In some cases the consumption of that product may favor its producer; therefore, through linked metabolic transformations, both can coexist and in effect cooperate to mutual benefit.

The fermentation reactions that occur during anaerobic decomposition of organic matter provide the most well-known examples of syntrophic metabolism by microbial consortia. Complex organic compounds are subject to sequential hydrolysis and fermentation by distinct groups and, ultimately, to anaerobic respiration or methanogenesis, with the entire process dependent on linked production and consumption of hydrogen (H_2) by different microbes (Madigan and Martinko 2006). Syntrophic interactions, however, can also couple other elemental cycles. For example, Boetius *et al.* (2000) identified a consortium of methanogens in marine sediments that occurred in microscopic clusters surrounded by sulfate reducers, effectively interacting to couple the C and S cycles through the anaerobic oxidation of methane. Similarly, Raghoebarsing *et al.* (2006) found a consortium of methanogens and denitrifiers that could also oxidize CH_4 anaerobically, thereby coupling the C and N cycles. The potential complexity of interspecies metabolic coupling is exemplified by a report that certain anaerobic methane oxidizers can fix N and share it with their SO_4^{2-}-reducing counterparts, thus coupling the S cycle with cycles of C and N (Dekas *et al.* 2009). These examples emphasize that microbial consortia are capable of coupling elemental cycles. Because many of the findings are so new, however, we know little about the ecosystem-level importance of the processes.

In syntrophy, microbes couple elemental cycles via their close spatial proximity (micrometers) and in rapid concurrent biochemical reactions. Yet the effects of this coupling extend broadly over larger spatial and temporal scales. This occurs when the dissimilatory transformations mediated by microbes produce reactive substances that in turn trigger a complex cascade of biogeochemical reactions, thereby indirectly affecting other elemental cycles. A hypothetical example of a wetland that dries periodically (Figure 6) illustrates the complexity of indirect biogeochemical coupling over larger space and time scales.

Wetland sediments tend to be anoxic because of their high biological O_2 demand and limited diffusive O_2 replacement, and over time they may accumulate reduced Fe compounds. When such wetland soils are drained and oxidized, Fe oxidizers produce poorly crystalline Fe oxyhydroxides (denoted as FeOOH). These minerals can accumulate, binding trace metals and P, and can be the main sink for P in soils and aquatic sediments, thereby regulating productivity in many lakes and wetlands (Smolders *et al.* 2006) and in highly weathered, Fe-rich soils (Sánchez 1976). Yet once these sediments or soils become anoxic, Fe reducers can carry out anaerobic respiration until the supply of Fe is depleted. After that point, SO_4^{2-} reduction may become the predominant form of anaerobic respiration, generating H_2S. Reduced forms of Fe react with H_2S to yield relatively insoluble iron sulfide minerals (FeS_x; Rickard and Morse 2005); in aquatic sediments, this FeS_x formation is often a semi-permanent sink for both elements and can further release bound P. However, the eventual oxidation of these FeS_x minerals upon desiccation or drainage of sediments can produce a pulse of sulfuric acid that lowers the pH of the sediments enough to enhance mineral weathering and mobilize trace metals, including potentially toxic forms of aluminum (Al). Sulfur-linked production of acidity and mobilization of toxic metals such as Al have been documented in tropical catchments of northern Australia, where this has been associated with freshwater fish kills (Hart and McKelvie 1986), as well as negative impacts on coastal marine biota where rivers drain into the Great Barrier Reef ecosystem (Powell and Martens 2004).

■ Conclusions and research needs

Microbes couple elemental cycles, both directly and indirectly, through their metabolic functions, thereby creating the foundation of what we know as biogeochemical cycles. Dissimilatory transformations of elements by microbes couple biogeochemical cycling beyond the tightly linked assimilation and remineralization of C, N, and P that are typically considered as coupled biogeochemical cycles. Through microbial metabolism based on redox reactions and syntrophic interactions, the Mn, Fe, and S cycles can influence N, C, and P cycling and indirectly regulate nutrient availability for net primary production, greenhouse-gas emissions, levels of contaminants, and natural toxins.

Over the past two decades, technological and methodological advances have increasingly allowed researchers to detect novel chemolithotrophic processes in nature, such as Feammox, anammox, S-driven denitrification, and anaerobic CH_4 oxidation; these findings have changed our understanding of how biogeochemical cycles are linked. Most of these pathways have not yet been detected in terrestrial ecosystems, despite thermodynamically favorable conditions for the reactions to occur. Future work on these processes should focus on quantifying rates and determining controls to understand the importance of these pathways in the biogeochemical cycles of terrestrial and aquatic ecosystems. Additionally, scientists should consider how these linkages will be affected by the escalating, simultaneous anthropogenic perturbation of multiple biogeochemical cycles, including C via fossil-fuel combustion, S via coal burning and acid deposition, and N via fertilizer production and agricultural practices. Furthermore, some of these novel microbial processes may prove to be tremendously valuable in bioreactor systems engineered to produce biofuels or to process wastes, as exemplified by the high level of interest in industrial-scale anammox bioreactors (Kuenen 2008). As biogeochemical and molecular biology techniques improve, more links among the major elemental cycles will likely be discovered, which will improve our appreciation of the diversity of microbes and metabolic pathways that mediate these links.

■ References

Beal EJ, House CH, and Orphan VJ. 2009. Manganese- and iron-dependent marine methane oxidation. *Science* **325**: 184–87.

Berntson GM and Aber JD. 2000. Fast nitrate immobilization in N saturated temperate forest soils. *Soil Biol Biochem* **32**: 151–56.

Boetius A, Ravenschlag K, Schubert CJ, *et al.* 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**: 623–26.

Brettar I and Rheinheimer G. 1991. Denitrification in the Central Baltic: evidence for H_2S oxidation as motor of denitrification at the oxic-anoxic interface. *Mar Ecol-Prog Ser* **77**: 157–69.

Broda E. 1977. Two kinds of lithotrophs missing in nature. *Z Allg Mikrobiol* **17**: 491–93.

Brunet RC and Garcia-Gil LJ. 1996. Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiol Ecol* **21**: 131–38.

Burgin AJ and Hamilton SK. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front Ecol Environ* **5**: 89–96.

Burgin AJ and Hamilton SK. 2008. NO_3^--driven SO_4^{2-} production in freshwater ecosystems: implications for N and S cycling. *Ecosystems* **11**: 908–22.

Chacon N, Silver WL, Dubinsky EA, and Cusack DF. 2006. Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. *Biogeochemistry* **78**: 67–84.

Chen XP, Zhu YG, Hong MN, *et al.* 2008. Effects of different forms of nitrogen fertilizers on arsenic uptake by rice plants. *Environ Toxicol Chem* **27**: 881–87.

Clement JC, Shrestha J, Ehrenfeld JG, and Jaffe PR. 2005. Ammonium oxidation coupled to dissimilatory reduction of iron under anaerobic conditions in wetland soils. *Soil Biol Biochem* **37**: 2323–28.

Colman BP, Fierer N, and Schimel JP. 2007. Abiotic nitrate incorporation in soil: is it real? *Biogeochemistry* **84**: 161–69.

Colman BP, Fierer N, and Schimel JP. 2008. Abiotic nitrate incorporation, anaerobic microsites, and the ferrous wheel. *Biogeochemistry* **91**: 223–27.

Conrad R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiol Rev* **60**: 609–40.

Dail DB, Davidson EA, and Chorover J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry* **54**: 131–46.

Dalsgaard T, Thamdrup B, and Canfield DE. 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. *Res Microbiol* **156**: 457–64.

Davidson EA, Chorover J, and Dail DB. 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. *Glob Change Biol* **9**: 228–36.

Davidson EA, Dail DB, and Chorover J. 2008. Iron interference in the quantification of nitrate in soil extracts and its effects on hypothesized abiotic immobilization of nitrate. *Biogeochemistry* **90**: 65–73.

Dekas AE, Poretsky RS, and Orphan VJ. 2009. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science* **326**: 422–26.

Dubilier N, Bergin C, and Lott C. 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nat Rev Microbiol* **6**: 725–40.

Dubinsky EA, Silver RS, and Firestone MK. 2010. Tropical forest soil microbial communities couple iron and carbon biogeochemistry. *Ecology*, doi:10.1890/09-1365.1.

Falkowski PG, Fenchel T, and Delong EF. 2008. The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**: 1034–39.

Fenchel T, King GM, and Blackburn TH. 1998. Bacterial biogeochemistry: the ecophysiology of mineral cycling. San Diego, CA: Academic.

Fossing H, Gallardo VA, Jørgensen BB, *et al.* 1995. Concentration and transport of nitrate by the mat-forming sulfur bacterium *Thioploca*. *Nature* **374**: 713–15.

Hart BT and McKelvie ID. 1986. Chemical limnology in Australia. In: De Decker P and Williams WD (Eds). Limnology in

Australia. Dordrecht, Netherlands, and Melbourne, Australia: Dr W Junk Publishers and CSIRO.

Hulth S, Aller RC, and Gilbert F. 1999. Coupled anoxic nitrification/manganese reduction in marine sediments. *Geochim Cosmochim Ac* **63**: 49–66.

Humbert S, Tarnawski S, Fromin N, *et al.* 2010. Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. *ISME J* **4**: 450–54.

Huygens D, Boeckx P, Templer P, *et al.* 2008. Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. *Nat Geosci* **1**: 543–48.

Jetten MSM, van Niftrik L, Strous M, *et al.* 2009. Biochemistry and molecular biology of anammox bacteria. *Crit Rev Biochem Mol* **44**: 65–84.

Knittel K and Boetius A. 2009. Anaerobic oxidation of methane: progress with an unknown process. *Annu Rev Microbiol* **63**: 311–34.

Kuenen JG. 2008. Anammox bacteria: from discovery to application. *Nat Rev Microbiol* **6**: 320–26.

Liptzin D and Silver WL. 2009. Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. *Soil Biol Biochem* **41**: 1691–702.

Lovley DR, Coates JD, Blunt-Harris EL, *et al.* 1996. Humic substances as electron acceptors for microbial respiration. *Nature* **382**: 445–48.

Luther GW, Sundby B, Lewis BL, *et al.* 1997. Interactions of manganese with the nitrogen cycle: alternative pathways to dinitrogen. *Geochim Cosmochim Ac* **61**: 4043–52.

Madigan MT and Martinko JM. 2006. Brock biology of microorganisms, 11th edn. Upper Saddle River, NJ: Prentice Hall.

Madsen EL. 2008. Environmental microbiology: from genomes to biogeochemistry. Oxford, UK: Wiley-Blackwell.

Martin W, Baross J, Kelley D, and Russell MJ. 2008. Hydrothermal vents and the origin of life. *Nat Rev Microbiol* **6**: 805–14.

Megonigal JP, Hines ME, and Visscher PT. 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In: Schlesinger WH (Ed). Biogeochemistry. Oxford, UK: Elsevier-Pergamon.

Nadelhoffer KJ, Downs MR, and Fry B. 1999. Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. *Ecol Appl* **9**: 72–86.

Payne EK, Burgin AJ, and Hamilton SK. 2009. Sediment nitrate manipulation using porewater equilibrators reveals potential for N and S coupling in freshwaters. *Aquat Microb Ecol* **54**: 233–41.

Perakis SS and Hedin LO. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. *Ecology* **82**: 2245–60.

Powell B and Martens M. 2004. A review of acid sulfate soil impacts, actions and policies that impact on water quality in Great Barrier Reef catchments, including a case study on remediation at East Trinity. *Mar Pollut Bull* **51**: 149–64.

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, *et al.* 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* **440**: 918–21.

Reeburgh WS. 2007. Oceanic methane biogeochemistry. *Chem Rev* **107**: 486–513.

Rickard D and Morse JW. 2005. Acid volatile sulfide (AVS). *Mar Chem* **97**: 141–97.

Sánchez PA. 1976. Properties and management of soils in the tropics. New York, NY: John Wiley and Sons.

Sawayama S. 2006. Possibility of anoxic ferric ammonium oxidation. *J Biosci Bioeng* **101**: 70–72.

Schlesinger WH, Cole JJ, Finzi AC, and Holland EA. 2011. Introduction to coupled biogeochemical cycles. *Front Ecol Environ* **9**: 5–8.

Schmidt BHM and Matzner E. 2009. Abiotic reaction of nitrite with dissolved organic carbon? Testing the ferrous wheel hypothesis. *Biogeochemistry* **93**: 291–96.

Schulz HN and Jørgensen BB. 2001. Big bacteria. *Annu Rev Microbiol* **55**: 105–37.

Schuur EAG and Matson PA. 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. *Oecologia* **128**: 431–42.

Seitzinger S, Harrison JA, Bohlke JK, *et al.* 2006. Denitrification across landscapes and waterscapes: a synthesis. *Ecol Appl* **16**: 2064–90.

Sexstone AJ, Revsbech NP, Parkin TB, and Tiedje JM. 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Sci Soc Am J* **49**: 645–51.

Shrestha J, Rich JJ, Ehrenfeld JG, and Jaffe PR. 2009. Oxidation of ammonium to nitrite under iron-reducing conditions in wetland soils: laboratory, field demonstrations, and push–pull rate determination. *Soil Sci* **174**: 156–64.

Silver WL, Lugo AE, and Keller M. 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry* **44**: 301–28.

Smolders AJP, Lamers LPM, Lucassen ECHET, *et al.* 2006. Internal eutrophication: how it works and what to do about it – a review. *Chem Ecol* **22**: 93–111.

Sterner RW and Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton, NJ: Princeton University Press.

Thamdrup B and Dalsgaard T. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Appl Environ Microb* **68**: 1312–18.

Ward BB, Devol AH, Rich JJ, *et al.* 2009. Denitrification as the dominant nitrogen loss process in the Arabian Sea. *Nature* **461**: 78–81.

Wang FY and Chapman PM. 1999. Biological implications of sulfide in sediment – a review focusing on sediment toxicity. *Environ Toxicol Chem* **18**: 2526–32.

Weber KA, Achenbach LA, and Coates JD. 2006a. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat Rev Microbiol* **4**: 752–64.

Weber KA, Urrutia MM, Churchill PF, *et al.* 2006b. Anaerobic redox cycling of iron by freshwater sediment microorganisms. *Environ Microbiol* **8**: 100–13.

White D. 2000. The physiology and biochemistry of prokaryotes. New York, NY: Oxford Press.