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Subsurface microbial activity: Beyond the thermodynamic ladder

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Abstract: An integration of the study of groundwater chemistry, numerical modeling, long-term bioreactor experiments, and in-situ sampling of activity growing microbes yields a portrait of subsurface anaerobe activity that differs sharply from conventional thinking. The chemical record of microbial activity on a groundwater's redox chemistry in a well-studied aquifer is surprisingly incomplete, casting doubt on current methods of classifying subsurface microbial activity in aquifers. We find no evidence to support a concept by which subsurface microbial activity is arranged according to a thermodynamic ladder, nor do we find backing for the theory of competitive exclusion. Instead, we find strong evidence that iron reducing and sulfate reducing bacteria, previously considered locked in competition, are in fact forming mutualistic relationships in which they tightly cooperate. Where a group of microbes such as the methanogens is excluded from an ecosystem, the explanation is more likely ecologic than thermodynamic.

Key Words : Anaerobic microbes, microbial activity, microbial ecology, aquifer redox chemistry

1. Introduction

Subsurface microorganisms draw the energy they need to live and reproduce from the groundwater in which they live, and in the process control many aspects of the water's redox chemistry. To capture energy from its environment, a microbe catalyzes the transfer of electrons from an initially reduced species to an initially oxidized species, releasing the electron donor in oxidized from, and the acceptor in reduced form. In this way, the microbe's activity leaves an imprint on the chemistry of its environment.

In anoxic aquifers, the subject of this paper, groundwater can in many cases be divided into zones on the basis of chemical composition. By conventional thinking, the segregation has been interpreted to reflect variation or zoning in the activity of anaerobic microbes within the aquifer (Jones et al., 1989). On a regional scale, for example, the Cretaceous Middendorf aquifer in South Carolina can be divided into three parts, one rich in dissolved iron, a second where groundwater holds little iron, and a third where methane accumulates in the flowing water.

The three chemical zones within the Middendorf are believed to represent the habitats, respectively, of iron reducing bacteria, sulfate reducing bacteria, and methanogens (Chapelle and Lovley, 1992). On a local scale, as a second example, arsenic concentration in the Cretaceous Mahomet aquifer in East Central Illinois was found to be controlled by the activity of sulfate reducing bacteria: where sulfate reducers are active, the arsenic concentration is invariably small (Kirk et al., 2004).

Chemical zoning in an aquifer, in other words, signifies the differentiation of microbial activity into discrete zones. The zones are commonly taken to be exclusionary, so that each is dominated by a single functional group of microbes, according to an energetic hierarchy known as the thermodynamic ladder. In this paper, we use the results of more than a decade of studies within our research group — ranging from field observation to long-term laboratory experiments to theory and quantitative modeling — to reflect on the role of thermodynamics in shaping microbial metabolic activity in subsurface environments.

2. Concept of a thermodynamic ladder

The idea that an energetic hierarchy among functional groups of microbes might cause aquifers to become zoned microbiologically arose from observations in the Cretaceous Middendorf aquifer in South Carolina, USA (Chapelle and Lovley, 1992). An upgradient zone there holding groundwater rich in iron is replaced along the direction of flow by a zone with low iron levels. The latter zone, in turn, gives way downgradient to a zone where dissolved methane builds up.

The three zones are believed to represent the dominance along flow of first bacterial iron reduction, then sulfate re-

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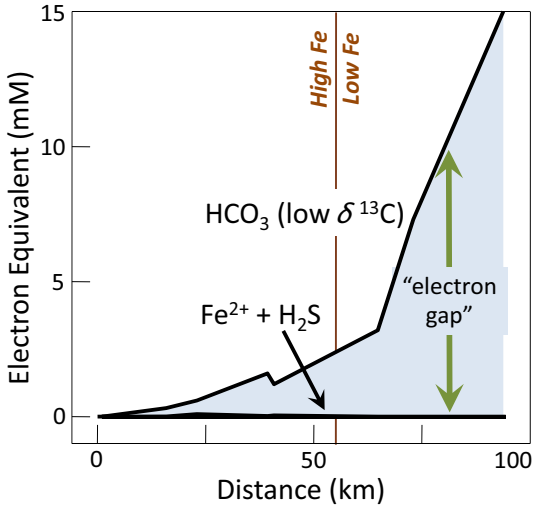


Fig. 1 The "electron gap" is the discrepancy between the record of the number of electrons donated, as reflected by the accumulation of biogenic inorganic carbon in groundwater along the Middendorf aquifer, and evidence of the number of electrons acccepted, as accounted for by ferrous iron and sulfide in solution (Park et al., 2006). Vertical line separates zones of iron-rich and iron-poor groundwater.

duction, and finally methanogenesis. Where ferric minerals exist, at neutral to acidic pH, an iron reducer's catabolic reaction is favored energetically relative to those of sulfate reducers and methanogens. Iron reduction, then, might be able to drive down electron donor concentrations (i.e., the levels of H_2 and small-molecule organic acids) to a point low enough that insufficient energy remains in the aquifer for sulfate reducers or methanogens to live. Similarly, sulfate reducers might employ the same strategy, known as competitive exclusion, to exclude methanogens.

When Park et al. (2006) looked at the redox chemistry of groundwater from the Middendorf aquifer, however, they found little evidence to support this concept. Along a flow path spanning the iron-rich and iron-poor zones, they found enough energy everywhere in the aquifer to drive forward the catabolic reactions of iron-reducers, sulfate reducers, and aceticlastic methanogens. This result calls into question the idea that thermodynamic exclusion might play a role in the aquifer's zoning.

Park et al. furthermore attempted to balance the record of electron donation in the aquifer with evidence of electron acceptance. As natural organic matter in the sedimentary section ferments, it liberates reduced species such as acetate that can donate electrons for microbial respiration. In this way, four electrons are made available for each carbon that oxidizes to carbonate. The electrons may then be accepted by sulfate reduction, which adds one sulfide to solution per eight electrons accepted, and by iron reduction, which yields an Fe^{2+} for each electron. Biogenic dissolved carbonate (carbonate with isotopically light carbon) accumulating in the flowing water, then, records the electrons donated up to that point in the flow, and the buildup of sulfide and ferrous iron reflects how the electrons were accepted.

Plotted in terms of electron equivalents (Fig. 1), the chemistry of Middendorf groundwater shows a pronounced "electron gap" across which electrons donated in the aquifer cannot be accounted for by evidence of acceptance: there is not nearly enough iron or sulfide in the water to balance the biogenic carbonate. We see that groundwater chemistry reveals an incomplete picture of the microbial processes occurring within an aquifer; it cannot be relied upon to characterize the microbial activity there, nor to argue that one functional group of microbes excludes, or even dominates other groups.

3. Thermodynamic control on microbial kinetics

Any evaluation of the role of energetics in the origin of aquifer zoning requires a thermodynamically consistent description of microbial kinetics. Rate laws in common use are derived considering only the forward rate of reaction and therefore cannot account for a thermodynamic drive or an equilibrium end point. Such laws may be applied to experiments where energy is available in abundance and the forward reaction rate overwhelms the reverse, but are not suitable for analysis of natural environments in which little chemical energy may be available.

Jin and Bethke (2007) derived a kinetic description of microbial metabolism that rigorously honors the requirements of thermodynamics. In their analysis, electron transfer through a microbe's transport chain is enzymatically coupled to the phosphorylation of ADP, adenosine diphosphate, to create ATP, adenosine triphosphate. The energy ΔG_U usable by the cell to drive forward its catabolic reaction is given

$$\Delta G_U = \Delta G_A - m\Delta G_P \qquad (1)$$

Here, ΔG_A is energy available in the environment to drive forward the cell's electron transfer reaction, or the negative of the reaction's free energy change at ambient conditions, such as $-\Delta G_r$ of sulfate reduction coupled to acetate oxidation. Variable m is the ATP number, the number of ATPs produced per turnover of the cell's catabolic reaction; and ΔG_P is the free energy change of ADP phosphorylation, about 50 kJ mol^{-1}.

A thermodynamic potential factor F_T defines the control of energetics on reaction rate. The factor, ranging from zero to one, is given as from the environment's usable energy ΔG_U as

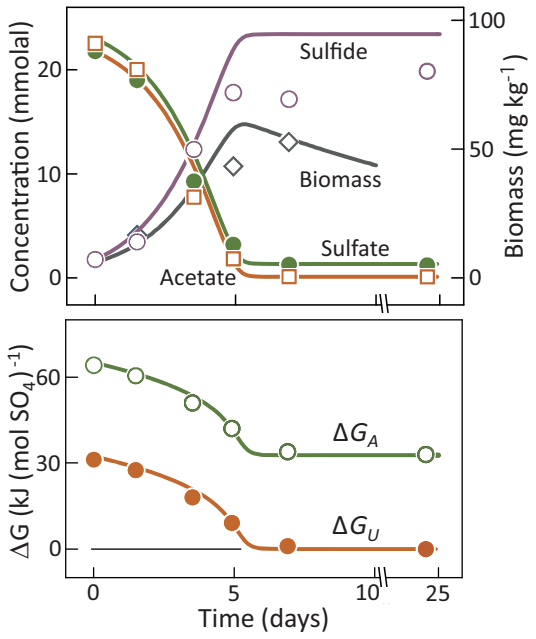


Fig. 2 Result of a batch reactor experiment tracing bacterial sulfate reduction in pure culture. The *Desulfobacter hydrogenophilus* bacteria transform sulfate and acetate to sulfide and carbonate, using the energy liberated to create biomass (Jin and Bethke, 2009). Symbols show measured concentrations (top plot) and the available ΔG_A and usable ΔG_U free energies in the experiment calculated from the measured concentrations (bottom plot), as functions of time elapsed. Lines show the result of a reaction model of the experiment calculated using The Geochemist's Workbench® software (Bethke, 2008). Reaction in the experiment ceases when the usable energy, and hence the thermodynamic drive vanishes, even though finite amounts of sulfate and acetate remain unreacted and the available energy is not exhausted.

$$F_T = 1 - \exp\left(-\frac{\Delta G_U}{\chi RT}\right) \tag{2}$$

Here χ is the average stoichiometric number, the number of times the rate limiting step occurs per reaction turnover; R is the gas constant; and T is absolute temperature.

The rate at which a microbe catalyzes its catabolic reaction varies with F_T according to

$$r = k_+ [X] F_D F_A F_T \tag{3}$$

Here, r is the net reaction rate, the difference between the forward and reverse rates; k_+ is the rate constant, which is commonly known from experiments; $[X]$ is biomass concentration; and F_D and F_A are kinetic terms related to electron acceptance and donation, the forms of which vary depending on the microbe in question, and its environment. The forms of F_D and F_T depend on the natures of the electron donating and accepting reactions for the microbe in question, and on which species in those reactions have concentrations in the microbe's environment that might vary over time (see, for example, Jin and Bethke, 2002; 2003) .

A notable point is that in an oligotrophic environment where available energy ΔG_A is little more than the cell's internal energy $m \cdot \Delta G_P$, the usable energy ΔG_U approaches zero, driving F_T and the reaction rate r to approach zero, as well. The point at which available and internal energy for a microbial catabolism are in balance, in other words, represents the catabolism's thermodynamic limit.

Jin and Bethke (2009) set out to test the prediction of a thermodynamic limit for the case of bacterial sulfate reduction. In a series of pure culture experiments, they found acetotrophic sulfate reduction proceeded until available energy ΔG_A dropped to $\sim$33 – 43 kJ (mol SO_4)$^{-1}$ and then stopped (Fig. 2), even though a finite amount of both acetate and sulfate remained unreacted. They further calculated available energy in a variety of natural environments in which previous studies had shown sulfate reduction to have ceased. In these cases, the available energy remaining was $\sim$40 – 56 kJ (mol SO_4)$^{-1}$. Energies in these ranges map by Eq. (1) to ATP numbers m of about $2/3$ – 1, in agreement with current understanding of the cellular physiology of sulfate reducers (Jin and Bethke, 2009).

4. Reactive transport modeling

Bethke et al. (2008) conducted a series of numerical experiments* to see how microbial communities might develop in the subsurface. In their reactive transport models, water containing a small amount of dissolved sulfate recharges a 200-km-long aquifer. Acetate is added along the aquifer at a slow, continuous rate, to represent the effect of natural organic matter fermenting within surrounding fine-grained sediments. In some experiments, the aquifer contains small amounts of goethite (ferric oxyhydroxide, FeOOH). The aquifer in the simulations is seeded with a few aceticlastic methanogens and a small number of sulfate reducing and iron reducing bacteria. The initially small populations can grow over the course of a run to colonize the aquifer and differentiate to create a microbial community.

Figure 3 shows the result of such a simulation containing no ferric iron, so only aceticlastic methanogens and acetotrophic sulfate reducers can grow, after the model has run to a steady state at which the rates of microbial growth and decay are in balance. Dissolved sulfate decreases in concentration as water flows away from the recharge point, because sulfate reducers use the molecule as an electron acceptor. Much of the acetate added to the aquifer is consumed as the electron donor for the bacteria; the remaining

*The input files used to create the varous numerical models cited in this paper are available from repositories, as noted in original publications cited.

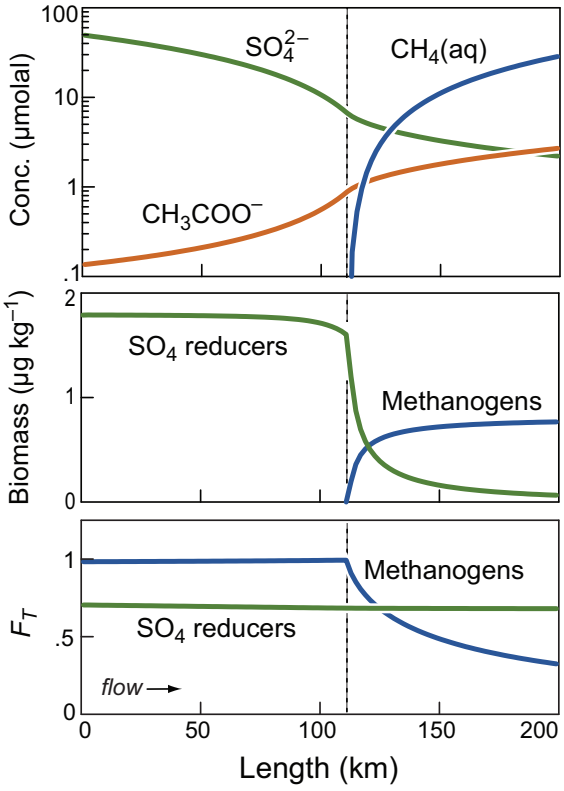


Fig. 3 Long-term solution to a reactive transport model in which sulfate reducers and aceticlastic methanogens within an aquifer compete for acetate (Bethke et al., 2008). Sulfate is present in the recharge water and acetate is supplied by the fermentation of natural organic matter in surrounding sediments. Diagrams show concentration of dissolved sulfate, acetate, and methane (top); biomass of the two functional groups of microbes (middle); and thermodynamic potential factor F_T for each (bottom).

mass accumulates along the direction of flow. The aceticlastic methanogens seeded in this upgradient portion of the aquifer, rather than growing into a population, have died, leaving a monoculture of sulfate reducers.

A little more than 100 km along the aquifer, acetate builds up to a level sufficient for aceticlastic methanogens to form a stable population. Past this point, methane accumulates in the flowing groundwater. This downgradient zone is colonized by a mixed community of methanogens and a lesser population of sulfate reducers.

At first glance, sulfate reducers might appear to dominate the upgradient zone by competitive exclusion, but the actual mechanism of exclusion is more interesting. As shown in Figure 3, the thermodynamic potential factor F_T for methanogens is close to unity, whereas F_T for the sulfate reducers is only about 0.7. Availability of usable energy, in other words, provides no impediment to the growth of methanogens, which are in fact thermodynamically favored over sulfate reducers. Thermodynamics, then, plays no role in the exclusion of methanogens in this zone.

The inability of methanogens to establish a stable community is instead an ecologic phenomenon. The requirement for maintaining a population is that the rate of cell growth must meet or exceed that of decay. Bethke et al. (2008) showed that for aceticlastic methanogens, this condition demands that the acetate concentration m_{Ac} satisfy the inequality

$$m_{Ac} \geq \frac{D}{Y} \cdot \frac{K_D}{k_+ F_T} \tag{4}$$

Here, Y is growth yield, K_D is the aceticlasts' half saturation constant, and k_+ is the rate constant; each of these values is determined experimentally. The decay constant D appropriate for a given natural environment can be estimated from Y and the biomass concentration $[X]$ there by assuming the population is in steady state (Bethke et al., 2008).

Methanogens in general grow slowly, have a small growth yield Y, and with respect to acetate are associated with large half-saturation constants K_D. As a result, the ecologic criterion for maintaining a population, as given by Eq. (4), is a relatively large acetate concentration m_{Ac}. The ecologic minimum concentration, notably, is roughly a factor of 30 larger than the thermodynamic limit given by Eq. (1). Simply put, aceticlastic methanogens have sufficient energy to grow everywhere in the simulation, but cannot establish a population until acetate accumulates to a level that allows cells to be replaced as quickly as they die. Failing to replace the cells that die, the population enters an extinction vortex and vanishes.

In a related simulation (Fig. 4), the upgradient third of the aquifer contains goethite, allowing iron reducers to grow there. This portion of the aquifer becomes colonized by a mixed community of a predominate population of sulfate reducers and a lesser population of iron reducers. The community produces both sulfide and ferrous iron, so the mineral mackinawite (FeS) can form according to

$$Fe^{2+} + HS^- \rightarrow FeS(s) + H^+ \tag{5}$$

The concentration of Fe^{2+} in the upgradient zone increases along the direction of flow, because the microbial community contributes more ferrous iron to solution than sulfide. The increment of Fe^{2+} in excess of sulfide cannot be taken up by mackinawite precipitation and hence builds up in the flowing groundwater.

It is notable that ferrous iron rather than sulfide builds up in solution, even though the iron reducing population is subordinate to the population of sulfate reducers, in terms of both biomass and reaction rate (Fig. 4). The accumulation of ferrous iron is due to the fact that iron reducers liberate eight Fe^{2+} ions

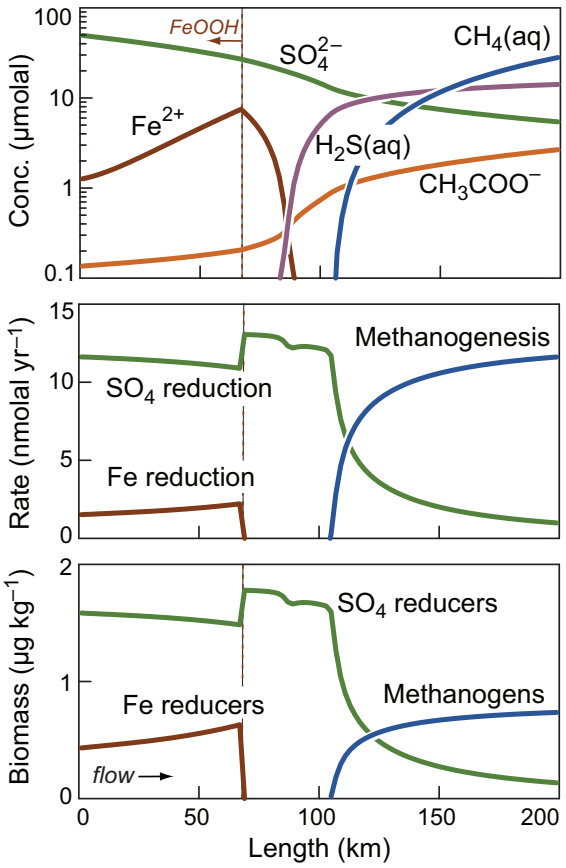


Fig. 4 Results of a reactive transport similar to Fig. 3, except that goethite (FeOOH) is present in sediments within the upgradient third of the aquifer (Bethke et al., 2008). Diagrams show dissolved concentration (top), reaction rates on a per acetate basis (middle), and biomass of each functional group (bottom).

$$CH_3COO^- + 8\,FeOOH(s) + 15\,H^+ \rightarrow 2\,HCO_3^- + 8\,Fe^{2+} + 12\,H_2O \quad (6)$$

per acetate consumed, whereas sulfate reducers

$$CH_3COO^- + SO_4^{2-} + H^+ \rightarrow 2\,HCO_3^- + H_2S(aq) \quad (7)$$

contribute a single sulfide. A groundwater high in iron, significantly, cannot be taken as evidence that a population of iron reducers dominates sulfate reducers in the subsurface community.

5. Bioreactor experiments

In a series of long-term (∼300 days) experiments in semi-continuous flow bioreactors (Kirk et al., 2010), a natural consortium containing iron reducing bacteria, sulfate reducing bacteria, and methanogens was allowed to grow in the presence of natural aquifer sediments, while fluid containing nutrients was added and reacted fluid removed. They conducted three experiments: one in which the feed fluid contained sulfate but the sediment held no ferric iron, a second in which the feed lacked sulfate but the sediment was amended with goethite, and a third that included both sulfate in the fluid and goethite in the sediment.

Bethke et al. (2011) analyzed the rates of sulfate reduction, iron reduction, and methanogenesis in the experiments, as well as the usable energy available to the three functional groups of microorganisms. The latter experiment, in which sulfate reduction and iron reduction can occur together, is of particular interest to us. As shown in Figure 5, sulfate concentration in the fluid decreased gradually for about the first 100 days, and acetate decreased in about two months to below detection, at which point the sole source of electron donor was the feed fluid.

There are several notable aspects to the results (Fig. 5). First, despite the advantage in available energy that iron reducers hold over sulfate reducers at neutral to acidic pH, sulfate reduction occurs 6 to 9 times more rapidly in the experiment than iron reduction. Second, iron reduction oc-

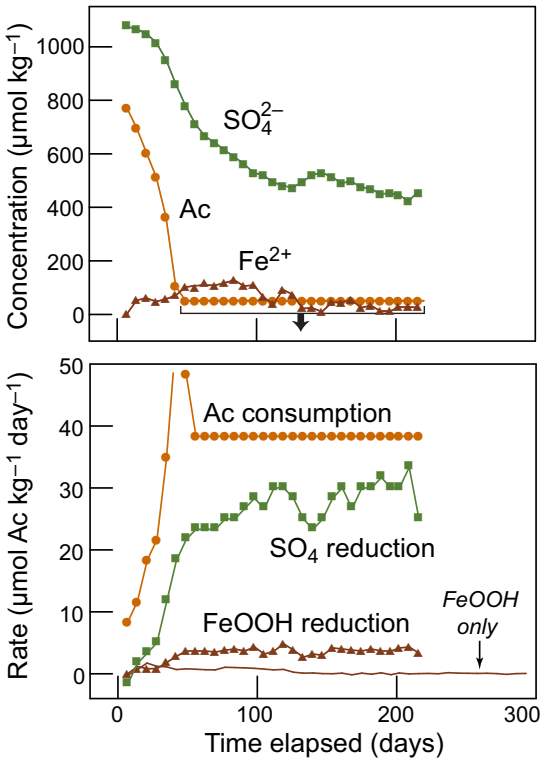


Fig. 5 Results of a long-term experimental study of microbial growth in a semi-continuous flow reactor conducted by Kirk et al. (2010). Sediment, along with its natural microbial community, and goethite (FeOOH) were allowed to react over time with aliquots of water containing dissolved sulfate and nutrients, as equal volumes of reacted water were removed. Diagrams show concentration of sulfate, acetate (Ac), and ferrous iron in water periodically withdrawn from the reactor (top) and rates on a per acetate basis of sulfate reduction and iron reduction, and the rate acetate is consumed by the microbial community (bottom). Solid line at base of bottom diagram shows rate of iron reduction in a parallel experiment that contained goethite, but not sulfate.

curs about 14 times more rapidly in this experiment than it did in the second experiment in the series, which contained goethite but no sulfate. In other words, when the iron reducers competed with sulfate reducers for acetate, they respired the acetate considerably more rapidly than they did in the absence of competition.

Third, and perhaps most notably, it is clear the iron reducers and sulfate reducers enter into a tight mutualistic relationship in which each functional group benefits the other. This point is demonstrated in Figure 6, which shows that the community adjusts after about one month to a condition in which sulfate reduction on a per-acetate basis is close to eight times as rapid as iron reduction. Since a sulfate reducer produces one sulfide ion per acetate, whereas an iron reducer produces eight Fe^{2+} ions, this ratio of reaction rates represents the point at which sulfide and ferrous iron are added to solution at the same rate, which by Reaction (5) is the stoichiometric ratio of mackinawite precipitation.

Bethke et al. (2011) suggested that iron reducers operating in isolation produce Fe^{2+} ions that sorb

$$\equiv \mathrm{FeOH} + \mathrm{Fe}^{2+} \rightarrow \equiv \mathrm{FeOFe}^{+} + \mathrm{H}^{+} \qquad (8)$$

onto the ferric surface, diminishing the its ability to accept electrons. Here, $\equiv$ FeOH and $\equiv$ $FeOFe^{+}$ represent uncomplexed and iron-complexed sites, respectively, on the ferric surface. An iron reducer producing one Fe^{2+} for each sulfide ion producing by a sulfate reducer, however, would likely precipitate mackinawite according to Reaction (5), thereby removing the Fe^{2+} from solution before it can sorb on the ferric surface by Reaction (8). The sulfate reducers, likewise, avoid by this relationship a buildup of dissolved sulfide, which might lead to product inhibition of its catabolic reaction.

Each of the functional groups, rather than competing and attempting to exclude the other, as suggested by the concept of a thermodynamic ladder, is in fact deriving such significant benefit from the other that the two catabolic reactions proceed in lockstep. The mutualistic relationship goes a long way toward explaining why iron reducers work more rapidly in the presence of sulfate reducers than in their absence.

6. In-situ sampling

Determining the nature of microbial activity in an aquifer is most commonly an indirect process. Activity may be interpreted on the basis of the chemical evolution of groundwater, assuming the water maintains a record of past microbial activity. As discussed above, however, little of the activity may in fact be recorded. Aquifer sediments may also be retrieved and the attached microbial community analyzed by molecular methods, but the whole community does not necessarily reflect microbial activity in the aquifer, because an unknown fraction of microbes may be dormant, or nearly so.

To address these problems, Flynn et al. (2012) undertook to measure microbial activity in the Pleistocene Mahomet aquifer in East Central Illinois, USA, using an in-

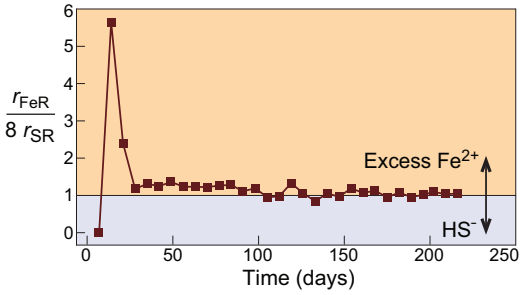


Fig. 6 Balance between iron reduction and sulfate reduction in the long-term flow-through reactor experiment conducted by Kirk et al. (2010), shown in Fig. 5, from Bethke et al. (2011). Bethke et al. (2011) calculated the rates of iron and sulfate reduction in the experiment from mass balance. When on a per-acetate basis the rate of iron reduction in the results equals eight times the rate of sulfate reduction, the microbial community generates Fe^{2+} and dissolved sulfide in an equal molar ratio, which is the stoichiometric ratio for mackinawite precipitation. After about one month, the rates approach the 1 : 8 ratio and maintain it through the remainder of the experiment. In this way, the two groups of microbes demonstrate mutualistic rather than competitive behavior.



Fig. 7 In-situ sampler, or "bug trap", used by Flynn et al. (2012) to retrieve the actively growing microbial community from wells completed in the Mahomet aquifer. Washed, autoclaved sediment from the aquifer is enclosed in a mesh bag, suspended on a fishing line, and lowered to screen level within the well; after a period of months, the sampler is retrieved and the biomass attached to the sediment analyzed.

situ sampling method. The authors suspended mesh bags of autoclaved aquifer sediment (Fig. 7) at the screens of 18 wells, so that ambient groundwater flowed across the "bug traps". After about 14 weeks, they retrieved the initially sterile traps and used molecular techniques to analyze the microbial community that had grown up attached to the sediment within them. In this way, they analyzed only active microbes and hence presumably derived a more direct characterization of microbial activity in the aquifer than might be possible by other techniques.

The Mahomet aquifer holds groundwater rich in dissolved iron, but poor in sulfide. As such, the aquifer falls squarely within the iron-reducing category, as proposed by Chapelle et al. (2009). Figure 8 shows the molecular analysis of bacteria retrieved from the bug traps, broken down by groundwater sulfate concentration. Even though iron reducers might be expected to dominate the community, genera of sulfate-reducing bacteria make up a strikingly significant fraction of the actively growing biomass sampled from the Mahomet. In wells with more than a negligible concentration of sulfate (> 0.03 mM), sulfate reducing bacteria were about equally numerous as iron reducing bacteria. Even at wells where water is nearly devoid of sulfate, sulfate reducers make up a significant fraction of the community (Fig. 8).

These results show that sulfate reducers in the Mahomet coexist with iron reducers, even though the aquifer is nominally iron reducing. Indeed, as already discussed in this paper, coexistence of a significant population of sulfate reducers in the presence of high-iron groundwater is not entirely surprising, in light of the fact that iron reducers contribute eight times as much Fe^{2+} to solution on a per-electron basis as sulfate reducers add sulfide.

In a broader sense, the study lends no support to the idea that a thermodynamic ladder exerts control over the microbial community, or to the concept of competitive exclusion of sulfate reducers by iron reducers. Instead, the evidence suggests that wherever sulfate is available in the aquifer, sulfate reducers and iron reducers live together, perhaps in a mutualistic relationship of the sort demonstrated by Bethke et al. (2011) and discussed in the previous section of this paper.

7. Discussion

We in this paper attempt to develop an understanding of the nature of subsurface microbial activity by integrating study of groundwater chemistry, numerical modeling,

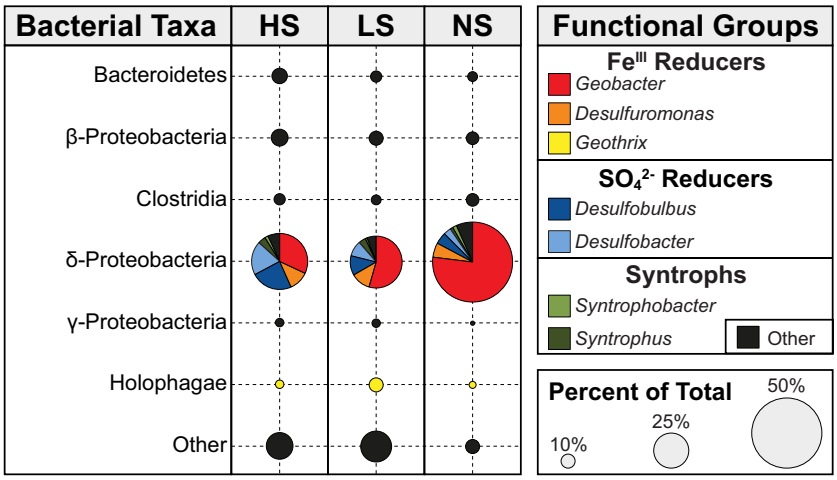


Fig. 8 Relative distribution of the bacterial populations that make up a series of natural communities sampled from the Mahomet aquifer of east-central Illinois, USA, from Flynn et al. (2012). The communities were obtained from 18 wells completed within the aquifer using the "bug traps" shown in Fig. 7. The traps were left in place for 14 weeks before being retrieved, at which point the bacterial community that had grown up attached to sediment in each trap was detached and analyzed by molecular methods. Data are grouped in the figure by groundwater sulfate concentration: column "HS" contains data from wells where sulfate concentration is > 0.2 mM; concentration for column "LS" is between that level and 0.03 mM; column "NS" is for data from wells with negligible sulfate, < 0.03 mM. For each taxonomic group, the diameter of the circle representing high, low, and negligible sulfate conditions shows the group's relative importance within the community; the radii of wedges within circles break out the proportions of molecular sequences attributed to iron reducers (red, orange, yellow), sulfate reducers (blue), synthrophs (green), and other classes of bacteria (black). The results show that, even though conditions in the Mahomet aquifer are considered by conventional criteria to be strongly iron reducing, the sulfate reducing bacteria are about equally numerous as iron reducing bacteria wherever measurable amounts of sulfate ("HS" and "LS") are found in the groundwater. Even where sulfate concentration is negligible ("NS"), sulfate reducers make up a significant fraction of the community.

bioreactor experiments, and the in-situ sampling of actively growing microbes from an aquifer. Taken together, these studies portray microbial communities and their relationship to ecosystem chemistry in a manner distinct from convention interpretations.

The nature of subsurface microbial activity has most commonly been inferred by comparing the redox chemistry of groundwater from place to place. The variation might be observed on a sub millimeter scale in soil, or over a range of hundreds of kilometers in a regional flow regime. A spot where groundwater is rich in iron, for example, would have been taken to reveal a community of iron reducing bacteria inhabiting an aquifer.

The Middendorf aquifer of South Carolina, USA, however, shows a large electron gap: groundwater in the aquifer records just a small fraction of the respiration occurring there (Fig. 1). In a reactive transport model (Fig. 4) and a bioreactor experiment (Fig. 5), furthermore, groundwater becomes enriched in iron, even though iron reduction in both the model and the experiment is subordinate to sulfate reduction. Clearly, the presence of dissolved iron cannot be taken as evidence of a subsurface community dominated by iron reducers.

As well, conventional thinking has held that the distribution of microbial populations in the subsurface falls along a thermodynamic ladder in which iron reducers hold an advantage over sulfate reducers, which in turn dominate methanogens. By the theory of competitive exclusion, wherever an advantaged population can live, it displaces other groups of microbes from the habitat by denying them the energy they need to live.

We see no evidence that subsurface communities behave in this manner. In the Middendorf, sufficient energy exists everywhere for iron reducers, sulfate reducers, and aceticlastic methanogens to live. In a reactive transport experiment, methanogens are excluded from part of an aquifer, but not by the lack of energy. Instead, these methanogens enter an extinction vortex because they fail to reproduce quickly enough to replace cell decay; their exclusion from the community is an ecologic rather than thermodynamic phenomenon.

In contrast to the conventional perspective, we find that microbial communities commonly exist as mixed populations, shaped as much by mutualism as competition. In the Middendorf aquifer, the electron gap (Fig. 1) might be explained by a community of coexisting iron reducers and sulfate reducers. If the Fe^{2+} and sulfide these groups add to solution were to precipitate as mackinawite, the groundwater would lose part of the record of electron acceptance. The microbial communities that grow up in the reactive transport modeling, furthermore, are as commonly mixed communities than monocultures.

Perhaps most striking is the evidence that iron reducers and sulfate reducers can establish a close mutualistic relationship. In a long-term bioreactor experiment (Fig. 6), the relationship between the two groups becomes so tightly balanced that the community produces Fe^{2+} and sulfide in a one-to-one ratio. Just as notably, the rate of iron reduction in the experiment was roughly 15 times faster than in a parallel experiment lacking sulfate. When the iron reducers had to compete with sulfate reducers for acetate, they actually worked more quickly than in the absence of competition.

In-situ sampling of actively growing microbes from the Mahomet aquifer, which is taken by conventional criteria to be strongly iron reducing, seems to confirm results of the bioreactor experiment. Despite its classification, the Mahomet wherever sampled is seen to have a significant population of active sulfate reducers. Given a small amount of sulfate in the groundwater, the sulfate reducers are roughly equivalent in population size to the iron reducers.

We argue that classifying microbial activity on the basis of an ecosystem's redox chemistry is obsolete, as is the concept that communities reflect an energetic hierarchy among functional groups of microbes. Instead, microbial populations distribute themselves according to ecologic phenomena that are of necessity subject to the constraints of thermodynamics, but do not directly reflect them.

Acknowledgments

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