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Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates

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Abstract

Microorganisms play an important role in the weathering of silicate minerals in many subsurface environments, but an unanswered question is whether the mineral plays an important role in the microbial ecology. Silicate minerals often contain nutrients necessary for microbial growth, but whether the microbial community benefits from their release during weathering is unclear. In this study, we used field and laboratory approaches to investigate microbial interactions with minerals and glasses containing beneficial nutrients and metals. Field experiments from a petroleum-contaminated aquifer, where silicate weathering is substantially accelerated in the contaminated zone, revealed that phosphorus (P) and iron (Fe)-bearing silicate glasses were preferentially colonized and weathered, while glasses without these elements were typically barren of colonizing microorganisms, corroborating previous studies using feldspars. In laboratory studies, we investigated microbial weathering of silicates and the release of nutrients using a model ligand-promoted pathway. A metal-chelating organic ligand 3,4 dihydroxybenzoic acid (3,4 DHBA) was used as a source of chelated ferric iron, and a carbon source, to investigate mineral weathering rate and microbial metabolism.

In the investigated aquifer, we hypothesize that microbes produce organic ligands to chelate metals, particularly Fe, for metabolic processes and also form stable complexes with Al and occasionally with Si. Further, the concentration of these ligands is apparently sufficient near an attached microorganism to destroy the silicate framework while releasing the nutrient of interest. In microcosms containing silicates and glasses with trace phosphate mineral inclusions, microbial biomass increased, indicating that the microbial community can use silicate-bound phosphate inclusions. The addition of a native microbial consortium to microcosms containing silicates or glasses with iron oxide inclusions correlated to accelerated weathering and release of Si into solution as well as the accelerated degradation of the model substrate 3,4 DHBA. We propose that silicate-bound P and Fe inclusions are bioavailable, and microorganisms may use organic ligands to dissolve the silicate matrix and access these otherwise limiting nutrients.

Keywords: Microbial weathering; Nutrient cycling; Phosphorus; Iron; Chelates

1. Introduction

Participation of bacteria in mineral weathering is now an accepted, even expected component of subsurface geochemistry. Microorganisms have been

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found at depths exceeding 3 km and at temperatures greater than 100 °C, and there is growing evidence that the biochemical functions of these organisms may be the driving force behind many low temperature mineral weathering reactions (e.g., Ehrlich, 1996; Priscu et al., 1999). A fundamental question in interpreting this interaction is whether the microorganism benefits by the dissolution of a mineral or, conversely, if weathering is simply a coincidental byproduct of basic biochemical functions. Our previous research on subsurface microbial colonization of silicates in a petroleum-contaminated aquifer suggested that microorganisms preferentially colonize and dissolve feldspars that contain phosphate and iron minerals as minor components, both of which are limiting nutrients for this microbial consortium (Bennett et al., 2001; Rogers et al., 1998). We also found that silicates without these elements are typically barren of colonizing microorganisms. These observations raise three basic questions about the nature of the microbe–mineral interaction. (1) Do microorganisms selectively target silicates containing specific inorganic nutrients that are limiting? (2) Are the silicate-bound nutrients bioavailable to the colonizing microbial population? (3) Does the microbial population benefit from release of nutrient from silicates? These questions recast the problem of interpreting the microbe–mineral interaction from one of simple geochemical consequence to an investigation of microbial ecology where the aquifer mineralogy is a fundamental part of the equation. This study presents evidence from laboratory experiments and field studies at a shallow, petroleum-contaminated aquifer that subsurface microorganisms selectively colonize and weather minerals and glasses for their nutritional constituents and that the microbial consortium benefits from the release of phosphorus and iron from silicates.

1.1. Microbial ecology and minerals as nutrient sources

Microorganisms in the shallow subsurface (to a depth of 50 m) have long been known to play a significant role in the cycling of carbon and nutrients as well as in mineral-weathering processes in the soil and unsaturated zones (e.g., Berthelin, 1988; Sposito, 1989; Ehrlich, 1996; and references therein). All microorganisms require electron donors and acceptors, the primary macronutrients nitrogen and phosphorus (P) and, to a lesser extent, iron (Fe) and a variety of micronutrients that can be species and habitat specific. In the typical groundwater environment, however, phosphorus and sometimes iron are often limiting, and microbial strategies that increase the bioavailability of critical nutrients will enhance the viability of the native population or consortium.

Phosphorus is a fundamental macronutrient needed by microorganisms for synthesis of nucleic acids, nucleotides, phosphoproteins, and phospholipids (e.g., Madigan et al., 2002); and the lack of bioavailable P can diminish cell growth and metabolic efficiency (e.g., Ghiorse and Wilson, 1988). Microbial populations in oligotrophic (nutrient limited) environments therefore must develop strategies to scavenge any available phosphorus from minerals by proton production to enhance mineral dissolution (Goldstein, 1986; Halder and Chakrabartty, 1993), iron chelation or reductive dissolution to release bound P (e.g., Duff et al., 1963; Jansson, 1987).

Like P, bioavailable Fe can be scarce in oxic groundwaters because of the low solubility of iron oxyhydroxides at neutral pH. Microorganisms use Fe as a component of cytochromes and iron–sulfur proteins, which are involved in cellular electron transport. In addition, some metal-respiring anaerobes derive energy from the Fe^{3+}–Fe^{2+} redox couple (+0.77 V) and use Fe^{3+} as a terminal electron acceptor (TEA) (Lovley et al., 1989; Madigan et al., 2002). Many microorganisms have developed strategies to dissolve mineral Fe to increase its bioavailability. Dissimilatory iron-reducing bacteria (DIRB), for example, use ferric iron as a TEA, and a ready supply of oxidized iron is required for respiration. DIRB can use Fe^{3+} from hydrous ferric oxide and hematite as a TEA (e.g., Roden and Zachara, 1996) as well as Fe^{3+} chelated by Fe(III)-specific organic ligands (Lovley and Woodward, 1996) or siderophores (Page, 1993). Siderophores react with iron oxyhydroxides and can dissolve Fe-bearing silicate minerals (Kalinowski et al., 2000; Liermann et al., 2000b) and glasses (Callot et al., 1987).

1.2. Microbial silicate weathering

Microorganisms can alter silicate solubility directly, when attached, by perturbing mineral–water equi-

libria and reaction dynamics at the point of attachment by producing proton, hydroxyl or metal-chelating metabolic byproducts (e.g., Bennett et al., 2001; Liermann et al., 2000a; Drever and Stillings, 1997; Brantely and Stillings, 1996). The chemical environment around a microorganism is often different from that of the bulk solution. For example, Barker et al. (1998) observed perturbations in pH near metabolizing cells using confocal microscopy, and many researchers measure significant chemical gradients in biofilms using microelectrodes (e.g., Yu and Bishop, 2001; Yu et al., 1998). These types of microenvironments can be highly reactive with respect to mineral surfaces and result in localized etching (e.g., Fisk et al., 1998; Callot et al., 1987). Furthermore, there are indications that microbes derive from silicate minerals and glasses both macronutrients and such trace nutrient metals as K (Valsami-Jones et al., 1998) as well as Fe, Ni, V and Mn (Brantley et al., 2001). To gain access to essential nutrients in silicate rock, microorganisms may take advantage of the removal of the silicate matrix that results from metabolic processes and the resultant release of interstitial metals or the exposure of nutrient-rich inclusions.

Microorganisms have been shown to accelerate the dissolution of a variety of silicates by the production of excess proton and organic ligands as well as hydroxyl (Aristovskaya et al., 1969) and extracellular polysaccharides (EPS) (Berthelin and Belgy, 1979; Malinovskaya et al., 1990; Welch et al., 1999). Microorganisms have also been shown to accelerate silicate dissolution by oxidation or reduction of metals in the mineral (Ivarson et al., 1978, 1980, 1981). Organic ligands may be especially important in circum-neutral pH environments, where the proton-promoted rate is at a minimum (Chou and Wollast, 1985; Ullman et al., 1996). These ligands can enhance silicate dissolution rates by decreasing pH, by forming framework-destabilizing surface complexes, or by complexing metals in solution (Bennett and Casey, 1994; Blake and Walter, 1996; Drever and Vance, 1994; Stillings et al., 1996; Welch and Ullman, 1993).

Although glasses are less resistant to chemical weathering than their crystalline counterparts, microorganisms can play a significant role in glass dissolution. Dissolution of glasses is of particular interest to the nuclear industry, where borosilicate glasses are proposed for long-term disposal of high-level radioactive waste (e.g., Robinson, 1962). In the absence of water, glasses are stable over geologic time periods, and their structure allows insertion of radionuclides. In aqueous environments, however, glasses transform into a thermodynamically stable assemblage of secondary phases (Abrajano et al., 1988; Bourcier, 1989; Grambow, 1985; Mendel, 1984). Several researchers have examined the dissolution of both natural (e.g., White, 1983; Staudigel and Hart, 1983; Callot et al., 1987; Crovisier et al., 1987; Thorseth et al., 1995; Fisk et al., 1998; Oelkers and Gislason, 2001; Techer et al., 2001) and synthetic glasses and found that silicate glasses develop a leached layer during dissolution (Advocat et al., 1998; Bourcier et al., 1992; Bunker et al., 1983, 1988; Leturcq et al., 1999; Rana and Douglas, 1961; Sterpenich and Libourel, 2001). During leaching, several reactions may occur simultaneously, including ion exchange, glass hydration and network hydrolysis (Bunker et al., 1983; Casey and Bunker, 1990).

Based on the results of both field and laboratory experiments using silicate minerals and manufactured glass, we propose that subsurface microorganisms colonize nutrient-bearing silicates and dissolve the silicate matrix to extract and use limiting inorganic nutrients. The microbial population responds to the availability of these nutrients by increasing biomass and increasing biodegradation. Silicate minerals (and glasses) may be an important and often overlooked source of nutrients to groundwater microorganisms. Mineralogy, therefore, may play a vital role in microbial abundance and viability in many subsurface environments.

2. Methods

We investigated the controls on microbial weathering both in the field and laboratory to determine if microorganisms take advantage of nutrients in silicate minerals and glasses. Field experiments used manufactured glasses containing P and Fe to examine the relationship between colonization, solid phase composition and weathering. Laboratory experiments using batch mineral dissolution reactors were performed to determine the abiotic rate of nutrient release from minerals and glasses with and without a model organic ligand. Live laboratory microcosm experiments

were used to compare mineral or glass weathering and rate of nutrient release in the presence of the native microbial consortium and to determine if microorganisms respond to nutrients released from silicates and glass. The model ligand was also used in live laboratory microcosms to serve as a mechanism to supply chelated Fe^{3+} and to investigate this ligand's influence on microbial metabolism and silicate weathering.

2.1. Site description

The study site is a petroleum-contaminated, shallow sand and gravel aquifer, located near Bemidji, MN, part of the U.S. Geological Survey's Toxic Substances Program. A floating pool of free-phase petroleum ~1 m thick collected on the water table after a petroleum pipeline rupture, and a plume of organic and inorganic solutes extends downgradient from the source (Hult, 1984; Baedecker et al., 1993). In the anaerobic part of the contaminated groundwater studied here, the microbial biomass is dominated by DIRB with less abundant fermenting bacteria and narrowly distributed methanogens (Bekins et al., 1999b). Dissolved aromatics are rapidly degraded coupled to Fe^{3+} reduction with secondary methanogenesis in the anoxic groundwater beneath and downgradient from the oil pool (Lovley et al., 1989; Baedecker et al., 1993; Bennett et al., 1993; Eganhouse et al., 1993; Revesz et al., 1995; Anderson, 1998; Rooney-Varga et al., 1999), and a variety of organic acids are produced as secondary metabolites (Cozzarelli et al., 1990, 1994). Field experiments were performed and groundwater used for laboratory experiments was collected in the anaerobic zone downgradient of the oil. Groundwater chemistry has been reported previously (Bennett et al., 2000), but, briefly, the water is anaerobic with a pH of 6.72, 1.46 mmol l^{-1} dissolved organic carbon (DOC), 0.4 mmol l^{-1} Fe^{2+} and 0.9 mmol l^{-1} Si. The water also contains 2.6 mmol l^{-1} Ca^{2+}, 0.8 mmol l^{-1} Mg^{2+}, 8.4 mmol l^{-1} HCO_3^- and <0.01 mmol l^{-1} of Al, K, Na, SO_4, NO_3 and PO_4.

2.2. Mineral and glass chemistry

A suite of silicates containing varying amounts of P and Fe, including anorthoclase (Wards no. 46E0575, Larvik, Norway), microcline (Wards no. 46E5125, Keystone, South Dakota) and plagioclase (Wards no. 46E0230, Ontario, Canada) were used to investigate microbial weathering and release of trace nutrients. Silicate rock specimens were characterized using light microscopy, scanning electron microscopy–electron backscattering spectroscopy, energy dispersive system (SEM–EDS; JEOL SEM with EDAX), electron microprobe analysis and trace metal and whole rock analysis as described previously (Rogers et al., 1998; Bennett et al., 2001) and summarized in Table 1. These rocks are referred to by their bulk mineralogy, but the microcline and anorthoclase contain minor mineral inclusions. Compositional analysis of the microcline yielded 1200 ppm of inorganic P, which occurs as Cl-bearing fluorapatite inclusions, while anorthoclase contained zoned Fe–Ti oxide inclusions (12,000 ppm $Fe^{(T)}$) with 1000 ppm P primarily as fluorapatite inclusions with some rare earth element (REE) phosphates (Rogers et al., 1998).

Glasses manufactured in our laboratory were used as artificial minerals to introduce nutrient and non-nutrient solid phases without the variability of natural minerals. Glasses are distinct compositionally, virtually homogenous and, unlike rock-forming minerals, their compositions can be controlled exactly. Because of their low melting temperatures (800–1300 °C) and high durability, Pyrex-type (borosilicate) glasses were used as the matrix to investigate positive controls on microbial attachment. Pyrex-type glasses containing mineral inclusions of apatite and goethite were manufactured to replicate the trace composition of the natural silicate minerals used in this study. These glasses were reasonable artificial rocks and were used to determine how spatial compositional heterogeneity relates to colonization and the ability of microorganisms to access silicate-bound nutrients.

Glass compositions were calculated using formulae from Lawrence Livermore National Laboratory (Bourcier, 1997, personal communication). Stock powder mixtures of Pyrex glass were homogenized for 20 min, and for glasses with mineral inclusions, the mineral chips (size range: 1–5 mm in diameter) were added, and the mixture was shaken for 5 more minutes. Glass compositions consist of Pyrex glass with no additions (Pyrex glass), 1% apatite (Ap glass), 1% goethite (Go glass) and 1% each of both apatite and goethite (ApGo glass) (Table 1). Glasses were then melted in a furnace at 950–1250 °C for 5 h in

Table 1
Compositions of silicates and manufactured glasses

Silicate[a]	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	B_2O_3	P_2O_5
Anorthoclase	60.63	19.08	4.41	0.93	3.04	6.62	3.99	0.93	–	0.24
Microcline	65.17	18.38	0.90	0.01	0.15	2.18	13.5	–	–	0.28
Plagioclase	59.80	20.87	1.07	0.08	2.37	6.69	7.37	–	–	–
Pyrex Gl.[b]	80.8	2.2	–	–	–	4.3	–	–	12.0	–
Apatite Gl.	80.8	2.2	–	–	–	4.3	–	–	12.0	1.0[c]
Goethite Gl.	80.8	2.2	1.0[d]	–	–	4.3	–	–	12.0	–
Ap + Goe Gl.	79.8	1.2	1.0[d]	–	–	4.3	–	–	6.0	1.0[c]

[a] Values are expressed as weight percent oxide. Analysis from Bennett et al. (2001) and Rogers et al. (1998).
[b] Values are expressed as mole percent.
[c] P_2O_5 as apatite.
[d] Fe_2O_3 as goethite.

platinum crucibles. After cooling to room temperature, the glass slug was removed from the crucible, and washed with distilled water.

2.3. Field microcosms

Field microcosm experiments have been described previously (e.g., Bennett et al., 1996; Hiebert and Bennett, 1992; Rogers et al., 1998) and were used to assess field microbial colonization as a function of glass composition. Microcosms consisted of sterile, polyethylene containers punctured to permit flow-through of groundwater and filled with sterile, glass chips (10- to 15-mm size fraction). Constructed microcosms were suspended into the screened portion of the well and left undisturbed for 9 months (Fig. 1). Groundwater samples were taken at the time of placement and removal of the microcosms. Additional microcosms were reserved in the laboratory for reference and control. Upon retrieval, glass chips were fixed in the field using a chemical critical point drying method (Nation, 1983; Vandevivere and Bevaye, 1993). This procedure was also used for laboratory microcosms. Chips were stub-mounted and gold sputter-coated for 30 s, then imaged using conventional SEM. At least 20 fields were investigated on each sample at a variety of magnifications. The patterns of colonization, extent of colonization, presence of attachment features and glycocalyx were noted, as well as changes in the mineral or glass surface.

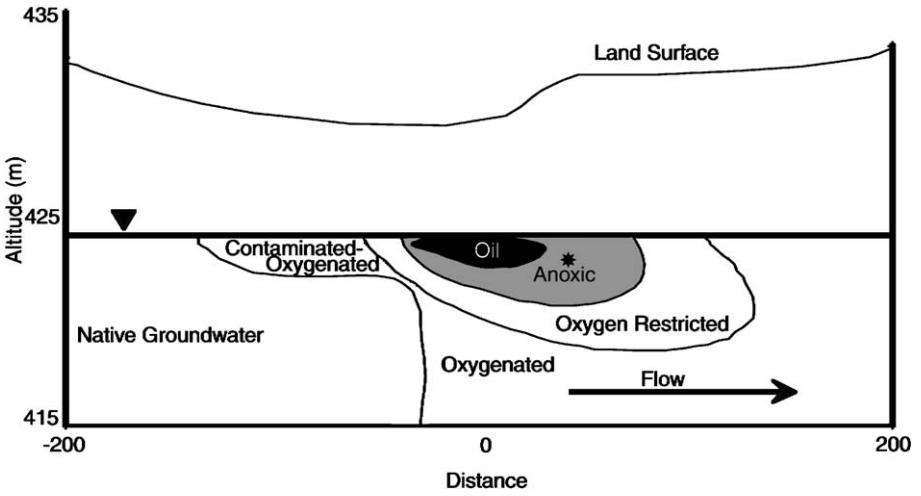

Fig. 1. Cross-section view of petroleum contaminated groundwater at the study site. Groundwater flow is from left to right and zonation of dissolved oxygen is shown. The study well, 532B, is indicated with a ✸.

2.4. Model ligand

Many organic ligands increase feldspar dissolution rate (e.g., Duff et al., 1963; Bennett, 1991, Welch and Ullman, 1993) by a variety of specific interactions, and many reactive ligands are present in contaminated groundwater. 3,4 Dihydroxybenzoic acid (DHBA) was identified as one of many ligands in the study aquifer present at low concentration, and it is a compound related to microbial siderophores, which are ferric–iron-specific ligands produced by microorganisms that sequester ferric iron in iron-limited environments (Neilands, 1993). We have used this ligand as a model compound (Bennett et al., 1998) to test the hypothesis that microbially derived organic ligands release nutrients from the silicate matrix.

The complexation of iron and silicon from solid phase by 3,4 DHBA was investigated to determine the stability and rate of reaction at circum-neutral pH and low temperature. Batch reactors were used to equilibrate stock solutions of 3,4 DHBA with fresh ferrihydrite and silica gel. 3,4 DHBA solutions were prepared with ultrapure water and reagent-grade material. All glassware and plastic were acid washed to prevent any iron contamination. Stability constants for 3,4 DBHA with several metals, including Al, are reported in the literature and summarized in Table 2.

Initially, the Fe(III)/3,4 DHBA complex was investigated as a function of pH using UV-difference spectroscopy (e.g., McBride et al., 1988) with a constant-temperature cell at 298 K (e.g., Kennedy and Powell, 1985). A solution of 1 mM 3,4 DHBA was mixed with 0.1 mM $FeCl_3$ and titrated with 0.1 M NaOH up to a pH of 11. As the pH increased, the samples developed distinctive hues, which occurred immediately and did not attenuate over time, changing from blue to purple to pink with increasing pH, indicative of the Fe(III)/3,4 DHBA chromophore. At pH 6.8, the solution was a deep purple color, with a lambda-max of 570 nm (the characteristic chromophore of the $Fe^{(III)}$/3,4 DHBA complex), and a calibration curve at pH 6.8 was prepared using an autotitrator in pH stat mode (Titrino).

Batch experiments were used to examine the interaction of organic ligand solutions with ferrihydrite and to determine solution complex stability. The ferrihydrite was prepared fresh before each experiment according to methods described by Grantham and Dove (1996). A 0.1 M $FeCl_3$ + 0.05 M NaCl solution was titrated to pH 5.5 with 0.1 M NaOH. The solid phase was washed repeatedly in distilled water and resuspended in 5 ml of DIW. Each reactor contained 30 ml of 1 mM or 0.5 mM 3,4 DHBA adjusted to pH 6.8 using 0.1 mol l^{-1} NaOH and 5 ml of the amorphous ferrihydrite. The solutions were mixed in a cell maintained at constant pH using an autotitrator (Metrohm Titrino) and allowed to sit overnight at 25 °C, and absorbance of each sample was measured. The reactors were left for an additional 5 days after which time absorbance was measured again. The complex concentration was determined using UV spectroscopy, while the concentration of Fe^{3+} was calculated using the volume of 0.1 N HCl titrated into the solution and the solubility constant for ferrihydroxide ($K = 10^{-38}$; Stumm and Morgan, 1996). This procedure was repeated at 40 °C using a constant temperature bath. Ionic strength was calculated from the solution composition and activity values for measured species are given in Table 3.

A similar experimental protocol was used for the 3,4 DHBA silica system, where stirred batch reactors were used to equilibrate 0.2 mmol l^{-1} and 0.5 mmol

Table 2
Stability constants of 1:1 complexes with simple organic acids at 25 °C

Siderophore	Fe (III)	Al(III)	Si	Fe(II)	Cu(II)	Ni(II)
Catechol[a]	10^{20}	10^{17}	no data	$10^{8}-10^{9.5}$	10^{14}	$10^{8}-10^{9.5}$
2,3 DHBA	$10^{21.4}$	no data	no data	no data	no data	no data
3,4 DHBA	$10^{16.5}$ *	$10^{5.77}$	$10^{2.9}$ *	no data	$10^{12.8}$	$10^{8.27}$
Citric acid	$10^{11.5}$	$10^{7.98}$	no data	$10^{4.4}$	$10^{5.9}$	$10^{5.4}$
Tropolone	$10^{10.5}$	no data	no data	$10^{5.97}$	$10^{8.35}$	$10^{5.97}$

[a] Values from Page (1993), Martell and Motekaitis (1989), and Martell and Smith (1974–89).
* Denotes that the value was calculated in this paper.

Table 3
Activities used for stability constant calculations

Species	Activity (298 K)	Activity (313 K)
Fe^{3+}	2.87×10^{-17}	2.92×10^{-17}
Fe^{3+}/3,4 DHBA	4.90×10^{-4}	2.50×10^{-4}
3,4 $DHBA^{-}$	4.92×10^{-4}	7.12×10^{-4}
$Si(OH)_4$	5.65×10^{-4}	**
$Si^{(IV)}$/3,4 DHBA	1.17×10^{-4}	**
3,4 $DHBA^{-}$	2.11×10^{-4}	**

** Denotes that the value was not calculated in this study.

l^{-1} of 3,4 DHBA with silica gel. A volume of 50 ml of solution was mixed with 14 g of silica gel for the 0.2 mmol l^{-1} concentration and 20 g of silica gel for the 0.5 mmol l^{-1} concentration. The solutions were adjusted to pH 5 using 0.1 M NaOH and were sampled every 7 days. At the end of 21 days, pH and solution DOC were measured on a filtered, unpreserved sample followed by wet oxidation of the volatile organic carbon and measurement of evolved CO_2 Dohrman DC-180 carbon analyzer, to insure that no biological activity occurred during the course of the experiment. Solutions were analyzed by scanning UV-difference spectroscopy between 200 and 350 nm; and total dissolved Si concentration was determined on a filtered acidified sample using inductively coupled plasma optical emission spectrometry (JY ICP-OES). The latter analysis was used to calculate the 3,4 DHBA stability constant at 25 °C (Table 3).

2.5. Laboratory microcosms

Batch dissolution experiments were performed to measure the abiotic release of orthophosphate, silica and iron from the silicate matrix. Microcline, anorthoclase and ApGo glass powders were prepared by crushing them in a sapphire mortar and pestle and sieving to 200–400 mesh. The powders were sonicated at low power to remove fines and surface areas were characterized with a Quantachrome Autosorb1 using a seven-point BET with nitrogen as the adsorbate gas.

The experiments were performed at pH 5 using two different buffers: a 1 mmol l^{-1} acetate ($pK_{a_1} = 4.7$) and a 1 mmol l^{-1} 3,4 DHBA ($pK_{a_1} = 4.4$) solution. The acetate solution was used as a control organic electrolyte compared to the chelating organic electrolyte, 3,4 DHBA. Solutions were cold sterilized by passing them through a 0.2-μm filter into steam-sterilized Teflon reactor vessels (121 °C for 45 min). Reactors were assembled with 0.2 g of the sterile mineral or glass powder per 200 ml of buffer solution and stirred at low speed at room temperature. Samples of 10 ml each were taken through sampling ports once a day and analyzed for pH, orthophosphate, and major cations and trace elements. Orthophosphate was measured using the stannous chloride method (Greenberg et al., 1992), and pH was measured with an electrode that had been soaked in an ammonium molybdate solution to remove contaminating P from pH 7 calibration solutions. Iron, silica and other major cations were measured by ICP–OES.

Laboratory microcosms containing a live microbial consortium were used to investigate whether microorganisms use the P and Fe released during silicate dissolution and what affect this had on the microbial population and silicate weathering rate. 3,4 DHBA was used in these experiments as a source of chelated iron as well as a stable substrate and potential rock-weathering ligand. The microcosm containers were constructed of sterile, nitrogen-purged serum bottles filled with 40 ml of a 50:50 mixture of anaerobic formation water and sterile deionized water. Four grams of sterile mineral or glass chips, including Ap glass, Go glass, ApGo glass, anorthoclase, microcline, quartz and plagioclase were then added to the serum bottles; four microcosms of each mineral or glass type and six nonmineral/nonglass controls were constructed. Aquifer sediments were collected anaerobically and aseptically using a freezing-shoe piston core barrel (Murphy and Herkelrath, 1996). The core was transferred to an anaerobic chamber in the field where it was homogenized, and then 70 g of sediment was added to 30 ml of diluted groundwater, along with 5 μl of Tween 80 (a nonionic surfactant) to dislodge adhering cells. The mixture was shaken and left for 3 h, then sonicated for 1 min. Each microcosm was inoculated with 1 ml of the resulting microbial cocktail. Two separate cores, located within 1 m of each other and in the same depth interval, were used for inoculation of the silicate and glass microcosms, respectively. The field-prepared microcosms were then injected with 1 ml of air to precipitate the dissolved ferrous iron as amorphous iron oxides.

3,4 DHBA powder (7.7 mg; final concentration in microcosm was 1 mM 3,4 DHBA) was then added to each microcosm in the anaerobic chamber to chelate the ferric iron for use by the microorganisms.

Microcosms were stored in the dark at 25 °C. The concentrations of 3,4 DHBA and its byproducts were measured on filtered, acidified samples by high-performance liquid chromatography (Waters HPLC) using a Supelcogel column (ID no. C-610H) with 0.1% H_3PO_4 eluent and UV detection at 220 nm. After 3 months, all microcosms were sampled and analyzed for ferrous iron by the bipyridine method (Skougstad et al., 1979) at 520 nm on a Perkin Elmer Lambda 6 spectrophotometer, cations and orthophosphate.

The microbial biomass in laboratory microcosms was determined by direct counts of cells using the fluorescent dye 4′, 6-diamidino-2-phenylindole (DAPI), which stains nucleic acids and thus makes microbes visible for counting using fluorescent microscopy. Microcosms were sonicated at low power for 25 s, and then a 1-ml sample was extracted. Samples were stained and prepared according to the methods of Yu et al. (1995), then imaged at 40 × on a Leica-inverted epifluorescent microscope attached to a Leica TCS4D scanning confocal laser. Six random fields of 150 × 150 μm were imaged digitally from each filter, then cells were counted using the image processing and analysis program Scion-Image (Scion). Because DAPI does not discriminate dead from living cells, an average number of cells was also determined for sterile controls, and these numbers were subtracted from values for live, experimental samples.

3. Results and discussion

3.1. Nutrient-driven colonization of glasses

Previous studies suggest that microorganisms preferentially colonize feldspars containing P and Fe, nutrients that are otherwise limiting in the aquifer (Rogers et al., 1998, 1999; Bennett et al., 2001), while leaving non-P- and non-Fe-bearing feldspars barren. Etching on nutrient-rich feldspar surfaces is associated only with attached microorganisms, and the extent of etching correlates directly to the extent of colonization. A summary of field colonization results (from Rogers et al., 1998; Bennett et al., 2001) is given here to serve as the basis for the laboratory studies presented in this study. Anorthoclase, containing both P and Fe^{3+}, was heavily colonized and the silicate matrix intensively etched. Microcline, which contains P, was colonized to a lesser extent and lightly etched. Plagioclase, however, contained neither P nor Fe and was barren of cells and no etching occurred.

To test the hypothesis that P and Fe promote microbial colonization on silicate surfaces, we used manufactured glasses doped with P and Fe. Like the silicate minerals, the native consortium colonized the nutrient-doped glasses. Heavy colonization was observed, in particular, on the apatite-containing glasses. Ap glass and ApGo glass both had glycocalyx covering the entire surface with groups of rods and some cocci (Fig. 2). The Go glass was moderately colonized primarily by rod-shaped cells, but little glycocalyx and no etching was observed. Scant colonization of the non-nutrient Pyrex glass was observed and no glycocalyx or dissolution was detected. Based on nutrient content, the colonization of glasses is similar to silicate minerals. Heavier colonization was observed on glasses that contain P than the non-nutrient control, but colonization of Go glass suggests that Fe also played a role in this interaction. One possibility is that the system was limited with respect to both P and Fe. Although there was sediment extractable Fe(III) in the aquifer sediments, Bekins et al. (1999a) found that DIRB were not using it in some areas, possibly because Fe(II) surface coatings on the surfaces inhibited reduction. These limitations may make P or Fe in silicate sources, such as those introduced into the aquifer in experimental microcosms, attractive to the native microorganisms.

The observation that surface cell density correlates to P content of the solid phase may be a result of chemotactic behavior, the result of growth after attachment, or a combination of the two. Another possibility is that the iron oxides have a positive surface charge that increases attachment on the sites of exposure. Cells may collect on the surface due to coulombic attraction, or DIRB may be attracted to Fe and attach more strongly to those sites (e.g., Lower et al., 2001). Once on the surface, DIRB may use the Fe as a TEA (Lovley and Woodward, 1996), and also take advantage of P, using it to increase their biomass.

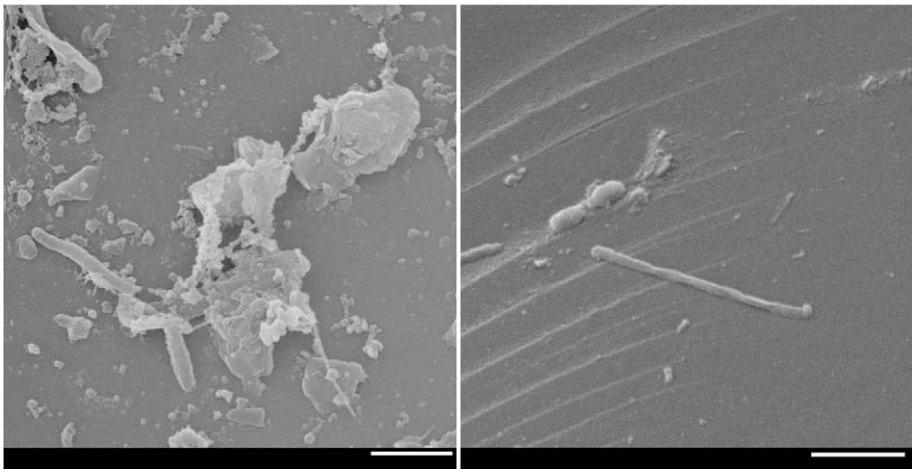

Fig. 2. SEM photomicrographs of glass surfaces from in situ microcosms. Glasses were reacted with the native groundwater at well 532B for 9 months. From left to right: ApGo glass and the non-nutrient Pyrex glass. ApGo glass has heavy colonization with abundant glycocalyx. Surface etching was not detected on any of the glasses although it is possible that glycocalyx may obscure dissolution features. The non-nutrient Pyrex glass has scant colonization by isolated cells with no discernable glycocalyx and no etching features.

3.2. Complex stability

Our experiments confirm that 3,4 DHBA forms a stable complex with Fe(III) derived from solid-phase, amorphous ferrihydroxide (Table 3). At equilibrium with the solid phase, the stability constant, β, was calculated with concentrations of Fe^{3+}, $Fe^{(III)}$/3,4 DHBA using the following equations:

$$Fe^{3+} + 3OH^- \Leftrightarrow Fe(OH)_{3(s)} \quad (1)$$

$$Fe^{3+} + 3,4DHBA^- \Leftrightarrow (Fe^{(III)}/3,4DHBA)^{2+} \quad (2)$$

$$\beta_{Fe} = \frac{(Fe^{(III)}/3,4DHBA)^{2+}}{(Fe^{3+})(3,4DHBA^-)} \quad (3)$$

The resulting conditional stability constant, calculated assuming a 1:1 complex stoichiometry, for $Fe^{(III)}$/3,4 DHBA was $\beta_{Fe(III)} = 10^{16.5}$ at 25 °C. This complex becomes slightly less stable as temperature increases, dropping to $10^{16.1}$ at 40 °C. This is a substantially stronger complex than the 3,4DHBA/Al complex from the literature (Table 2) and is in line with the predictions of the Irving–Williams series (Irving and Williams, 1953). UV-difference analysis of the 3,4 DHBA/Al system shows a strong chromophore at 325 nm.

3,4 DHBA also formed a stable complex with dissolved silica after equilibration with solid amorphous silica (Table 3). A weak chromophore at 240 nm was identified in the UV-difference experiment, indicating a charge–transfer complex occurring between silicic acid and the organic ligand. The stability constant was calculated from the difference in total solubility of amorphous silica in water compared to the organic ligand solution, i.e.:

$$Si(OH)_4 + 3,4DHBA^{-1} \Leftrightarrow ((OH)_2SiO_2/3,4DHBA)^{-1} + 2H_2O \quad (4)$$

$$\beta = \frac{(Si^{(IV)}/3,4DHBA)^{-1}}{(Si(OH)_4)(3,4DHBA^-)} \quad (5)$$

The resulting conditional stability constant, again assuming a 1:1 complex stoichiometry, is $\beta_{Si} = 10^{2.4}$ at 25 °C.

3,4 DHBA forms a much stronger complex with Fe than Si, with Al between the two extremes, but is still capable of chelating these important silicate framework-forming metals. The accelerated silicate dissolution observed in the study aquifer occurs at circumneutral pH, where proton-promoted dissolution is at a minimum. Therefore, it is likely that a ligand-promoted mechanism is responsible for the observed disso-

lution (e.g., Ullman et al., 1996; Welch and Ullman, 1993). Although 3,4 DHBA has lower stability constants than other ligands, it is an appropriate ligand to use in this study because of its ability to complex with Fe^{3+}, Si^{4+} and Al^{3+}, presence in the study aquifer and potential role as a carbon substrate for the native anaerobic consortium. 3,4 DHBA may mobilize Fe^{3+} for DIRB and increase the dissolution rate of silicates by forming framework destabilizing surface complexes with aluminum and, to a lesser extent, silica.

3.3. Weathering, release and utilization of nutrients

Abiotic release rates of P, Fe and Si from silicates were determined to compare with laboratory microcosm experiments in which the active microbial community might consume or transform these constituents. The design of each experiment, surface areas, final concentration of P, Fe and Si, and bulk dissolution rates are listed in Table 4. Mass transfer of P, Fe and Si are expressed as μmol m^{-2} of mineral or glass and are summarized in Figs. 3–5. The bulk rate of dissolution (J) was calculated as d(P, Fe, Si)/d*t* over the linear portion of each mass–transfer curve.

Abiotic batch dissolution experiments using both acetate and the model ligand, 3,4 DHBA, indicate that the minerals and glasses and their inclusions act as independent phases (i.e., P and Fe release are not dependent on Si removal; Figs. 3–5). The ratio of Si to P in the solid phase was much greater than in solution (Table 4), likely because P is not present as a matrix element but rather is released from included apatite crystals. Apparently, the surface expression of inclusions is large enough that dissolution is independent of the weathering of the silicate matrix on the relatively short time scale investigated. The 3,4 DHBA electrolyte stimulates the mass transfer of Si and Fe due to ligand-promoted dissolution. Both mass transfer and bulk dissolution rates increase in the presence of 3,4 DHBA compared to acetate buffer for both feldspars and glass.

Laboratory microcosm reactors containing the active microbial consortium were initially P-limited, contained an initial source of iron as Fe^{3+}/3,4 DHBA, but no source of phosphate other than the mineral/glass-bound apatite. Tween 80, the nonionic surfactant used to remove microorganisms from sediment, contains phosphate; however, the final concentration of phosphate in solution from this source did not exceed 1 μmol l^{-1}, which is the approximate concentration in the study aquifer.

In laboratory microcosms, only P-bearing minerals and glasses had a net release of orthophosphate, with ApGo glass releasing the most P followed by the Ap glass, microcline and anorthoclase (Fig. 6). Release of silica from mineral experiments was detected primarily in P- and Fe-bearing minerals, while silica was released in all glass microcosms (Fig. 7). The microbial consortium reacted to the presence of Fe^{3+}/3,4 DBHA by reducing the chelated Fe^{3+} to Fe^{2+} after 24 h. In all microcosms, ferrous iron increased compared to the blank, except for microcline, which was slightly lower than the

Table 4
Summary of experimental conditions and final results from bulk dissolution experiments at 25 °C and pH 5

Silicate[a]	SA[b]	Si/P[c]	Electrolyte[d]	Bulk rate[e]	Final P[f]	Bulk Rate	Final Fe	Bulk Rate	Final Si	Si/P[g]
Anor	0.43	298	Acetate	4.05×10^{-7}	0.259	3.53×10^{-5}	7.8	9.25×10^{-5}	54.4	210
			3,4 DHBA	1.42×10^{-5}	0.759	8.55×10^{-5}	28.0	1.01×10^{-4}	91.1	120
Mic	0.23	274	Acetate	2.22×10^{-5}	0.936	3.32×10^{-6}	0.2	2.27×10^{-4}	98.8	106
			3,4 DHBA	2.34×10^{-5}	0.939	2.14×10^{-5}	2.94	3.38×10^{-4}	108.6	116
ApGo	0.26	94	Acetate	8.21×10^{-5}	1.25	4.95×10^{-5}	0.3	6.05×10^{-5}	11.9	10
			3,4 DHBA	5.20×10^{-5}	3.40	8.51×10^{-5}	12.58	6.65×10^{-5}	19.68	7

[a] Experimental run time = 159 h. Anor is anorthoclase, Mic is microcline and ApGo is ApGo glass.
[b] Surface area of the solid expressed as $m^2\ g^{-1}$.
[c] Molar ratio of Si to P in solid phase mineral or glass.
[d] Electrolyte concentrations were 1 mmol l^{-1}.
[e] Dissolution rate as μmol $m^{-2}\ s^{-1}$ mass transfer of P, Fe or Si.
[f] Final concentration of P/Fe/Si expressed as μmol l^{-1}.
[g] Molar ratio of Si to P in solution at end of experiment.

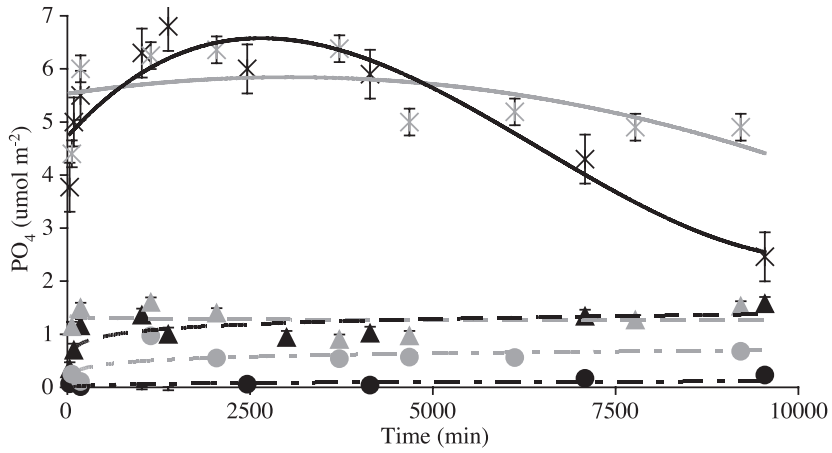

Fig. 3. Abiotic release of phosphate from microcline (▲), anorthoclase (●) and ApGo glass (×) over time in batch reactors containing 1 mM acetate (black) buffer or 1 mM 3,4 DHBA buffer (gray) at pH 5. ApGo glass has the highest rate of mass transfer followed by microcline and anorthoclase.

blank (Fig. 8). Anorthoclase and the ApGo, Ap and Go glasses had the highest concentrations of Fe^{2+}. Degradation of 3,4 DHBA was detected in microcosms after 21 days, with the formation of an intermediate degradation product tentatively identified by HPLC as catechol (e.g., He and Wiegel, 1996). While degradation of catechol occurred, it did so very slowly and not until all of the 3,4 DHBA had been used. The percent removal of 3,4 DHBA (expressed as removal of 3,4 DHBA + catechol) (Fig. 9) varied with mineral or glass composition.

There is mass transfer of P from microcline at pH 5, while little transfer occurred from anorthoclase (Fig. 3; Table 4). This likely is due to the form of phosphate present in each mineral. The microcline contains fluorapatite, while anorthoclase contains some fluorapatite but also some REE phosphates, which are much less soluble than fluorapatite (e.g.,

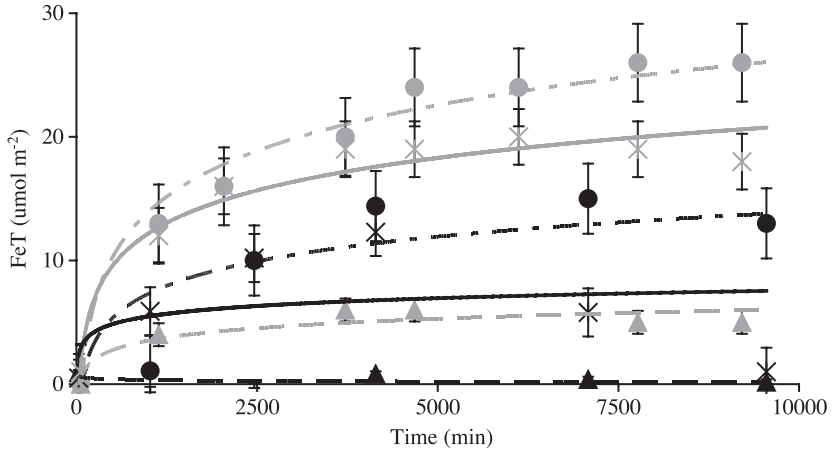

Fig. 4. Abiotic release of iron from microcline (▲), anorthoclase (●) and ApGo glass (×) over time in batch reactors containing 1 mM acetate buffer (black) or 1 mM 3,4 DHBA buffer (gray) at pH 5. The iron-bearing silicates, anorthoclase and ApGo glass have a higher rate of mass transfer than does microcline. The 3,4 DHBA buffer increases mass transfer of iron due to its chelating ability.

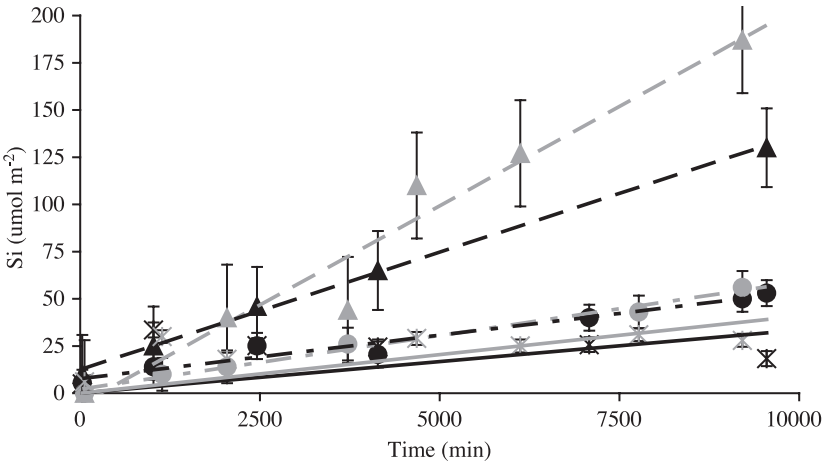

Fig. 5. Abiotic release of silicon from microcline (▲), anorthoclase (●) and ApGo glass (×) over time in batch reactors containing 1 mM acetate buffer (black) or 1 mM 3,4 DHBA buffer (gray) at pH 5. Microcline has the highest mass transfer of silicon followed by anorthoclase and ApGo glass. The 3,4 DHBA buffer increases mass transfer of silicon due to its chelating ability.

Vieillard and Tardy, 1984). Oelkers and Poitrasson (2002) reported steady-state dissolution rates for the REE phosphate monazite at 70 °C that were six to eight orders of magnitude slower than corresponding values for basaltic glass (Guy and Schott, 1989) and found that dissolution minima occurred at near neutral pH. The weathering of apatite at neutral pH is surface controlled (Christoffersen et al., 1978), and the kinetics of dissolution increase in the presence of some organic acids (Welch et al., 2002; Margolis and Moreno, 1992). In the present study, the presence of 3,4 DHBA did not appear to change the rate of P dissolution from the glass or silicates studied, suggesting that 3,4 DHBA is not particularly reactive with fluorapatite. The bulk dissolution rate of P from anorthoclase (Table 4) increased, however. One possibility is that 3,4 DHBA chelates REE and the increased release is ligand-promoted REE phosphate dissolution. Another possibility is that some phosphate is sorbed to surface-exposed iron oxides and is released as the 3,4 DHBA chelates ferric iron, as evidenced by the increased bulk dissolution rate of Fe from anorthoclase. In general, the silicate-associated phosphate bioavailability will vary with the

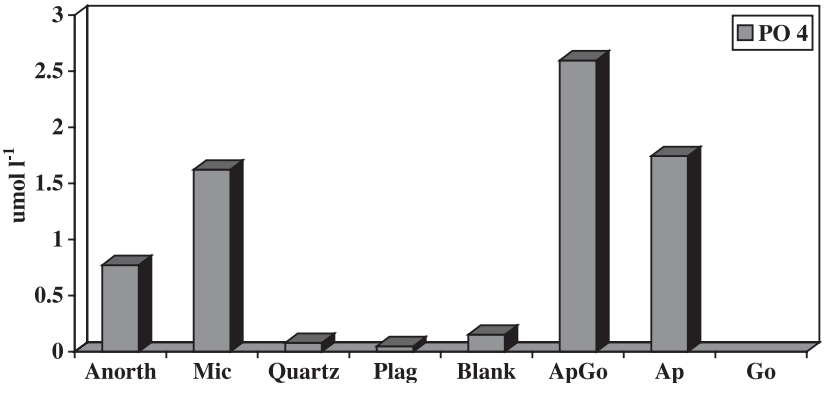

Fig. 6. Phosphate concentrations in laboratory microcosms containing 3,4 DHBA. Concentration is expressed as umol PO_4^{3-} m^{-2} of silicate mineral or glass, with the sterile control subtracted. Phosphate release was detected in microcosms that contained P-bearing silicates.

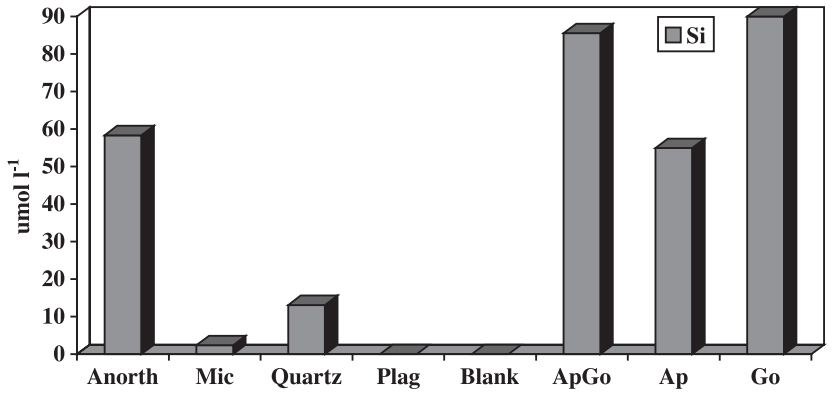

Fig. 7. Silicon concentrations in laboratory microcosms containing 3,4 DHBA. Concentration is expressed as μmol Si m^{-2} of silicate mineral or glass, with the sterile control subtracted. Silicon release was highest in microcosms that contained Fe-bearing silicates.

abundance of surface-exposed apatite in the silicate matrix as well as with phosphate mineral composition.

In live microcosms, phosphate concentrations were highest for ApGo glass, while less phosphate was released from Ap glass, microcline and anorthoclase (Fig. 6). Release rates for P in live microcosms are similar to those observed in the abiotic experiments (Fig. 3), although the pH is ~ 1.5 units higher in the live experiments. Overall, release appears to have been affected very little by the presence of metabolizing cells or their metabolic byproducts.

While microorganisms do not appear to significantly increase the concentration of dissolved P, microbial biomass measurements in laboratory microcosms reveal that cells are actively utilizing P from glass and minerals. Biomass was highest in microcosms containing P-bearing silicates and glasses. Biomass in Go glass microcosms increased slightly, but to a lesser degree than biomass in microcosms with Ap-bearing glasses and silicates. While biomass in glass microcosms was less than the overall biomass in mineral microcosms, the same trend was observed and the difference is likely to have been due to a smaller initial inoculant in these microcosms. Biomass increases only in microcosms that contain P-bearing silicates, indicating that growth was stimulated by the silicate-derived P, but it is not clear whether the cells are significantly increasing or decreasing the release of P from the silicate matrix on the time scale observed. It is possible that microorganisms promote P release, but it is utilized by the cells and therefore not detected. Over longer periods, the microorgan-

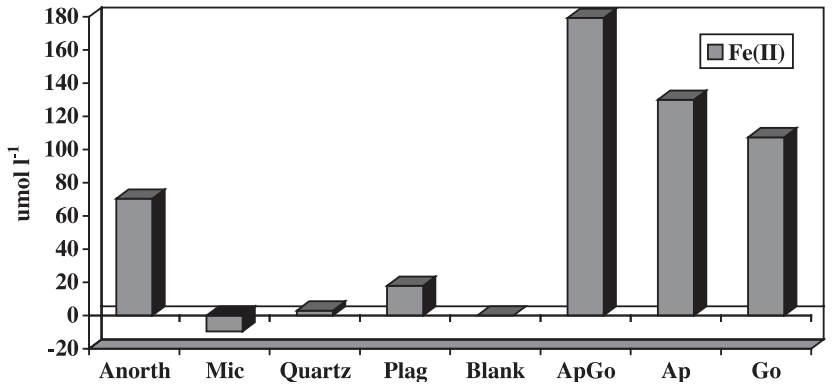

Fig. 8. Ferrous iron concentrations in laboratory microcosms containing 3,4 DHBA. Concentration is expressed as μmol Fe^{2+} l^{-1}, with the bottle blank subtracted. Microcosms contained an initial source of chelated Fe^{3+}. Additional Fe^{2+} is likely derived from silicate-bound iron oxide inclusions.

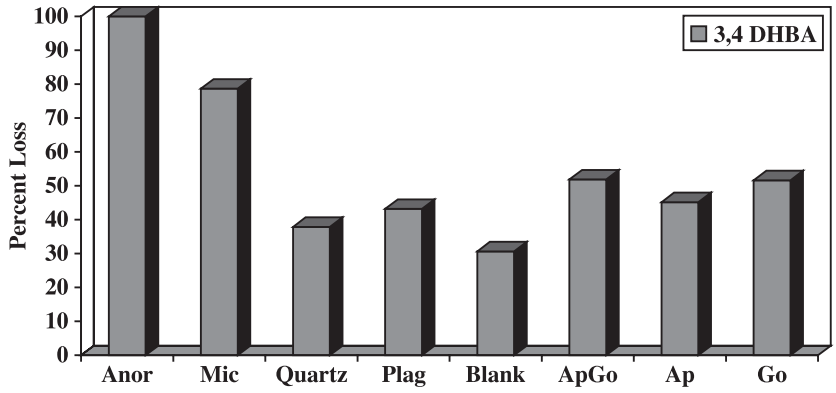

Fig. 9. Biodegradation in lab microcosms expressed as percent loss of 3,4 DHBA + catechol (its degradation intermediate) less the sterile control. Removal of 3,4 DHBA was stimulated by the presence of P- or Fe-bearing silicates, suggesting that the microbial community is actively accessing and utilizing nutrient inclusions.

isms' ability to dissolve the silicate matrix and expose fresh surface area of apatite may allow them to continue taking advantage of P release.

The observed increase in biomass also correlates with increased removal of 3,4 DHBA (Figs. 9 and 10); more 3,4 DHBA was removed from microcosms containing either P or Fe in silicate form. While biomass is one controlling factor in the degradation of carbon substrate (e.g., Monod, 1949), Fe-bearing silicates also increased the removal of 3,4 DBHA evidenced by the higher rate of 3,4 DHBA removal in the microcosms containing Fe-bearing silicates (Fig. 9). This finding suggests that the microbial consortium is capable not only of accessing P from silicates but also may use silicate-bound iron oxides as a TEA for the metabolism of organic carbon (Table 5).

Table 5
Summary of results for laboratory studies

	P or Fe	Biomass (Fig. 10)	Weathering (Fig. 7)	Biodegradation (Fig. 9)
Silicates				
Anorthoclase[a]	yes	+++[b]	+++	+++
Microcline[c]	yes	++	–[b]	++
Plagioclase[d]	no	–	–	+
Nutrient glass				
Ap+Goe[a]	yes	+++	+++	+++
Apatite[c]	yes	++	++	++
Goethite[e]	yes	+	+++	+++
Pyrex[e]	no	–	–	+

[a] Carbon + microbes + P and Fe → degradation product + Fe^{2+} + biomass.

[b] Relative amount of biomass, weathering (silica release) and biodegradation are indicated by + through +++, where + indicates that the feature was observed but to a lesser degree and ++ indicates a moderate degree, and +++ was the highest degree observed. (–) Indicates that the feature was absent. Refer to Figs. 7, 9 and 10 for absolute values for biomass, release of silica and removal of 3,4 DHBA. Ranges are defined separately for mineral experiments and glass experiments.

[c] Carbon + microbes + P → biomass.

[d] Carbon + microbes → minimal activity and metabolism.

[e] Carbon + consortium + Fe^{3+} → degradation product + Fe^{2+}.

Microbial activity also influenced the release of Fe and Si from the silicate matrix in microcosms containing active cells (Figs. 7 and 8). In abiotic experiments, microcline dissolution was faster than that of both anorthoclase and ApGo glass, but in microbially active experiments, we observed a reversal in the order of dissolution. While an increase in biomass occurred in microcosms containing microcline, there was no corresponding increase in Si concentration. Therefore, microbial biomass is not the sole control on mineral and glass dissolution. One possibility is that there are more attached cells in the anorthoclase, ApGo and Go glass microcosms, while more planktonic cells occur in the other microcosms. Researchers have observed a strong correlation between surface etching and proximal attached cells suggesting that a reactive microenvironment may exist around the metabolizing cell that is responsible for accelerated dissolution (e.g., Bennett et al., 2001;

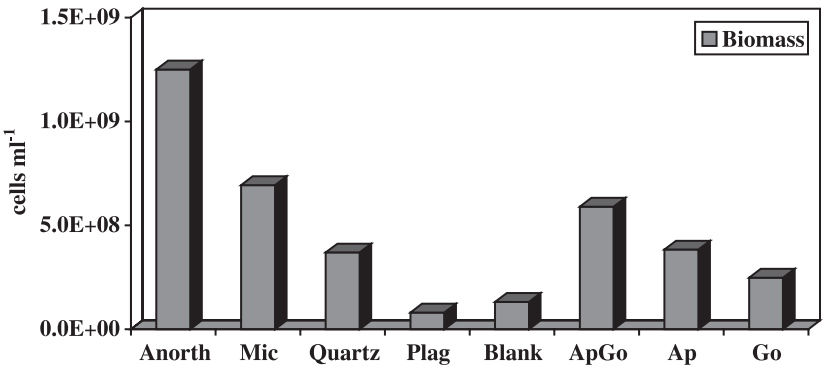

Fig. 10. Microbial biomass in lab microcosms. Biomass is expressed as cells ml^{-1} less the sterile control. The microbial community is apparently accessing silicate-bound P, which stimulates cell growth.

Liermann et al., 2000a). Other researchers have observed that DIRB attach to iron surfaces and reduce iron only near the point of attachment due to a membrane-bound reductase (Grantham and Dove, 1996; Lovley et al., 1989). Surface-exposed iron oxyhydroxides on anorthoclase and the ApGo and Go glasses, therefore, may promote attachment by this fraction of the mixed consortium. Furthermore, the coincident release of Fe(II) and Si suggests that DIRB are active in these microcosms and therefore may play a role in silicate dissolution, possibly by degrading aromatics to secondary metabolites capable of chelating Si and Al.

In situ, intermediate metabolites from degradation of toluene and benzene include reactive ligands that are detectable in micromolar quantities but may be more concentrated around metabolizing cells. In live microcosms 3,4 DHBA was degraded to catechol, a strong Si chelator and high concentrations of catechol at the mineral surface may have caused intense ligand-promoted dissolution of the silicate surface. Reduction of iron appears to be intimately linked to the mobilization of Si, and aromatics are degraded primarily through iron reduction at the study site (Anderson, 1998; Rooney-Varga et al., 1999). DIRB, therefore, may be responsible for producing a variety of secondary metabolites that are ultimately responsible for silicate dissolution. While attached cells appear to benefit from silicate dissolution, it is still unclear whether this interaction is incidental to metabolism or if ligands are produced specifically to mobilize nutrients from resistant silicates.

4. Summary and conclusions

Microorganisms preferentially colonize and weather silicates that contain the limiting nutrients P and Fe, while leaving similar non-nutrient silicates uncolonized and unweathered. Not only do microorganisms preferentially attach to nutrient-bearing silicates, but silicate-bound nutrients are bioavailable to the colonizing cells. The microbial consortium benefits from the release of nutrients by using Fe and P and increasing biomass and biodegradation rate, and this effect is intensified when both P and Fe are present. Furthermore, colonizing cells dissolve the silicate matrix by producing ferric-specific iron chelators. These ligands also form stable bidentate complexes with Si and Al, effectively removing these metals from the crystal lattice and increasing dissolution rate. Silicates may be a convenient source of nutrients, such as P and Fe, which are vital to microorganisms, and silicate weathering in nutrient limited environments may be controlled exclusively by microbial processes. The results of this study show that this interaction is restricted to only those surfaces that offer nutritional value to the native microbial consortium.

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