Microbiological and Chemical Aspects of Corrosion of Sheet Steel in the Duluth-Superior Harbor

A thesis submitted to the faculty of the graduate school of the University of Minnesota

By

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Dedication

This thesis is dedicated to my father, who always made an effort to get all of his children to take an active interest in math and science. Whether it was taking me to the Nobel Conference, driving us three hours to tour a nuclear power plant, enrolling us in university math programs like project YES and later UMTYMP, helping with school science projects, forwarding newspaper articles of interest to us, or just sitting at home talking about science, you were an important influence and a good role model. I'm very grateful for all that you've done.

Abstract

Steel structures in the Duluth-Superior harbor show unusual patterns of corrosion characterized by raised blister-like nodules called tubercles and pitted steel. This corrosion phenomenon could possibly decrease the integrity and the lifespan of the structures. Microbiological and chemical factors that may be influencing corrosion processes were examined in this study from 2007-2009. A laboratory microcosm experiment was designed to examine several microbiology and water quality parameters. Terminal-restriction fragment length polymorphism (T-RFLP) analyses showed that bacterial communities on steel were different from one another in microcosm treatments with different types of water (e.g. autoclaved Duluth-Superior harbor water vs. unaltered Duluth-Superior harbor water and Lake Superior water vs. Duluth-Superior harbor water). Coupons with different bacterial communities also showed differences in sulfate-reducing bacterial abundance and iron-oxidizing bacterial abundance. Further, coupons with higher abundances of sulfate-reducing bacteria and iron-oxidizing bacteria had higher inverse polarization resistance values. Inverse polarization resistance is frequently used to approximate the instantaneous rate of corrosion. Mass lost and pit depth were measured on steel in the microcosm, and these two measurements showed a positive correlation. Microbiological and corrosion measurements were also performed on steel in the Duluth-Superior harbor, and these measurements were compared to steel in the microcosm experiment. These experiments showed correlations among water chemistry, bacterial community, and populations of specific bacteria that are associated with corrosion in other ecosystems.

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List of Selected Abbreviations

Full title	Abbreviation
Duluth-Superior harbor	DSH
Microbiologically influenced corrosion	MIC
Terminal-restriction fragment length polymorphism	T-RFLP
Sulfate-reducing bacteria	SRB
Iron-oxidizing bacteria	FeOB
Polarization Resistance	R_p
Linear Polarization Resistance	LPR
High-Density Polyethylene	HDPE
Dissimilatory Sulfite Reductase	*dsr*
Colony-forming units	CFU

Chapter I

General Introduction and Overview

Corrosion, Iron-oxidizing Bacteria, and the Duluth-Superior harbor

Sheet steel structures in the Duluth-Superior harbor (located at the Twin Ports of Duluth, MN and Superior, WI, USA) show an unusual pattern of corrosion (Marsh et al. 2005). Raised blister-like nodules called tubercles appear on the surface of the steel. These tubercles cover pits of localized metal loss, and in extreme cases, these pits may entirely perforate the steel (Larsen 2008). Damage by pitting and perforation compromises the integrity of the steel and could possibly decrease the lifespan of the structures. The loss of infrastructure due to the corrosion phenomenon may affect up to 20 km of structures in the Duluth-Superior harbor (Marsh et al. 2005; MitMan 2006). The cost to replace these structures was recently estimated at $200-$250 million (Larsen 2008; Hicks 2009), meaning that the issue is of great economic interest to the shipping industry and the Duluth-Superior metropolitan area as a whole. The principle goal of my research was to examine how microbiology and water quality may affect the corrosion of the structures. This introduction will describe some general aspects of corrosion, review important features of iron-oxidizing bacteria and sulfate-reducing bacteria, indicate how these bacteria have been implicated in corrosion in other environments, and discuss how some of these phenomena may be involved in corrosion of steel in the Duluth-Superior harbor.

Corrosion of steel structures is a spontaneous, continuous process in aqueous environments. Iron atoms (Fe^{o}) lose two electrons, and these electrons remain on the surface of the metal, while ferrous ions (Fe^{+2}) diffuse into the water immediately adjacent

to the structure (Whitman et al. 1924; Tait 1994). Although the negative charge on the surface of the metal and the positive charge of the Fe^{+2} ions are both offset to some extent by dipoles of the water molecules, these opposite charges are still attracted to one another, causing the ions to remain in close proximity to the surface of the metal (Tait 1994). Corrosion reactions proceed in the forward direction when the electrons on the metal surface reduce ions or molecules in the water (H^+, O_2, etc.) (Hamilton 1985). Reduction of these electrochemically active species decreases the negative charge of the steel surface, causing the Fe^{+2} ions to freely diffuse into the water (Swaddle 1997). This conversion of $Fe^o(s)$ into $Fe^{+2}(aq)$ followed by loss of electrical attraction to the metal surface is the principle step in metal loss by corrosion.

In aerobic freshwater near neutral pH, Fe^{+2} tends to react with oxygen and hydroxide to form iron oxides, iron hydroxides, and iron oxyhydroxies ($Fe_2(OH)_3$, FeO(OH), Fe_3O_4, Fe_2O_3, and other similar species) (Swaddle 1997; Madigan et al. 2006). These oxidized iron species (Fe(III) oxide and hydroxide species) are much less soluble in water than Fe^{+2} or corresponding Fe(II) species (Weast 1973; Swaddle 1997) and tend to precipitate from the bulk medium. While the layer of oxidized iron does not form a passive film (as occurs in stainless steels, Hamilton 1985; Little and Lee 2007), the oxide layer does substantially reduce the rate of corrosion, as less of the surface area of the structure is bare steel that is exposed to water (Whitman et al. 1924).

pH has an important effect on corrosion reactions. At lower pH, Fe^{+2} reacts with oxygen at a much slower rate (Singer and Stumm 1970), resulting in slower production of iron oxide species. This is due to the difference in the redox potential of the ferric/ferrous iron couple at neutral and acidic pH (Ehrenreich and Widdel 1994 and references

therein). Further, protons are an excellent electrochemically active species that are readily reduced in corrosion reactions. Neutral pH necessarily has a substantially lower concentration of protons. Also, Fe^{+3} corrosion products are more soluble in acid (Swaddle 1997). Thus, steel structures tend to corrode faster at lower pH.

In some environments, iron-oxidizing bacteria (FeOB) can oxidize ferrous iron to ferric iron. The nature of iron-oxidizing bacteria was of some debate through the earlier part of the 20th century (Pringsheim 1949). Low pH environments in which Fe^{+2} does not readily donate electrons to O_2, such as acidic mine waters, were one of the first places that this phenomenon was recognized to have substantial ecological implications (Colmer and Hinkle 1947). However, other types of iron-oxidizing bacteria also thrive at neutral pH in aerobic environments, and still other species of FeOB grow in the absence of oxygen (for example, photoautrophic and anaerobic nitrate-dependent FeOB, Ehrenreich and Widdel 1994; Hafenbradl et al. 1996). The aerobic FeOB growing at neutral pH are microaerophilic, growing in areas where oxygen concentrations are reduced (reviewed in Emerson and Weiss 2004). Lower oxygen concentrations are necessary for the bacteria to compete with the abiotic rate of ferrous iron oxidation. Some of the most common locations of microaerophilic FeOB include wetland sediments, water distribution pipes, and groundwater seeps (Emerson and Weiss 2004). Some iron-oxidizing bacteria produce a sheath of extracellular polymeric substances (EPS), and iron oxides have been shown to be deposited in the sheath (reviewed in van Veen et al. 1978). These FeOB may also excrete an enzyme involved in iron oxidation (Corstjens et al. 1992). While iron-oxidizing bacteria at circumneutral pH may not substantially increase the rate at which Fe^{+2} is converted to Fe^{+3} on a geological scale (Weber et al. 2006), FeOB do grow on

steel structures found in water at circumneutral pH. The FeOB attached to steel structures may influence patterns of corrosion in a phenomenon known as microbiologically influenced corrosion (MIC).

As its name suggests, microbiologically influenced corrosion occurs when microorganisms are responsible for an increase in corrosive damage to a metal structure. Microorganisms can directly metabolize metal (e.g. iron oxidation), or the metabolism may produce a waste byproduct that is corrosive (e.g. sulfate reduction, Hamilton 2003). Alternatively, the bacteria may create a unique biochemical environment that causes corrosion reactions that wouldn't normally be predicted, and the microbiological presence indirectly results in a localized attack on the metal (Little et al. 1992). Bacteria normally promote corrosion in a complex symbiosis, involving many different species of bacteria engaging in multiple corrosive actions (Hamilton 1985). MIC has been recognized as a problem for many decades in a variety of environments. One of the most common places for MIC activity is enclosed pipes that move water, including those found in power plants, fire-suppression sprinkler systems, and municipal water distribution systems (Borenstein and Lindsay 1988, 1994). MIC has also been recognized as a factor in the degradation of steel structures like docks (Beech and Campbell 2008). Microbial biofilms consisting of a consortium of bacteria grow by attaching to metal structures. These biofilms form in virtually all natural environments (Little et al. 1991).

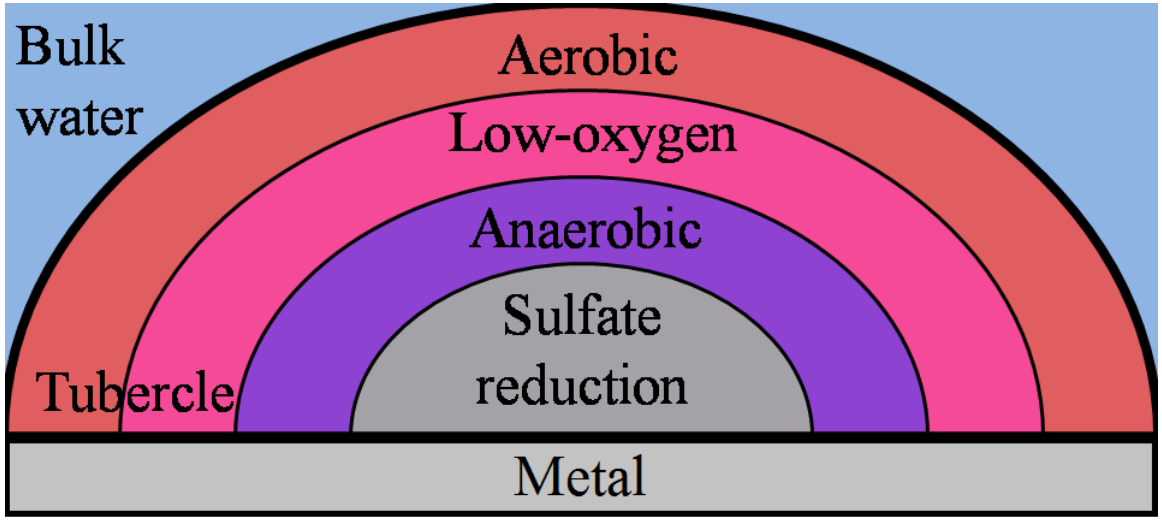
Iron-oxidizing bacteria can be an important factor in microbiologically influenced corrosion (Chamritski et al. 2004; Pitonzo et al. 2004). FeOB have been recognized in MIC for about 50 years (Shipley 1961). In order to establish a microaerophilic environment, FeOB and other synergistic bacteria may create an oxygen concentration

cell (Figure 1), reducing the concentration of oxygen in their immediate environment (Little and Lee 2007). FeOB can colonize steel surfaces within a matter of weeks (Rao et al. 2000). As the FeOB develop, they deposit insoluble iron oxides and iron hydroxides in a manner mediated by enzymes (Corstjens et al. 1992), a process that can form tubercles on the steel (Little and Lee 2009). The area under the tubercles is normally devoid of oxygen and can become an anode in a galvanic double with the adjacent (oxygenated) steel (Hamilton 1985), causing pitting corrosion of the area under the tubercle. A decrease in pH frequently accompanies the formation of the anode under the tubercle, further increasing the rate of corrosion (Little and Lee 2007). Accelerated corrosion involving FeOB and tubercles can be exacerbated by the presence of chloride. Iron present at the anode forms corrosive iron chlorides (Little and Lee 2007). Iron chlorides are very soluble in water (Weast 1973).

In previous work, Hicks (2009) isolated an iron-oxidizing bacterium from corroded areas (tubercles) of steel structures in the Duluth-Superior harbor. This bacterium is most similar in genetic identity to strain ES-1 reported by Emerson and Moyer (1997), a strain that has been tentatively named *Sideroxydans lithotrophicus*. This bacterium was not isolated from a nearby site where no tubercles were observed. *Sideroxydans lithotrophicus* gains energy by oxidizing Fe(II) to Fe(III), and it grows at neutral pH under microaerophilic conditions (Emerson and Moyer 1997).

Perhaps the most widely established cause of MIC is sulfate-reducing bacteria (SRB). These bacteria gain energy by oxidizing organic compounds or H_2 and using the sulfate ion as the terminal electron acceptor (Holt et al. 1994). The final product of their metabolism is H_2S and water. SRB are obligate anaerobes, but they are found in many

Figure 1. A diagram of the differential aeration cell model of corrosion tubercles. The outer aerobic area contains aerobic heterotrophic bacteria, and the low-oxygen middle area contains various facultative anaerobic bacteria and microaerophilic bacteria, including iron-oxidizing bacteria. The inner areas contain fermentative bacteria and anerobes like sulfate-reducing bacteria. Concept for figure from Hamilton (1985).


Bulk
water
Aerobic
Low-oxygen
Anaerobic
Sulfate
reduction
Tubercle
Metal

aerobic environments in local anaerobic niches (Dilling and Cypionka 1990). When growing on a steel surface, SRB are normally found in a biofilm where oxygen is excluded from the metal-biofilm interface (Hamilton 2003). Oxygen is typically at a lower concentration only within a few millimeters of the metal surface (Little and Lee 2007). The production of H_2S increases the corrosion rate of iron as iron sulfide minerals form near the surface of the metal (Wikjort et al. 1980), covering the surface in a layer of black sulfide precipitate. Depending on the exact conditions of the MIC system, FeS can conduct electrons and cause the steel to become an anode (Hamilton 2003). In addition to galvanic currents, SRB have been implicated in corrosion through sulfide-induced stress cracking, hydrogen-induced cracking, and production of extracellular polymeric substances (EPS) (Little and Lee 2007). EPS are an important component of biofilms, aiding in the adhesion of cells to metal surfaces (Decho 2000). EPS bind to metals and tend to increase corrosion rates by increasing the rate of the cathodic reaction (Little and Lee 2007).

One widely recognized form of MIC is accelerated low-water corrosion (ALWC). The mechanism of ALWC has not been fully elucidated, but a microbiological sulfur metabolism consisting of both SRB and sulfur-oxidizing bacteria is generally recognized as one of the most important contributors (Maritime Navigation Commission 2006; Beech and Campbell 2008). ALWC occurs on metal structures in marine environments at water level at low tide, and this corrosion appears to occur regardless of geographical location (Beech and Campbell 2008). Corrosion products are loosely attached orange and black material, and the surface under the corrosion product becomes pitted (Gubner 1998). Some estimates indicate that more than 10% of ports in western Europe may be

affected by ALWC (Gubner 1998).

Investigation of the corrosion process in the Duluth-Superior harbor is important because it is a basic science issue, but it also has a clear practical application. Corrosion costs in the United States were estimated at $276 billion in 2001 (US DOT 2002) and costs are generally estimated at 3% of United States GDP (Swaddle 1997). The cost of replacing all of the affected structures in the Duluth-Superior harbor has been estimated at $200-250 million (Larsen 2008; Hicks 2009), a cost that is comparable to the shipping industry's entire annual economic impact on the Duluth-Superior metropolitan area (Duluth Seaway Port Authority 2001). A better understanding of the nature of this corrosion problem may contribute to mitigation of some of the damages to the steel structures. Further, some reports have suggested that other western Lake Superior harbors have incurred similar corrosion problems (Sharrow, J., Bergeron, D., Clark, G., pers. comm., unreferenced; see Notes). Therefore, information learned from the Duluth-Superior harbor may also be relevant in other Lake Superior harbors.

Chapter II

Microbiological and Chemical Aspects of Corrosion of Sheet Steel in the Duluth-Superior harbor

Introduction

Sheet steel structures in the Duluth-Superior harbor (DSH) on Lake Superior are reported to show an unusual and possibly accelerated rate of corrosion (Marsh et al. 2005; Bushman 2006). Many structures have developed large orange blister-like, raised tubercles. Corrosion tubercles are mounds of corrosion products and deposits that cap localized regions of metal loss. Under these tubercles, deep pits may form in the steel, and at its most severe, the corrosion can leave large holes that perforate the sheet steel of docks and bulkheads (Larsen 2008). Up to 20 km of structures may be affected by this type of corrosion in the DSH. Several other harbors in western Lake Superior are also reported to show a similar corrosion phenomenon (Sharrow, J. and G. Clark, pers. comm., unreferenced; see Notes). An expert panel on corrosion published a report (Marsh et al. 2005) in which they suggested several factors that may influence corrosion in the Duluth-Superior harbor, including water quality and microbiology.

It is clear that corroding steel structures in the DSH are covered by complex microbial biofilms that contain bacteria of the type responsible for corrosion of steel in other environments (Hicks 2009). Several types of bacteria have been implicated in corrosion of steel in other environments, including iron-oxidizing bacteria (Xu et al. 2007). Hicks (2009) isolated an iron-oxidizing bacterium tentatively identified as *Sideroxydans lithotrophicus* and has detected DNA from this organism at corroded sites in the DSH. Also, electron microscopy has shown that large numbers of bacteria, possibly iron-

oxidizers, grow on the underside of tubercles and associate with amorphous iron hydroxides (Ray et al. 2009). In addition to iron-oxidizing bacteria (FeOB), sulfate-reducing bacteria (SRB) have been implicated in corrosion in other environments (Hamilton 1985; Little et al. 2000). The concentration of sulfate varies with location in the Duluth-Superior harbor, and some areas with the highest dissolved sulfate concentrations also show the most aggressive corrosion and highest corrosion rates (Hicks 2009).

The pattern of corrosion in the Duluth-Superior harbor correlates with dissolved sulfate concentration (Hicks 2009), the presence of iron-oxidizing bacteria (Ray et al. 2009), and the abundance of the dissimilatory sulfite reductase gene from SRB found on the surface of corroding steel (Hicks et al. unpublished observation). This information suggests that microbes are associated with this corrosion, possibly through a phenomenon known as microbiologically influenced corrosion (Little and Lee 2007; Xu et al. 2007). Microbiologically influenced corrosion (MIC) is rarely caused by a single microbial group, but more often by consortia of microbes including iron-oxidizing and sulfate-reducing bacteria (Rao et al. 2000; Starosvetsky et al. 2001). While data from prior field research (Hicks 2009) lead us to suspect that MIC may be responsible for the corrosion patterns seen in this harbor, the data did not provide conclusive evidence. Thus, a laboratory experiment using microcosms was designed to test the effects of water quality and microbes in this corrosion process.

Specifically, the goal of the experiment was to determine if the metabolism of microbial biofilms attached to steel structures in the harbor affects the corrosion of sheet steel material identical to that used to construct docks and bulkheads in the Duluth-

Superior harbor. Overall bacterial communities between microcosm treatments were also compared. These measurements were used to examine the microbiological and corrosive differences between steel coupons immersed in Lake Superior water and Duluth-Superior harbor water (Table 1). These differences were also examined between coupons in harbor water with and without added sulfate, and in harbor water that was autoclaved and harbor water that was not autoclaved. Finally, these differences were examined between two sets of coupons in autoclaved harbor water, one in which the coupons were sprayed with iron-oxidizing bacteria. I hypothesized that coupons in harbor water would corrode more and have more prokaryotes of all types than those in lake water, and that more sulfate-reducing bacteria would be present on coupons in the harbor water with added sulfate than those in unaltered harbor water. I also hypothesized that coupons in autoclaved harbor water would have fewer prokaryotes of all types and would be less corroded than coupons in unaltered harbor water, and that coupons with iron-oxidizing bacteria sprayed on them would have more iron-oxidizing bacteria and be more corroded than corresponding coupons without the iron-oxidizing bacteria spray.

In addition to the laboratory experiment, an experiment in the harbor was also designed as a control for the laboratory experiment. The harbor experiment was carried out in conjunction with the Duluth Seaway Port Authority and the United States Army Corps of Engineers. Through these experiments, the corrosion rates and pitting of sheet steel were measured, as well as the abundance of total prokaryotes, iron-oxidizing bacteria, and sulfate-reducing bacteria.

Table 1. Experimental treatments used to investigate the corrosion of sheet steel coupons in aquatic microcosms.

Treatment	Description
1	Autoclaved harbor water
2	Unaltered harbor water
3	Unaltered Lake Superior water
4	Harbor water supplemented with sodium sulfate (30 mg SO_4^{2-} L^{-1})
5	Autoclaved harbor water + *S. lithotrophicus* inoculums

Harbor water was obtained from the Great Lakes Aquarium water intake in the Duluth-Superior Harbor and the Lake Superior water was obtained from the Lakewood water intake for the city of Duluth, MN, which is along the north shore of Lake Superior. *S. lithotrophicus* is an iron-oxidizing bacterium that was isolated from corroding steel structures in the Duluth-Superior harbor (Hicks 2009).

Methods

Microcosm Experiment

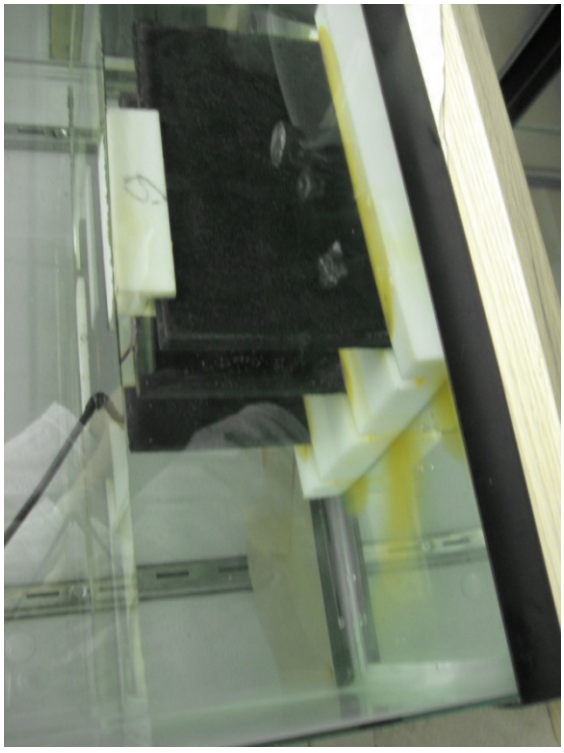
The effect of five experimental treatments on the corrosion of the steel coupons was examined (Table 1). This study combined measurements of corrosion of experimental steel coupons (instantaneous corrosion rate, area covered by tubercles, and corrosion mass lost and pit depth) with an investigation of bacteria that colonized and developed on these coupons in each experimental treatment.

Microcosm Construction

Experimental microcosms (Figure 2a) constructed from 10-gallon glass aquaria (Aqueon Glass, 50.8 cm × 25.4 cm × 30.5 cm) were used to test the corrosive effects of various chemical and microbiological treatments (Table 1). Each microcosm was equipped with an aquarium pump (Aquarium Systems Mini-Jet 404) to constantly circulate the water (~ 2 L hr^{-1}), and covered with a piece of acrylic (0.2 cm × 50.8 cm × 25.4 cm) with one corner cut out to allow gas exchange. The microcosms and covers were washed with soapy water, then rinsed with hydrochloric acid (10% by volume) and with Milli-Q deoionized water. The aquarium pumps were disinfected by soaking in 70% ethanol before treatments were established and when these treatments were renewed. All microcosms were incubated in the dark at 13°C (the average water temperature in the Duluth-Superior harbor during the ice-free season, Ksoll 2006) in a variable temperature room (Figure 2b).

Figure 2. (a) Photograph of a microcosm aquarium. Three coupons were held in place and electrically isolated from one another in the high density polyethylene (HDPE) holder. (b) Photograph of replicate experimental microcosms in the variable temperature room. (c) Photograph of a steel coupon before it was used in an experimental microcosm. Note the HDPE handle and the electrical wire on the top. (d) Close-up image of the wire brazed to a steel coupon. The wire was covered with orange epoxy to electrically isolate the wire, but a small amount was removed from the brazed area for this photograph.

(a)



(b)

(c)

(d)

Steel Coupons

Each experimental microcosm (Figure 2a) contained three steel coupons (Figure 2c). Steel coupons (19.1 cm × 11.7 cm × 1.25 cm) identical in composition to the sheet steel used to construct about 90% of the docks and bulkheads in the Duluth-Superior harbor (Chad Scott, AMI Consulting Engineers, pers. comm., unreferenced; see Notes) were cut from cold rolled sheet steel (ASTM A328, a low carbon steel). The Hallett Dock Company (Duluth, MN) donated this sheet steel after replacing a portion of their corroded docks in 2007. The steel coupons were washed with soapy water, lightly brushed, and rinsed with Milli-Q water to remove any loose material. Each coupon was designated with a unique number, weighed (each coupon weighed about 2 kg before the experiment), and then wrapped in aluminum foil and autoclaved before being randomly assigned to a microcosm and specific experimental treatment. All coupons were fitted with a high-density polyethylene (HDPE) handle (10.2 cm × 3.8 cm × 2.5 cm) to facilitate handling of the coupon (Figure 2c). Each microcosm had an HDPE coupon holder (25 cm × 20 cm × 2.5 cm) with three grooves (25 cm × 1.3 cm) to hold three steel coupons upright and keep them electrically isolated from one another (Figure 2a). Like the aquaria, the HDPE handles and holders were washed with soapy water, and then rinsed with dilute hydrochloric acid (10% by volume) and Milli-Q water.

One steel coupon in each microcosm was modified (Figure 2d) to measure polarization resistance in order to estimate the instantaneous corrosion rate. Biofilm on the surface of these coupons was not sampled each month, but all biofilm material was removed for analysis at the termination of the experiment. A copper wire (12 gauge) was brazed to the top edge of these coupons next to the HDPE handle so as not to interfere

with the formation of corrosion products on the face of the coupons. The plastic insulation was stripped from the top 5 cm of the wire to allow electrical contact with the device measuring polarization resistance (this area was not submerged). The brazed surface was covered with an epoxy glue to protect it from corrosion. Thus, the steel coupon and not the wire served as the working electrode.

Experimental Treatments

Duplicate microcosms of five treatments were constructed and filled with water (20 liters) of each treatment type (Table 1). The first experimental treatment consisted of autoclaved harbor water, which served as a "diminshed" biological treatment but not a truly abiotic control because these were not closed microcosms. Harbor water was collected from the intake of the Great Lakes Aquarium in Duluth, MN. Water in this treatment was autoclaved for 60 min at 121°C. The second treatment contained unaltered harbor water. The third treatment contained water from the nearshore zone of Lake Superior, collected northeast of Duluth from the Lakewood City Water Intake Station, which serves as the water supply for Duluth, MN. Lakewood is approximately 20 km from the harbor and is unlikely to be significantly influenced by water from the harbor. Both the harbor water and lake water were available from their respective sources throughout the year, even when ice prevented direct access to the harbor and Lake Superior. The fourth treatment contained unaltered harbor water that was supplemented with sodium sulfate (30 mg SO_4^{2-} L^{-1}). Measurements by Trace Analytical Labs (Muskegon, Michigan) in September 2006 indicated that the sulfate concentration was as high as 30 mg L^{-1} in the Duluth-Superior harbor. The sulfate concentration was raised to

a level that was at least double this amount by adding an additional 30 mg L^{-1} sulfate to water in this treatment to decrease the chance that the sulfate concentration limited the growth of sulfate-reducing bacteria. The final treatment contained autoclaved harbor water and steel coupons that were inoculated with an iron-oxidizing bacterium, which was isolated from corroding steel in the Duluth-Superior harbor and tentatively identified as *Sideroxydans lithotrophicus* by partial sequencing of its 16S rRNA gene (data not shown). This bacterial strain (HD506_02) was grown in FeS gradient culture tubes (Emerson and Moyer 1997), then the growth bands were extracted from multiple tubes (~10-12 ml) and used to inoculate the steel coupons with an "atomizer" spray bottle (~2 ml for each coupon).

Treatments in each aquatic microcosm were renewed monthly (within a few days of sampling) to prevent fouling (i.e. the buildup of decomposition products in the water) that was expected because the microcosms are closed systems (Ksoll et al. 2007). The steel coupons were temporarily removed and placed in a clean HDPE holder within a clean, empty aquarium. Water was emptied from each microcosm and then the microcosms, covers, and pumps were cleaned as described earlier. New water of the appropriate treatment was placed in each microcosm and then the steel coupons (unaltered during treatment renewal) were reintroduced. Fresh *S. lithotrophicus* inoculums were sprayed on coupons from the autoclaved harbor water + *S. lithotrophicus* treatment before these steel coupons were returned to their respective microcosms.

An additional unreplicated treatment was added later in the experiment. A third microcosm of unaltered harbor water was used to mimic the effect of ice on coupons. The Duluth-Superior harbor accumulates several feet of ice during the winter, and the

movement of ice may damage steel structures and remove tubercles and other rust that is found on the structures. In this scour microcosm, all corrosion product and biofilm material were scraped from each month starting at the end of month 7 and continuing through the end of the experiment. Month 7 was chosen to simulate the amount of time that structures in the harbor are affected by ice annually.

Sampling

The experiment was started on May 15, 2008 and continued for 12 months. The polarization resistance of modified steel coupons was measured semimonthly. Each month, biofilm and corrosion products were collected from the surface (about 20-30 cm^2) of two steel coupons in each microcosm (those not used for polarization resistance measurements). Biofilm and corrosion products on each coupon were scraped into a sterile 50 ml plastic tray using an acrylic scraper. After scraping, this area on each coupon was rinsed into the same tray with Milli-Q water. This scraped area was then scrubbed with a toothbrush, and rinsed again with Milli-Q water. This combined sample was transferred to a sterile 15 ml plastic centrifuge tube and centrifuged (2000*g*; 5 min.) Water overlying the sample was decanted, leaving the biofilm and corrosion products, which were stored briefly at 4°C. Subsamples of this material were distributed and used for counting total prokaryotic cells and for culture-based estimates of iron-oxidizing bacterial abundance. The remainder of the material was frozen (-20°C) for later extraction of DNA for molecular analyses.

Microbiological Analyses

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) Analysis. Each month, DNA was extracted from a subsample (approximately 0.5 g) of biofilm from each steel coupon using a PowerSoil DNA kit (MoBio Laboratories), which uses bead beating to help lyse cells. The DNA from these subsamples was frozen (-80°C). DNA collected after 4, 8, and 12 months was used to compare the overall similarity of bacterial communities in the experimental treatments by terminal-restriction fragment length polymorphism (T-RFLP) analysis (Liu et al. 1997). PCR amplification of the DNA extracts was performed with the 27 forward (27F; 5'-AGAGTTTGATCCTGGCTCAG-3') primer and the 1492 reverse (1492R; 5'-GGTTACCTTGTTACGACTT-3') primer, which are specific for the bacterial 16S rRNA gene (Lane 1991). The 27F primer was labeled with a 6-FAM fluorescent tag (Integrated DNA Technologies, Coralville, IA). Illustra PuReTaq Ready-To-Go PCR beads were used for the PCR reaction according to the manufacturer's instructions. DNA template was amplified in a BioRad DNA Engine Peltier Thermal Cycler under calculated control parameters. Following a 4 minute 94°C initial denaturation step, 22 cycles of PCR were carried out under the following conditions: 94°C denaturation for 30 seconds, 55°C annealing for 30 seconds, and 72°C extension for 75 seconds. A final 7 minute polishing extension at 72°C ended the cycle. PCR products were examined on a 1.5% agarose gel and cleaned using the Ultraclean PCR clean-up kit (MoBio Laboratories).

PCR products from each reaction were enzymatically digested using the *Hae*III and *Msp*I restriction enzymes (Promega) at 37°C for 3 hours. The digestion was terminated by incubation at 65°C for 15 minutes. Digested PCR products were precipitated in

ethanol, dried using a Speed-Vac (Savant SVC 100), and resuspended in nuclease-free sterile water. The terminal-restriction fragments (T-RFs) were separated at the BioMedical Genomics Center (BMGC) of the University of Minnesota. A solution containing 20% (v v^{-1}) T-RFs in water, 1.8% (v v^{-1}) MapMarker 1000 molecular weight standard (BioVentures), and 78.2% (v v^{-1}) Hi-Di Formamide (ABI) was heated at 95°C for 5 minutes and cooled on ice for 2 minutes. The T-RFs were detected with an ABI PRISM 3730xl DNA Analyzer at the BMGC.

After electrophoresis, T-RFs between 50 and 1000 bp were compared using the BioNumerics program version 6.1 (Applied Maths, Austin, TX). Bands were defined as peaks that were greater than 1% of the greatest peak height, and band classes were generated with a 0.5% position tolerance. Band class assignments were visually examined and manually redefined in some cases in order to assign bands to appropriate classes. After generating band classes, a dendrogram was created using the unweighted pair group method with arithmetic mean (UPGMA) method and cluster analysis using a Pearson correlation similarity matrix. MANOVA analysis was used to test whether nodes in the dendrograms represented treatment groups that were significantly different from one another based on the similarity of the bacterial biofilm communities on the sheet steel coupons. MANOVA was performed using principal components analysis (PCA) to determine the significant variability with a significance threshold of 95%. Two treatment groups were considered significantly different if the p-value was less than 0.05.

Total Prokaryotic Cell Abundance. Weighed subsamples (approximately 0.02 g) of biofilm and corrosion product were collected each month from all steel coupons, preserved with formaldehyde (2% final concentration), and stored at 4°C until cells were

stained with DAPI and counted using epifluorescence microscopy and UV illumination (Porter and Feig 1980). An aliquot of each subsample was stained for 5 min and then filtered onto a black polycarbonate filter (Poretics, 0.22 µm pore size). Fluorescent prokaryotic cells were counted (1,000 × total magnification) in at least 10 fields for each subsample (n=4 per treatment) and these counts were converted to prokaryotic cells per square centimeter of steel surface. The arithmetic mean was reported because the coupons did not differ in bacterial abundance by orders of magnitude.

Quantitative PCR of Dissimilatory Sulfite Reductase (*dsr*A) Gene. Microcosm DNA extracts were also used to quantitatively amplify the dissimilatory sulfite reductase (*dsr*A) gene to estimate the abundance of sulfate-reducing bacteria. Quantitative PCR (qPCR) was used to determine the number of copies of the *dsr*A gene on steel coupons in the different treatments. The *dsr*A-specific forward (DSR-1F+) and reverse (DSR-R) PCR primers (10 µM) developed by Kondo et al. (2004) were used to amplify a 221 bp PCR product. Reaction mixtures (25 µl) contained 12.5 µl Brilliant II SYBR Master Mix (Stratagene), 0.5 µl of each forward and reverse PCR primer (400 nM final concentrations), 20 µg of BSA, 10 ng of DNA template, and nuclease-free sterile water (Schippers and Neretin 2006). qPCR was performed using a Corbett Research RotorGene 3000 thermal cycler. The qPCR conditions were: 95°C for 10 min, and 40 cycles of 95°C for 15 sec, 60°C for 1 min, and a data acquisition step at 85°C for 15 sec. Accumulation of newly amplified DNA was tracked by the increase in fluorescence due to the binding of the SYBR green fluorescent dye to double-stranded PCR products. A melting curve analysis was performed at the end of the PCR reactions to check for PCR specificity and primer-dimer formation. Standard curves ranging from 8 to $8x10^9$ copies

of the *dsr*A gene (2 ag to 2 pg of genomic DNA) constructed with *Desulfovibrio vulgaris* genomic DNA (ATCC 29579D-5) were used to calculate the Ct value and subsequently the number of *dsr*A gene copies in biofilm DNA samples. Samples without DNA template (blanks) were used as a control to check for contamination of the qPCR reactions. The geometric mean of the number of *dsr*A copies per cm^2 was reported because abundance measurements differed by orders of magnitude and values were log-transformed to make statistical comparisons.

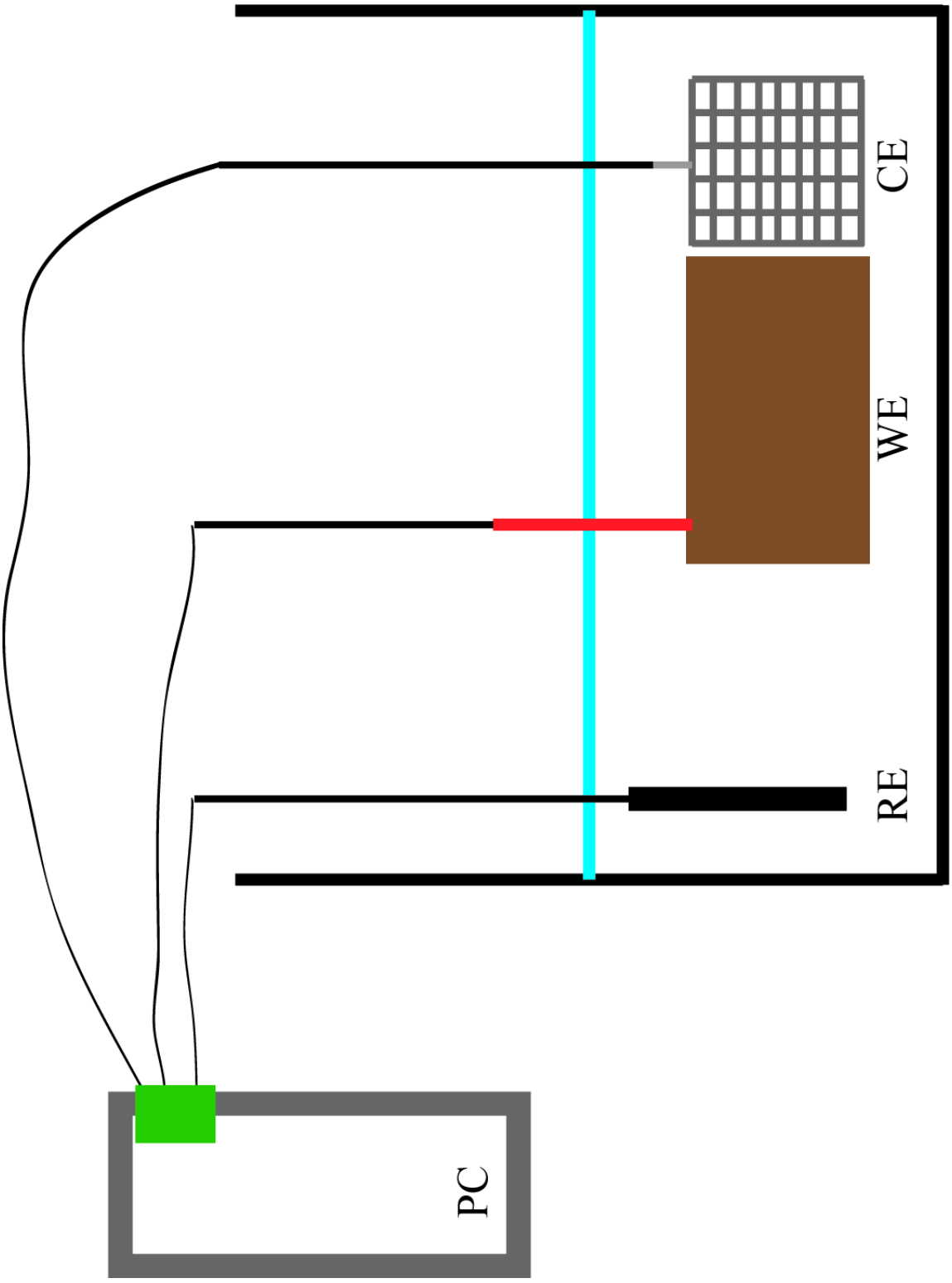
FeOB Dilution Cultures. Bimonthly, a biofilm subsample (0.02 g) from one steel coupon in each replicate microcosm of each treatment was diluted with 300 µl of sterile modified Wolfe's mineral medium (MWMM) in a sterile 96-well microplate. Only one sample was taken from each microcosm for effort and cost reasons. Then, ten-fold serial dilutions were made of this solution up to a 10^{-6} dilution. Iron gradient culture tubes containing a soft agarose gel of MWMM overlying an iron (II) sulfide plug (Emerson and Moyer 1997) were inoculated with a portion of these ten-fold serial dilutions to estimate the abundance of iron-oxidizing bacteria by a dilution-to-extinction technique. These gradient culture tubes were incubated at room temperature in the dark. After 4 weeks, the tubes were examined for growth of iron-oxidizing bacteria. The presence of cells in the rust-colored bands, which indicated the possible growth of iron-oxidizing bacteria, was verified by epifluorescence microscopy. Tubes with the highest dilution that showed cells were used to estimate the abundance of iron-oxidizing bacteria (i.e., iron-oxidizer colony-forming units (CFU) per square centimeter of steel surface). Abundances differed by orders of magnitude between treatments, so the geometric mean was calculated.

Corrosion Analyses

Linear Polarization Resistance. Polarization resistance (Scully 2000) was measured semimonthly in one steel coupon in each experimental microcosm (i.e., duplicate measurements per treatment). The steel coupon was the working electrode, and a mercury/mercury sulfate saturated calomel electrode was the reference electrode. A platinum-niobium mesh screen was the counter electrode. The reference electrode was placed 5 cm into the water near the coupon, while the counter electrode was immersed in the water immediately adjacent to the steel coupon (Figure 3). Polarization resistance was determined by connecting these electrodes to a PC computer with the Gamry Framework software. Electrical potential was fixed at 80 interval points between -10 mV and +10 mV of the open-circuit potential, and the current density passing through the circuit was measured at each of these points. The inverse of polarization resistance (R_p^{-1}) is frequently used to estimate instantaneous corrosion rate (Scully 2000). The arithmetic mean of measurements for the entire experiment was calculated for each coupon, and these means were statistically compared.

Corrosion Tubercle Area. Monthly digital photographs were made of both sides of the steel coupons that were used for polarization resistance measurements. Prints of these images were used to measure the area covered by orange corrosion tubercles. The image of the coupon was cut out and weighed. Afterwards, areas that were covered by corrosion tubercles were removed with an X-Acto knife, and the image was reweighed to estimate the percentage of the coupon area that was covered by orange corrosion

Figure 3. A diagram of the instrumental setup for polarization resistance measurements made during the microcosm experiment. The steel coupon was the working electrode (WE), and a mercury/mercury sulfate saturated calomel electrode was the reference electrode (RE). A platinum-niobium mesh screen was the counter electrode (CE). These electrodes were wired to a PC computer (PC) with the Gamry Framework software.


CE
WE
RE
PC

tubercles in each treatment. Values were averaged from both sides of the same coupon in each replicate microcosm from each treatment.

Coupon Mass Lost and Corrosion Pit Depths. After the experiment was terminated in May 2009, each steel coupon was cleaned by soaking in 6N HCl with 0.35% hexamethylenetetramine (ASTM International 2003). After 20 minutes, the coupons were rinsed in tap water and lightly scrubbed with a test tube brush. If corrosion products remained, then the coupon was placed in the solution for another 5 min, rinsed and brushed again. The steel coupons from each treatment (n=6) were then air dried and reweighed to determine the mass lost due to corrosion, a measure of uniform corrosion. Two coupons from each treatment (one per microcosm) were shipped to the Naval Research Laboratory at the Stennis Space Center in Bay St. Louis, MS, where pit profiles and the maximum depth of five randomly chosen pits (a measure of localized corrosion) were measured on each side of each steel coupon using a Microphotonics Nanovea PS50 non-contact optical profiler and a 3.5 mm optical laser pen as in Ray et al. (2009).

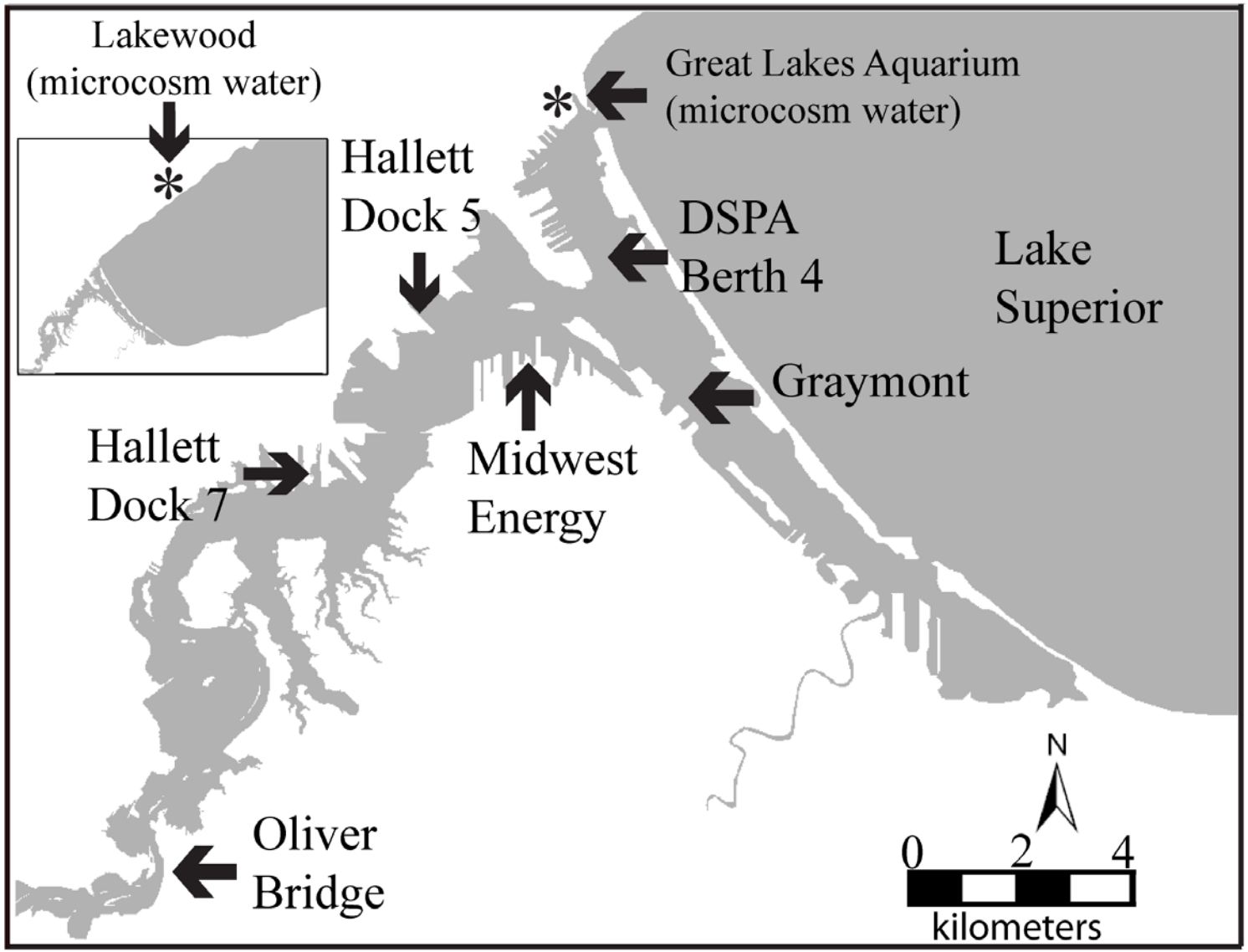
Harbor Coupons

Steel coupons were placed at several sites in the Duluth-Superior harbor in collaboration with the Duluth Seaway Port Authority and the United States Army Corps of Engineers. These harbor coupons were identical in composition to the coupons used in the microcosm experiments but were 0.95 cm thick instead of 1.25 cm thick. These coupons were placed in the harbor without washing or any other modification. At each site, coupons were placed in a steel sample tray which was welded to an adjacent steel structure by AMI Consulting Engineers (Superior, WI) as described in American Welding

Society specification D3.6. The top of the tray was 1 meter below the Lake Superior International Great Lakes Datum water level. Coupons were electrically isolated from one another and from the adjacent steel structure using HDPE spacers similar to those used in the microcosm.

A set of eight coupons was installed at six sites (Figure 4) on October 3-17, 2006. One coupon from each site was removed on August 14 2007 (after 10 months), and a second from each site was removed on October 8, 2008 (after 24 months), and coupons from select sites were removed on November 5, 2009 (after 37 months). Mass lost, pit depth, total bacterial abundance, FeOB abundance, and SRB abundance were measured as described previously for steel coupons in the microcosm experiment. Unfortunately, there were no coupons placed anywhere in Lake Superior. This decision was made by collaborators at the U.S. Army Corps of Engineers who focused their monitoring on the Duluth-Superior Harbor.

Figure 4. Map showing sites where sheet steel coupons were incubated from October 2006 to November 2009 in the Duluth-Superior harbor, near Duluth, MN. The asterisks (*) mark the sites in where water was collected for the experimental microcosm treatments. The insert shows the location of the Lakewood water intake station for the city of Duluth, which is along the north shore of Lake Superior approximately 20 km from the harbor. Water for the Lake Superior microcosms was collected at this location.


Lakewood
(microcosm water)
Great Lakes Aquarium
(microcosm water)
Hallett
Dock 5
DSPA
Berth 4
Lake
Superior
Graymont
Hallett
Dock 7
Midwest
Energy
Oliver
Bridge
N
0
2
4
kilometers

Results

Microbiological Communities in the Microcosm Experiments

The similarity of bacterial community DNA on the steel coupons in the microcosm experiment tended to cluster by treatment (Figures 5–7). Regardless of the enzyme chosen to digest the PCR products, the DNA from bacterial communities on coupons from the unaltered harbor water and harbor water supplemented with sodium sulfate tended to be similar and was usually different from bacterial community DNA in the autoclaved harbor water and iron-oxidizing bacterial treatments, which also tended to be similar. These two groups of treatments (i.e., the live versus autoclaved harbor water treatments) were often different (Figures 5–6). Sufficient DNA could not be extracted from the Lake Superior water treatment after 4 or 8 months, so amplification of 16S rDNA was not possible. However, after 12 months, DNA from the bacterial communities on replicate coupons incubated in Lake Superior water clustered together and was different in composition than DNA from bacterial communities on coupons from the other treatments (Figure 7). After one year, this bacterial community was different ($p<0.05$) from bacterial communities in the other treatments when the *Hae*III enzyme was used to digest the bacterial 16S rDNA PCR fragments (Figure 7b).

Extracellular polymer substances (EPS) produced by microorganisms were visibly more common on coupons in both treatments containing “raw” harbor water (i.e. the two treatments that did not contain autoclaved harbor water) than on steel coupons in the other experimental treatments. Mucus-like material often dripped from steel coupons in the harbor water treatments that were not autoclaved when they were sampled.

Figure 5. Similarity of DNA from bacterial communities after four months on steel coupons in the five experimental treatments based upon terminal restriction fragment length polymorphism (T-RFLP) analysis of the bacterial 16S rRNA gene. Terminal restriction fragments for these analyses were generated by digestions with *Hae*III (a) and *Msp*I (b) restriction enzymes. The treatment type, replicate microcosm, and the replicate are shown for each microcosm coupon. Branches in the dendrograms marked with an asterisk (*) indicate that the two clusters at this branching point were significantly different ($p<0.05$).

Key: A=autoclaved Duluth-Superior harbor water, H=unaltered harbor water, L=Lake Superior water, S=harbor water supplemented with sodium sulfate, F=autoclaved harbor water + *S. lithotrophicus* iron-oxidizing bacteria. Tanks A and B are replicate microcosms and replicates 1 and 2 are replicate coupons within these tanks. Tank C is the unreplicated “scour” microcosm, a third microcosm treatment of unaltered harbor water before coupons were scoured in this treatment after 7 months.

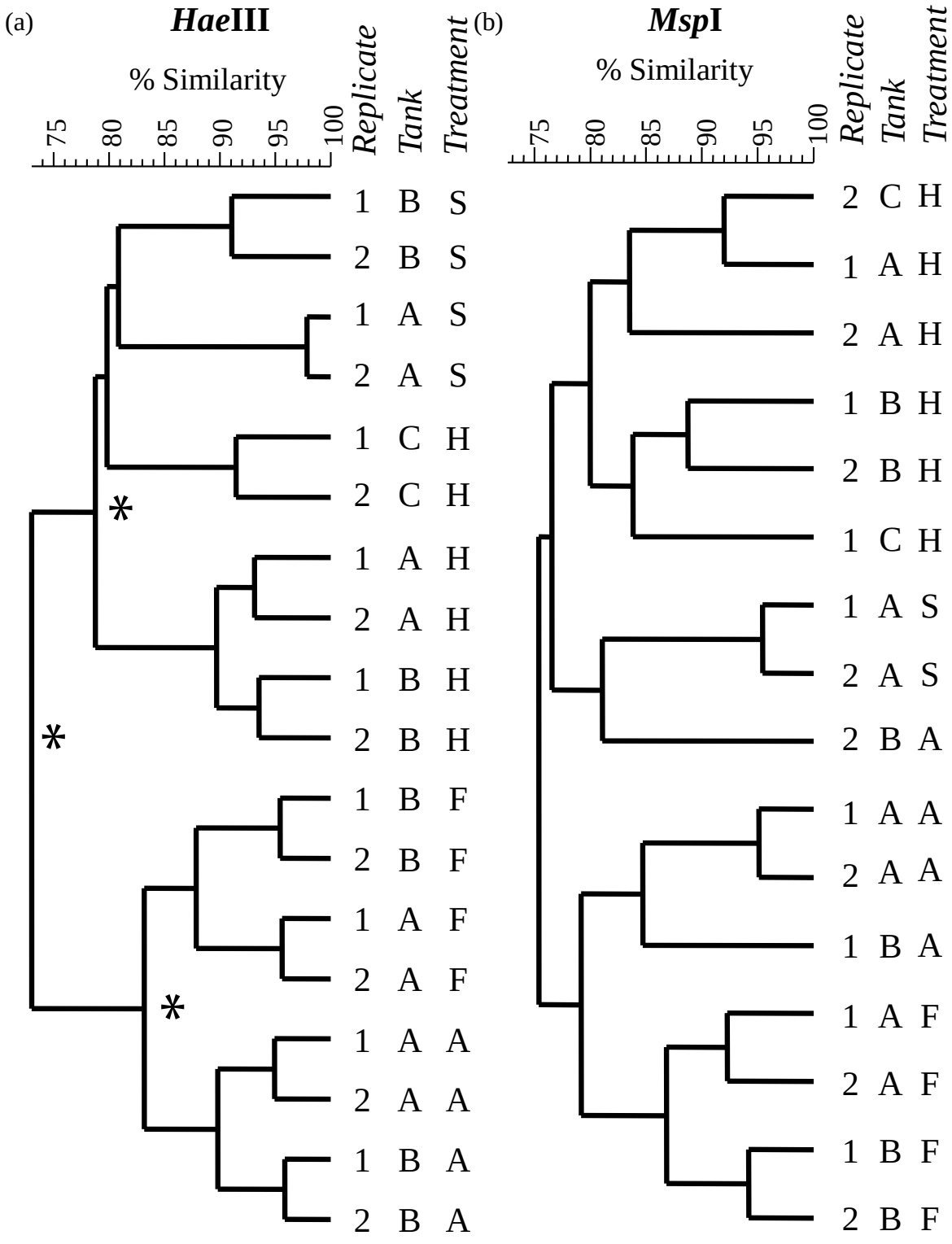

(a)
HaeIII
% Similarity
75
80
85
90
95
100
Replicate
Tank
Treatment
1 B S
2 B S
1 A S
2 A S
1 C H
2 C H
1 A H
2 A H
1 B H
2 B H
1 B F
2 B F
1 A F
2 A F
1 A A
2 A A
1 B A
2 B A
*
*
*
(b)
MspI
% Similarity
75
80
85
90
95
100
Replicate
Tank
Treatment
2 C H
1 A H
2 A H
1 B H
2 B H
1 C H
1 A S
2 A S
2 B A
1 A A
2 A A
1 B A
1 A F
2 A F
1 B F
2 B F

Figure 6. Similarity of DNA from bacterial communities after eight months on steel coupons in the five experimental treatments based upon terminal restriction fragment length polymorphism (T-RFLP) analysis of the bacterial 16S rRNA gene. Terminal restriction fragments for these analyses were generated by digestions with *Hae*III (a) and *Msp*I (b) restriction enzymes. The treatment type, replicate microcosm, and the replicate are shown for each microcosm coupon. Branches in the dendrograms marked with an asterisk (*) indicate that the two clusters at this branching point were significantly different ($p<0.05$).

Key: A=autoclaved Duluth-Superior harbor water, H=unaltered harbor water, L=Lake Superior water, S=harbor water supplemented with sodium sulfate, F=autoclaved harbor water + *S. lithotrophicus* iron-oxidizing bacteria. Tanks A and B are replicate microcosms and replicates 1 and 2 are replicate coupons within these tanks.

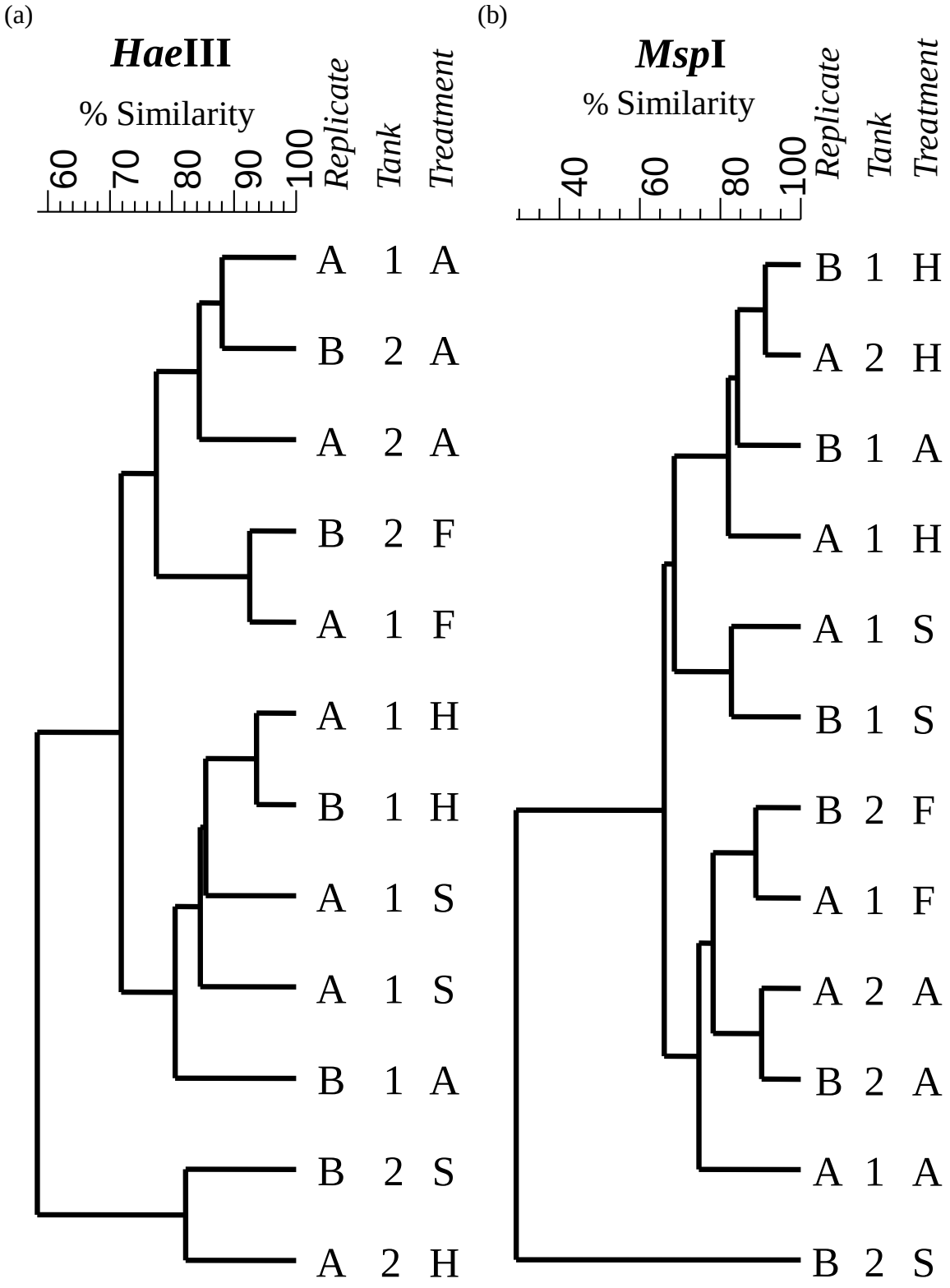
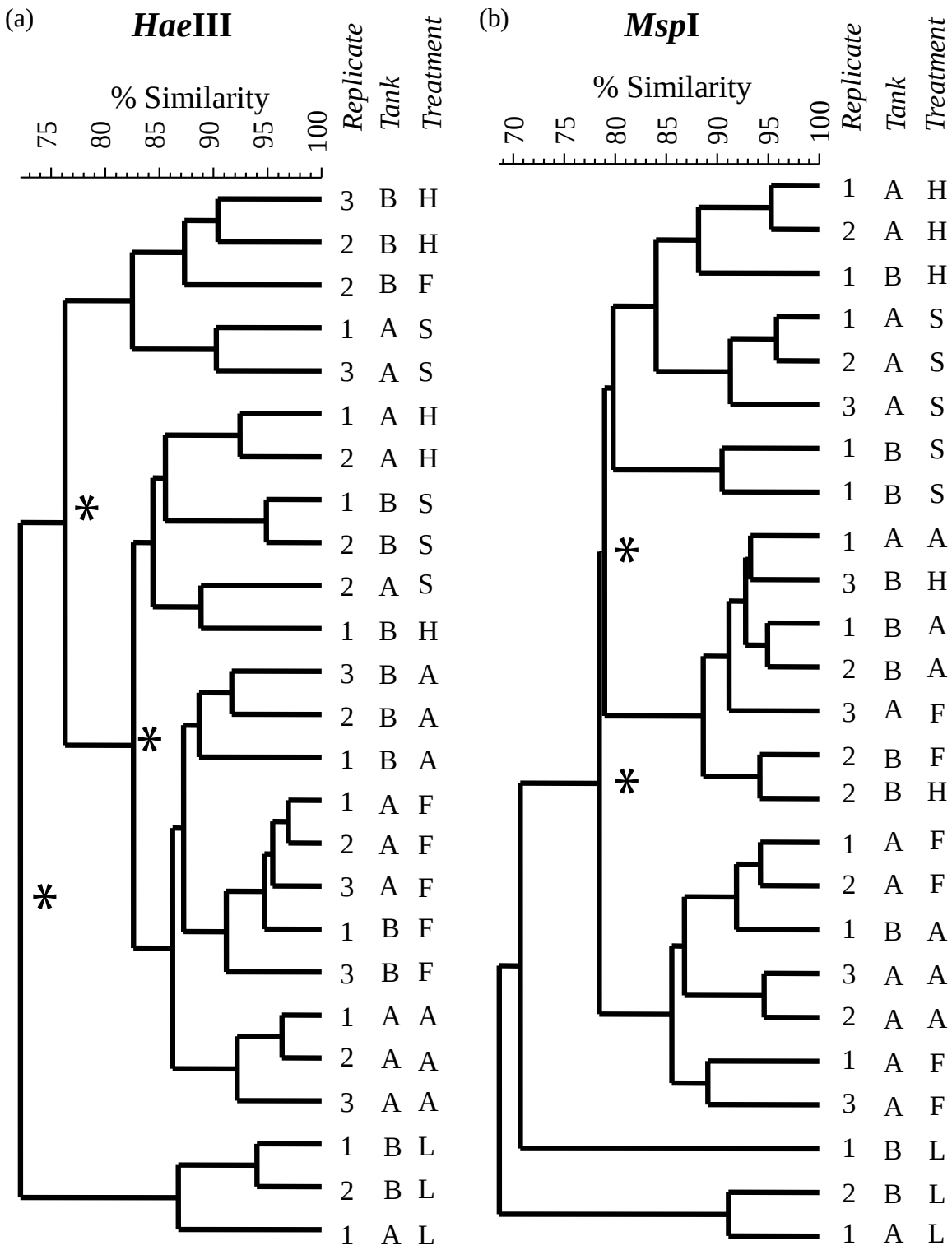

(a)
HaeIII
% Similarity
60
70
80
90
100
Replicate
Tank
Treatment
A 1 A
B 2 A
A 2 A
B 2 F
A 1 F
A 1 H
B 1 H
A 1 S
A 1 S
B 1 A
B 2 S
A 2 H
(b)
MspI
% Similarity
40
60
80
100
Replicate
Tank
Treatment
B 1 H
A 2 H
B 1 A
A 1 H
A 1 S
B 1 S
B 2 F
A 1 F
A 2 A
B 2 A
A 1 A
B 2 S

Figure 7. Similarity of DNA from bacterial communities on steel coupons after one year in the five experimental treatments based upon terminal restriction fragment length polymorphism (T-RFLP) analysis of the bacterial 16S rRNA gene. Terminal restriction fragments for these analyses were generated by digestions with *Hae*III (a) and *Msp*I (b) restriction enzymes. The treatment type, replicate microcosm, and the replicate are shown for each microcosm coupon. Branches in the dendrograms marked with an asterisk (*) indicate that the two clusters at this branching point were significantly different ($p<0.05$). Key: A=autoclaved Duluth-Superior harbor water, H=unaltered harbor water, L=Lake Superior water, S=harbor water supplemented with sodium sulfate, F=autoclaved harbor water + *S. lithotrophicus* iron-oxidizing bacteria. Tanks A and B are replicate microcosms and replicates 1 to 3 are replicate coupons within these tanks.


(a)
*Hae*III
% Similarity
75
80
85
90
95
100
Replicate
Tank
Treatment
3 B H
2 B H
2 B F
1 A S
3 A S
1 A H
2 A H
1 B S
2 B S
2 A S
1 B H
3 B A
2 B A
1 B A
1 A F
2 A F
3 A F
1 B F
3 B F
1 A A
2 A A
3 A A
1 B L
2 B L
1 A L
(b)
*Msp*I
% Similarity
70
75
80
85
90
95
100
Replicate
Tank
Treatment
1 A H
2 A H
1 B H
1 A S
2 A S
3 A S
1 B S
1 B S
1 A A
3 B H
1 B A
2 B A
3 A F
2 B F
2 B H
1 A F
2 A F
1 B A
3 A A
2 A A
1 A F
3 A F
1 B L
2 B L
1 A L

The number of total prokaryotic cells on the surfaces of steel coupons ranged from 1×10^7 cells cm^{-2} to almost 7×10^7 cells cm^{-2}. The abundance increased in all treatments during the first six months and then decreased in several treatments by the end of the experiment (Figure 8). In most months, there was a difference ($p<0.05$) in the abundance of total prokaryotic cells on the steel coupons in at least two treatments, but these were often different treatments. There was little difference in the number of prokaryotic cells on coupons between treatments when the entire duration of the experiment was considered. When cell abundances were averaged over the entire experiment, there were more prokaryotic cells on coupons (cm^{-2}) in the harbor water supplemented with sodium sulfate treatment than in the autoclaved harbor water + *S. lithotrophicus* treatment ($p<0.05$). Otherwise, there was no difference in prokaryotic cell abundance between the other treatments.

The abundances of sulfate-reducing bacteria and iron-oxidizing bacteria on steel coupons were different in the various experimental treatments. The abundance of the dissimilatory sulfite reductase gene (*dsr*A) was used as a proxy for the abundance of sulfate reducing bacteria (Figure 9). The abundance of this gene on steel coupons increased several orders of magnitude during the course of the experiment to over 10^6 copies cm^{-2}. After five months, coupons in harbor water (with or without added sulfate) had more copies of the *dsr*A gene than coupons exposed to Lake Superior water ($p<0.05$). Usually, there were 100- to 1,000-fold fewer copies of this gene on coupons immersed in Lake Superior compared to Duluth-Superior harbor water (Figure 9).

Figure 8. Abundance of prokaryotic cells on steel coupons in five treatments over the duration of the microcosm experiment. The mean and standard error of four measurements from two steel coupons in duplicate microcosms are shown (n=4). Key: □ = autoclaved Duluth-Superior harbor water, x = unaltered harbor water, ▲= Lake Superior water, ■ = harbor water supplemented with sodium sulfate, Δ = autoclaved harbor water + *S. lithotrophicus* iron-oxidizing bacteria.

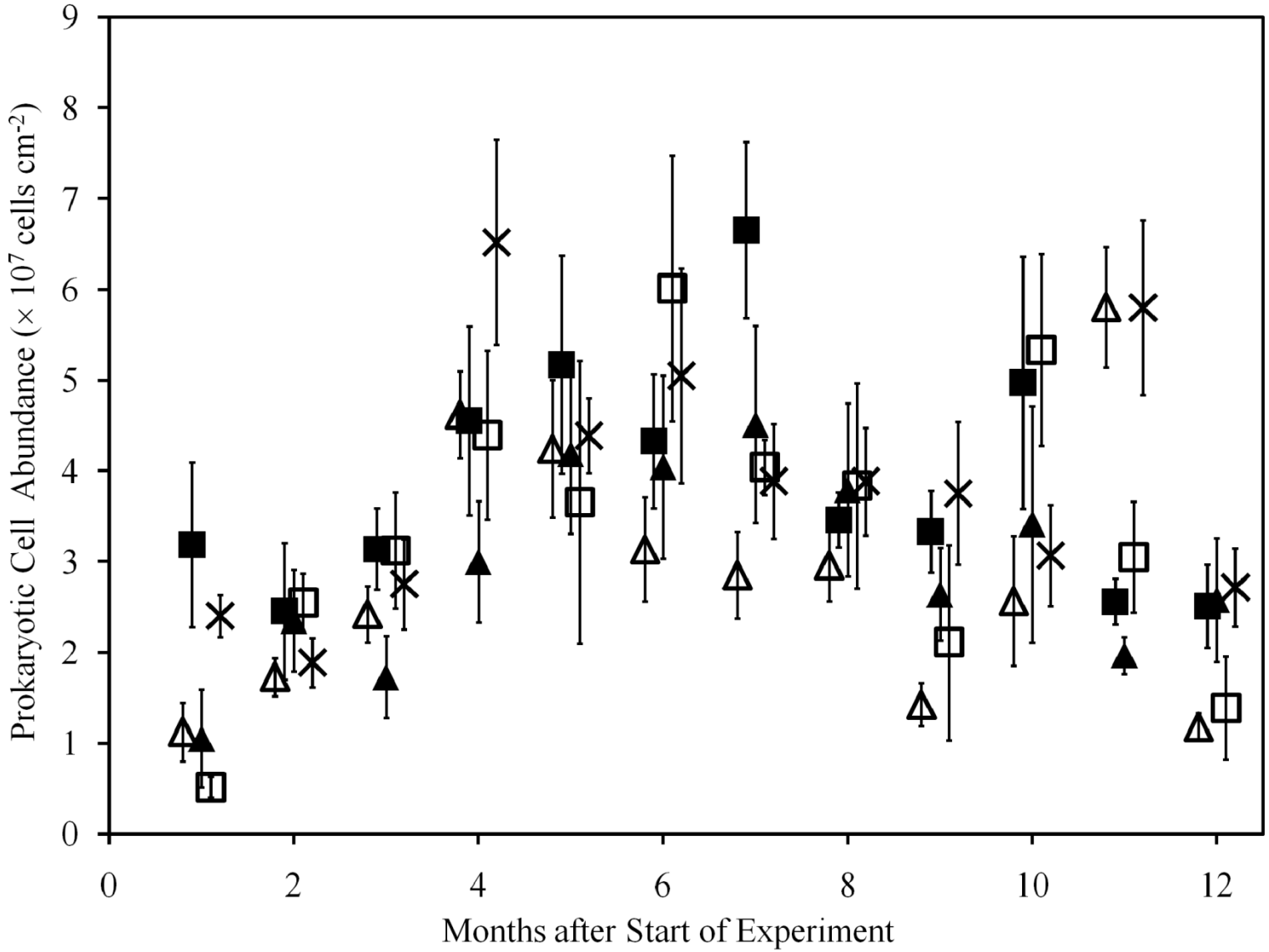
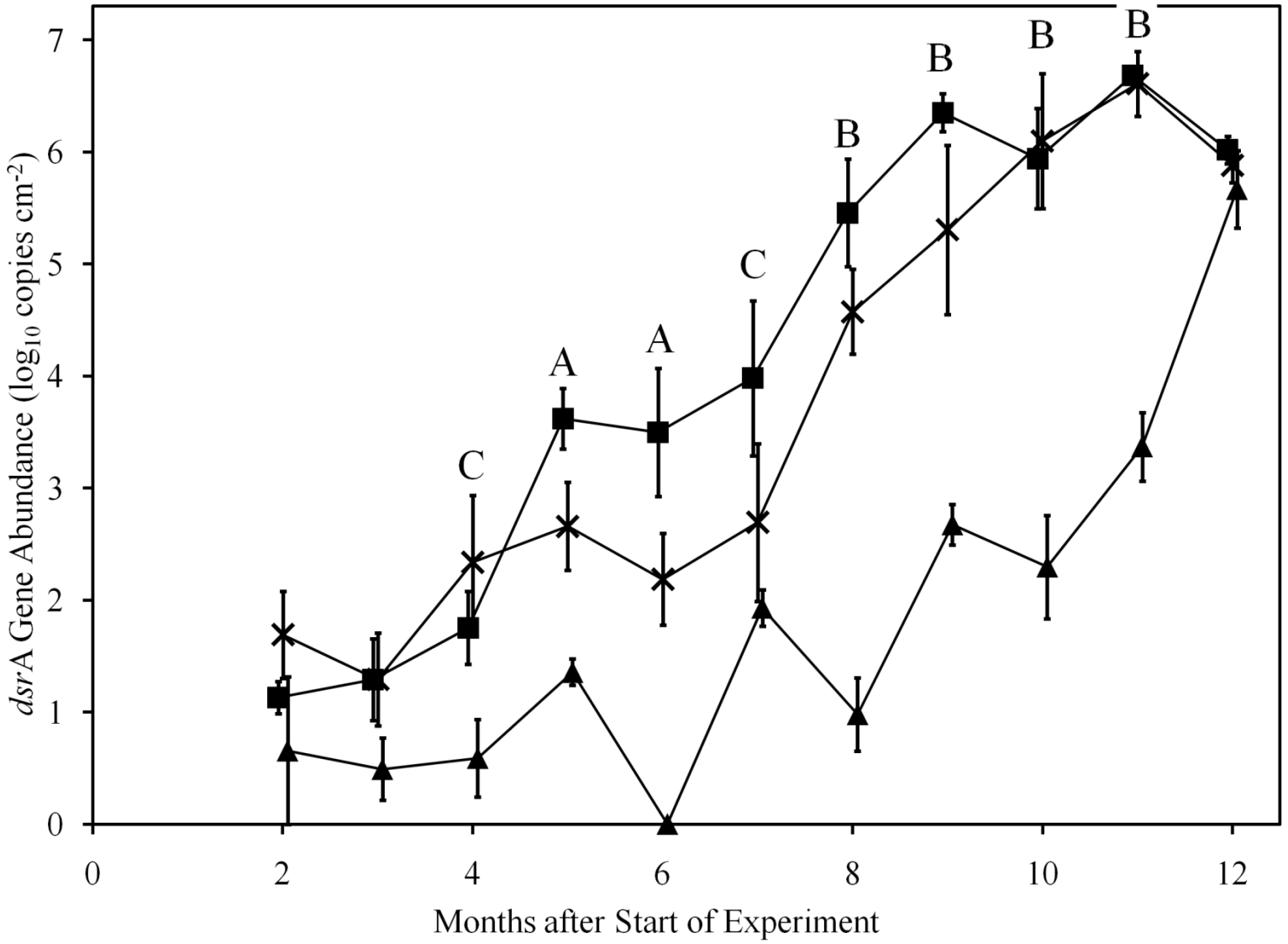

Prokaryotic Cell Abundance (× 10^7 cells cm^-2)
0
1
2
3
4
5
6
7
8
9
0
2
4
6
8
10
12
Months after Start of Experiment

Figure 9. Abundance of the dissimilatory sulfite reductase gene (*dsr*A) used to estimate the abundance of sulfate-reducing bacteria (SRB) on steel coupons in three of the experimental treatments. Mean values and the standard errors are shown (n=4; duplicate coupons in duplicate treatment microcosms). Letters above the lines indicate a significant difference between all three treatments (A), between all harbor and Lake Superior treatments (B), or between one harbor treatment and the Lake Superior treatment (C) at particular points during the experiment. Key: x = unaltered Duluth-Superior harbor water, ▲= Lake Superior water, ■ = harbor water supplemented with sodium sulfate.


dsrA Gene Abundance (log10 copies cm-2)
Months after Start of Experiment
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
C
A
A
C
B
B
B
B

The autoclaved harbor water treatments (with or without FeOB inoculum) had similar abundances to those of the other harbor water treatments (data not shown).

Depending on the treatment, the number of iron-oxidizing bacteria increased from near zero to over 100,000 CFU cm^{-2} of steel coupon during the first four to six months of the experiment (Figure 10). After eight months, there were more iron-oxidizing bacteria on coupons in the unaltered harbor water and Lake Superior water treatments than in the treatments containing autoclaved harbor water (either with or without *S. lithotrophicus*; $p<0.05$). At the end of the experiment, there were more iron-oxidizing bacteria on coupons immersed in harbor water (unaltered or supplemented with sodium sulfate) than in any of the other treatments ($p<0.05$).

Measures of Coupon Corrosion in Microcosms

The inverse polarization resistance (R_p^{-1}; units=Ω^{-1} cm^{-2} where Ω=ohms) varied from month to month, (Figure 11) ranging from less than 1×10^{-5} $ohms^{-1}$ cm^{-2} to over 12×10^{-5} Ω^{-1} cm^{-2}. When R_p^{-1} values were averaged over the experiment for each treatment, the highest mean R_p^{-1} (6.7×10^{-5} Ω^{-1} cm^{-2}) was observed in steel coupons from the unaltered harbor water supplemented with sodium sulfate treatment ($p<0.05$), and the second highest value occurred in the unaltered harbor water treatment (4.9×10^{-5} Ω^{-1} cm^{-2}). The R_p^{-1} values of coupons in the autoclaved harbor water treatments (with or without the *S. lithotrophicus* inoculum) were similar (4.1 to 4.3×10^{-5} Ω^{-1} cm^{-2}; $p>0.05$) and lower than the rates observed in the other harbor water treatments, but higher ($p<0.05$) than the mean R_p^{-1} of steel coupons measured in Lake Superior water (1.6×10^{-5} Ω^{-1} cm^{-2}).

Figure 10. Iron-oxidizing bacterial abundances on the surface of steel coupons in five experimental treatments during the microcosm experiment. Mean values and standard errors are shown for measurements in duplicate microcosms (n=2). There was no difference between treatments unless it is indicated by letters above the bars at each sample point. In these cases, treatments with the same letters were not statistically different. ND = FeOB abundance not determined for this treatment.

Key:

 = autoclaved Duluth-Superior harbor water

 = unaltered harbor water

 = Lake Superior water

 = harbor water supplemented with sodium sulfate

 = autoclaved harbor water + S. lithotrophicus iron-oxidizing bacteria

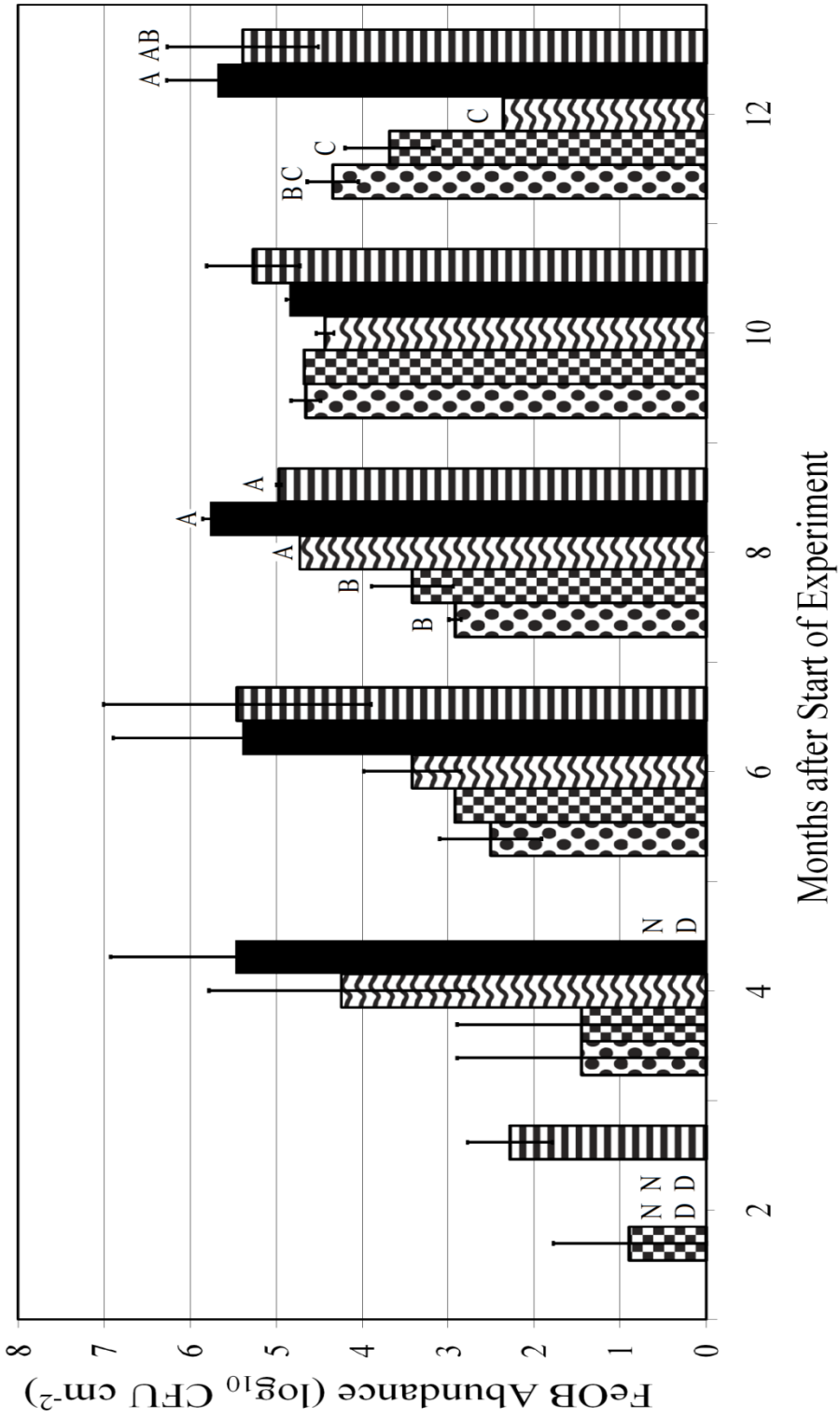
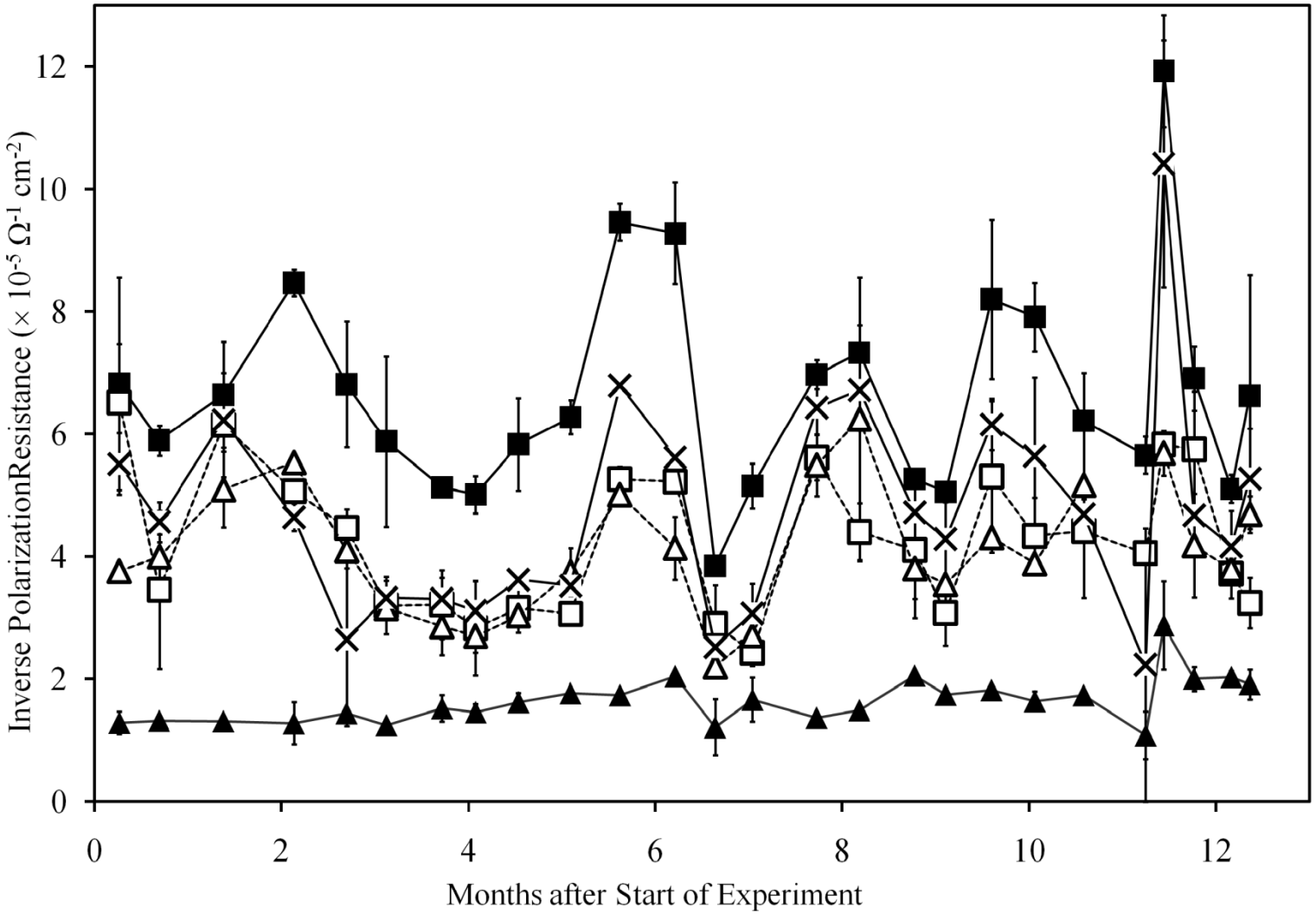

FeOB Abundance (log10 CFU cm-2)
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
Months after Start of Experiment
N
D
A
B
BC
C
A AB

Figure 11. Inverse polarization resistance (R_p^{-1}) measured in steel coupons for five treatments in the experimental microcosms. Inverse polarization resistance is proportional to the instantaneous corrosion rate. The mean and standard error are shown for measurements of coupons in duplicate microcosms of the same treatment (n=2). Key: □ = autoclaved Duluth-Superior harbor water, x = unaltered harbor water, ▲= Lake Superior water ■ = harbor water supplemented with sodium sulfate, Δ = autoclaved harbor water + *S. lithotrophicus* iron-oxidizing bacteria.


Inverse PolarizationResistance (× 10-5 Ω-1 cm-2)
0
2
4
6
8
10
12
Months after Start of Experiment

The cumulative corrosion measurements showed different trends than the instantaneous corrosion rate. In each treatment except the sulfate treatment, there was no difference in the area covered by orange tubercles on the steel coupons at 2 and 12 months (Table 2). Similarly, there was no difference between any of the treatments. At the end of the experiment, more mass had been lost from steel coupons immersed in Lake Superior water than from coupons in autoclaved harbor water ($p<0.05$). Other than this difference, the mass of steel lost from coupons was not different in the various treatments (Table 2), although the Lake Superior water coupons were close to having lost more mass than the other treatments ($0.05<p<0.10$). The depth of pits in the steel coupons, a measure of localized corrosion, was different in the various treatments at the end of the experiment ($p<0.05$, Table 2). Steel coupons in Lake Superior and unaltered harbor water had the deepest corrosion pits (558 ± 32 µm and 526 ± 19 µm, respectively) when compared to the other treatments ($p<0.05$). However, the pit depths in these two treatments were indistinguishable ($p>0.05$). Pit depths were different ($p<0.05$) and progressively shallower on steel coupons incubated in unaltered harbor water supplemented with sodium sulfate (458 µm) and autoclaved harbor water (411 µm). The average depth of corrosion pits was correlated with average mass lost from the experimental coupons (Figure 12).

Role of Ice Scouring

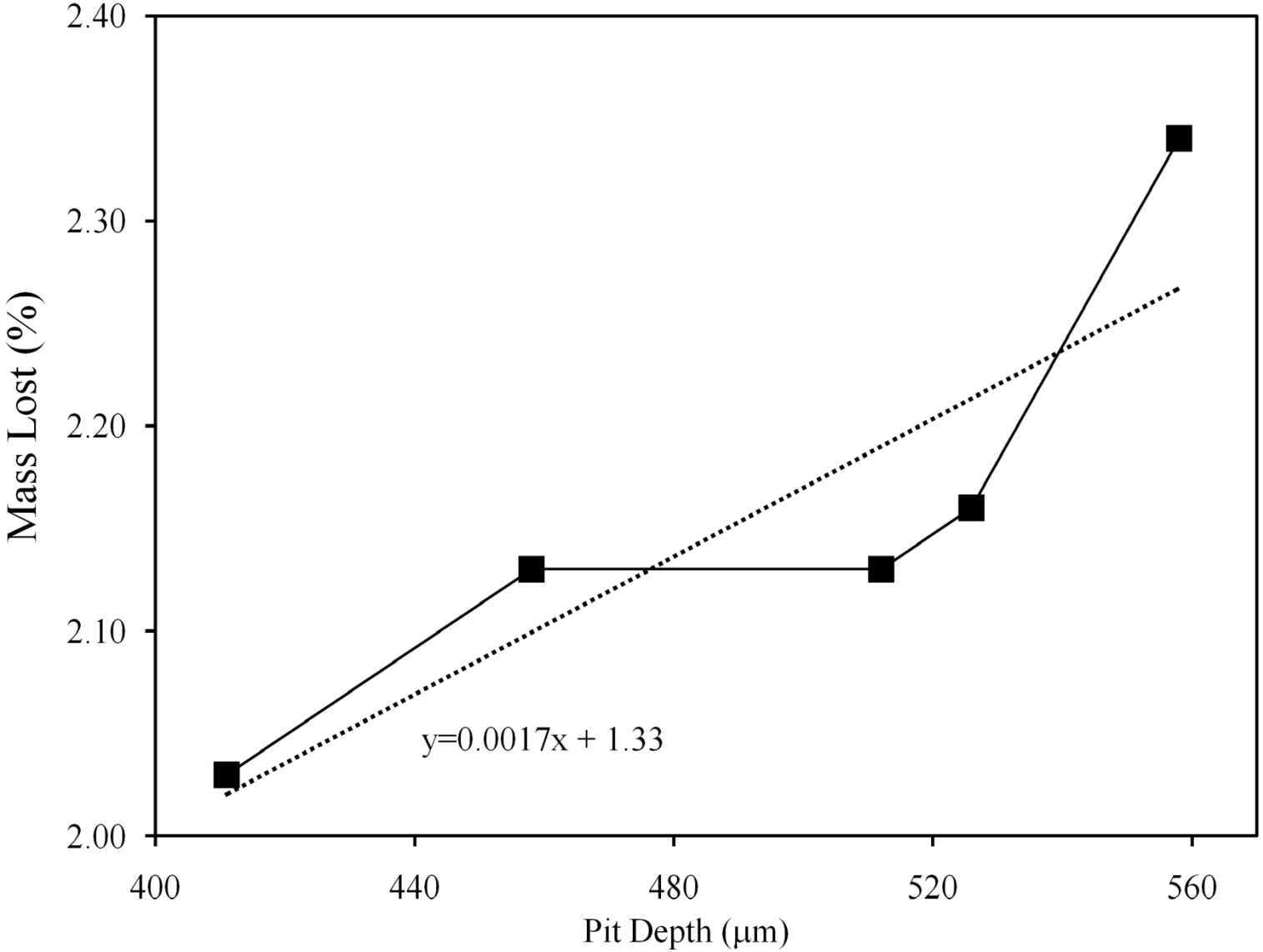
The treatment designed to mimic the effect of ice scouring had some similarities to the unaltered harbor water treatment, but there were some notable differences. The DNA of the bacterial community from the scour treatment was similar ($p>0.05$) to the DNA from

Table 2. Three measurements of cumulative corrosion for steel coupons in the five experimental treatments.

Treatment	**Area covered by tubercles (%)**		**Mass Lost (%/year)**	**Pit Depth (µm)**
	Month 2	**End of experiment**	**End of experiment**	**End of experiment**
Autoclaved harbor water	48^{a} (6)	46^{a} (3)	2.03^{b} (0.10)	411^{d} (13)
Unaltered harbor water	42^{a} (4)	48^{a} (7)	$2.16^{a,b}$ (0.11)	$526^{a,b}$ (19)
Lake Superior water	46^{a} (3)	45^{a} (11)	2.34^{a} (0.04)	558^{a} (32)
Harbor water + Na_2SO_4	37^{a} (2)	54^{a} (4)	$2.13^{a,b}$ (0.06)	458^{c} (10)
Autoclaved harbor water + *S. lithotrophicus*	41^{a} (4)	51^{a} (5)	$2.13^{a,b}$ (0.10)	512^{b} (36)

The area covered by tubercles is the mean of measurements made on two coupons in each treatment (n=2) and the mass lost is an average for three coupons in duplicate treatment microcosms (n=6). The pit depth is the mean of the deepest depth measured for five corrosion pits on each side of experimental coupons from two experimental microcosms of the same treatment (n=20). Standard errors of the mean are shown in parentheses. Means with the same letter superscript were not significantly different.

Figure 12. Relationship between mass lost and pit depths in steel coupons in the five experimental treatments after 12 months. Mean pit depth was positively correlated with the average mass lost from coupons in each treatment (r^2=0.78; p=0.05). The dotted line is the best fit line for relationship (Mass Lost (%) = 0.0017 × [(Pit Depth (μm)] + 1.33).


2.40
2.30
2.20
2.10
2.00
Mass Lost (%)
y=0.0017x + 1.33
400
440
480
520
560
Pit Depth (μm)

bacterial communities that developed on coupons in unaltered harbor water by month 12 (Figure 13), even though these communities were not periodically disturbed after month 7 like those in the "scour" treatment. Prokaryotic cell abundances comparisons were made between the scour and unaltered harbor water treatments. These comparisons examined the abundances after 8-12 months for both treatments because the growth of cells after scouring is a proliferation of cells that are already colonized to the steel, not an initial colonization. The abundance of iron-oxidizing bacteria was similar ($p>0.05$) between the scour and unaltered harbor water treatments (Table 3). However, the abundance of sulfate-reducing bacteria was smaller ($p<0.05$) on scour treatment coupons than on unaltered harbor water treatment coupons. There were fewer total prokaryotic cells ($p<0.05$) per square cm on scour treatment coupons than on unaltered harbor water treatment coupons in all months after month 7 when the scour treatment was started (Table 3). There was not a significant difference ($p>0.05$) between these treatments with respect to mass lost or pit depth (Table 3).

Harbor Coupons

There were differences in the mass lost from coupons incubated at different sites in the Duluth-Superior harbor ($p<0.05$). Less mass was lost from steel coupons that were farther upstream from the entries to this harbor (Table 4). The microbiological measurements showed that the coupon at the Graymont site in Superior Bay had the most total prokaryotic cells and a higher abundance of iron-oxidizing bacteria than most of the other coupons. Hallett Dock 5 and Midwest Energy coupons in St Louis Bay had lower abundances of FeOB. However, the Duluth Seaway Port Authority Berth 4 (DSPAB4)

Figure 13. Similarity of DNA from bacterial communities on steel coupons incubated in the unaltered harbor water (H) and scour (Sc) treatments based on terminal restriction fragment length polymorphism (T-RFLP) analysis of the bacterial 16S rRNA gene. The similarity of bacterial communities in these treatments was compared after one year at the end of the experiment. Terminal restriction fragments for these analyses were generated by digestions with *Hae*III (a) and *Msp*I (b) restriction enzymes. The treatment type, replicate microcosm, and the replicate are shown for each microcosm coupon. None of the clusters in either dendrogram were significantly different.

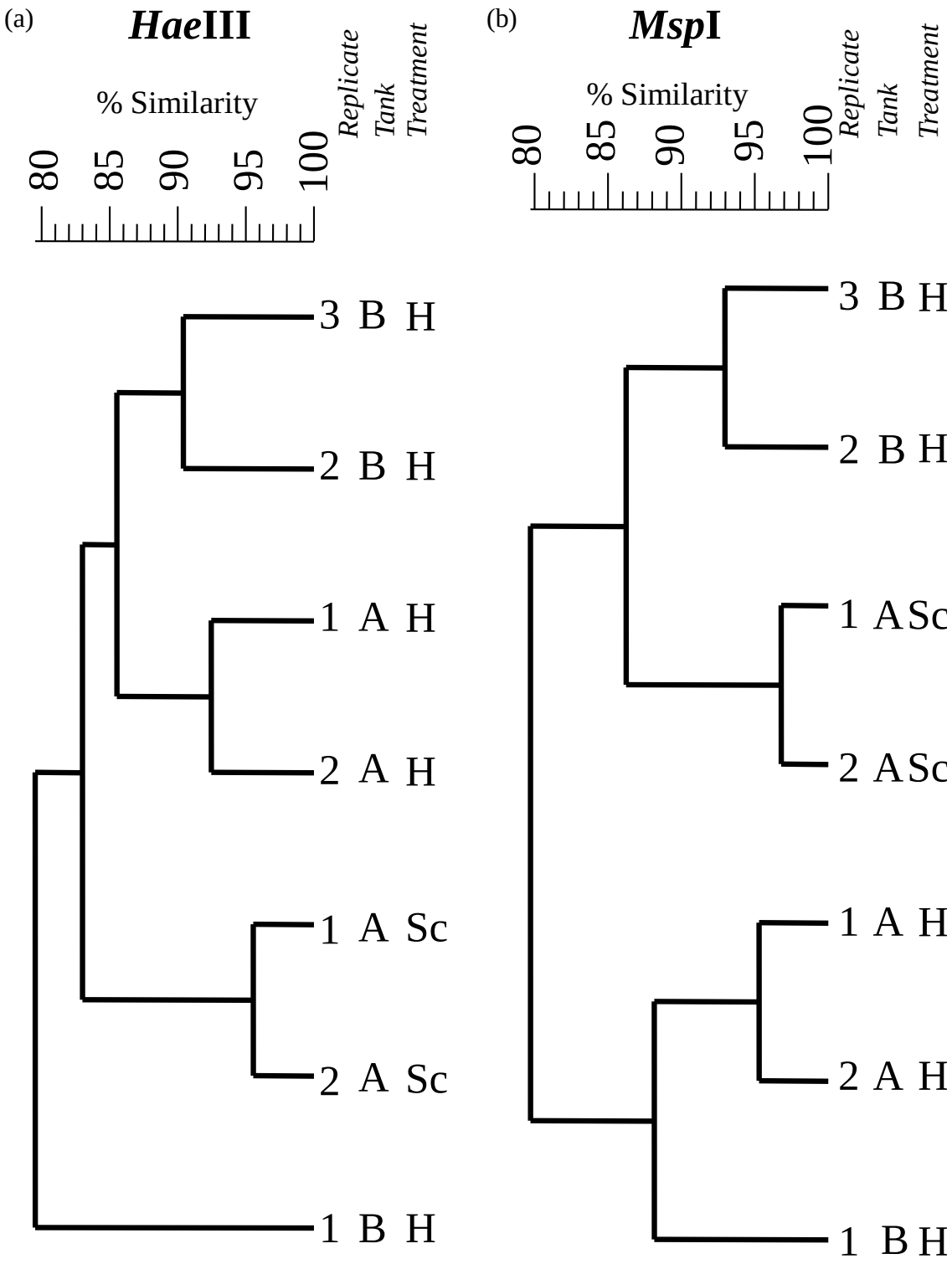

(a)
*Hae*III
% Similarity
80
85
90
95
100
Replicate
Tank
Treatment
3 B H
2 B H
1 A H
2 A H
1 A Sc
2 A Sc
1 B H
(b)
*Msp*I
% Similarity
80
85
90
95
100
Replicate
Tank
Treatment
3 B H
2 B H
1 A Sc
2 A Sc
1 A H
2 A H
1 B H

Table 3. Microbiological and corrosion data for steel coupons from the unaltered harbor water and "scour" treatments in the microcosm experiment.

Treatment	Prokaryotic Cell Abundance ($\times 10^6$ cm^{-2})	FeOB Abundance ($\times 10^4$ cm^{-2})	SRB Abundance ($\times 10^4$ cm^{-2})	Mass Lost (% year^{-1})	Pit depth (μm)	Polarization Resistance ($\times 10^{-5}$ R_p^{-1})
Scour	3.4(0.7)[b]	4[a]	4.5[b]	2.07 (0.13)[a]	526 (19)[a]	4.1
Unaltered Harbor water	38(5)[a]	20[a]*	49[a]	2.16 (0.11)[a]	471 (9)[a]	5.5 (0.5)

Microbiological measurements and polarization resistance measurements are the average of data from months 8 to 12 in the microcosms, and mass lost and pit depth are the average of measurements made at the end of the experiment. Inverse polarization resistance is shown for one coupon in each microcosm (n=1 for scour treatment; n=2 for unaltered harbor water treatment). Geometric means are given for FeOB and SRB abundances, while arithmetic means are shown for the other measurements (see methods for details). Means with the same letter superscript were not significantly different. The standard error of the mean is shown in parentheses for arithmetic means.

*FeOB abundance has only one significant figure.

Table 4. Corrosion and microbiological data for steel coupons incubated at six sites in the Duluth-Superior harbor from October 2006 to November 2009 (see Figure 4 for exact locations). These data are compared to similar data from steel coupons incubated in unaltered harbor water during the microcosm experiment.

Site	Mass Lost (g $year^{-1}$)	Prokaryotic Cell Abundance ($\times 10^7$ cm^{-2})	SRB Abundance ($\times 10^4$ cm^{-2})	FeOB Abundance ($\times 10^4$ cm^{-2})
Graymont	31(1)[b]	15	4.1	60*****
DSPA Berth 4	28(1)[c]	7.7	63	40*****
Hallett Dock 5	26(1)[c,d]	8.8	1.9	5
Midwest Energy	24(1)[d,e]	7.3	7.3	4
Hallett Dock 7	20(1)[e]	6.9	7.4	ND
Oliver Bridge	20(1)[e]	11	5.0	ND
Mean	25(2)	10(1)[a]	7.1[b]	15[a]
Microcosms (Unaltered harbor water treatment after 12 months)	46(2)[a]	4(1)[b]	76[a]	20[a]*****

(*Additional information on following page*)

(*Table 4 continued*)

For coupons placed in the harbor, the mass lost per year is the average mass lost from all coupons removed from each site (n=3 to 5 depending on site) and all microbiological data are measurements made on a single coupon from each site. For the microcosm coupons, the mass lost is the average of mass lost from all six coupons at the end of the experiment (after 12 months) and the means of microbiological data are shown. Mass lost and prokaryotic cell abundance values are arithmetic means, while the SRB and FeOB abundance values are geometric means (see methods for further details). Means with the same letter superscript were not significantly different. The standard error of the mean is shown in parentheses for arithmetic means. ND=not determined.

*****FeOB abundance has only one significant figure.

(*Harbor coupons results continued*)

coupon in Superior Bay had a comparable abundance of FeOB to the Graymont coupon. The DSPAB4 coupon also had the highest abundance of sulfate-reducing bacteria (5-fold to 10-fold higher than the other harbor sites). Unfortunately, these measurements were not replicated so statistical comparisons between sites cannot be made.

Discussion

In their initial report on corrosion in the Duluth-Superior Harbor, Marsh et al. (2005) suggested several possible factors in the corrosion of sheet steel pilings in the Duluth-Superior harbor. In particular, they suggested that water chemistry and microbiology were possible factors that needed further review. Therefore, we designed a laboratory microcosm experiment to determine the potential effects of a variety of microbiology and water quality parameters on the corrosion of steel identical to the steel that is used in construction of most of the docks and bulkheads used in the Duluth-Superior harbor. Hicks (2009) noted that sulfate and chloride ion concentrations were highest in areas of the harbor where corrosion issues appeared most acute. Work by Larson and Skold (1958) indicated that sulfate and chloride are two ions most closely related to the potential for corrosion in freshwater environments. Further, sulfate-reducing bacteria and iron-oxidizing bacteria have been implicated in corrosion in other environments (Hamilton 1985; Chamritski et al. 2004; Little and Lee 2009). This thesis focuses on the effects of microbiology and water chemistry in corrosion processes in the Duluth-Superior harbor.

The microbial communities and bulk chemistry of water used in the treatments may influence the composition of bacterial communities on the coupons. Terminal-restriction fragment length polymorphism (T-RFLP) measurements comparing bacterial community similarity showed that communities on coupons were different from one another in treatments with different types of water (Figures 5–7). The bacterial communities on coupons in the Lake Superior water formed a distinct cluster from the communities on coupons in the harbor water treatments. These communities were

significantly different from communities on other coupons in other treatments when the *Hae*III enzyme was used to digest T-RFLP PCR products. Further, communities on coupons in the two treatments using live harbor water (the unaltered harbor water and the harbor water plus sulfate treatments) formed a cluster, and this cluster was significantly different from the other harbor water treatments in most cases. The bacteria that colonized coupons in the autoclaved harbor water treatment had to come from aerosol contamination and the handling of coupons during sampling and treatment renewal. Bacteria had colonized the coupons during the first month of the experiment (before any sampling or treatment renewal), so bacteria from aerosols must have been responsible for the colonization of the coupons in the autoclaved treatment during the first month. In the FeOB treatment, some of the bacteria that colonized the coupons may have been from the *S. lithotrophicus* culture that was sprayed onto the coupons. However, the water was also autoclaved in this treatment, so once again any bacteria from the air and introduced by sampling and the treatment renewal process could have colonized the water and coupons. It is unclear whether colonization of bacteria from the air and sampling process was responsible for the apparent decrease in the difference between communities of the autoclaved and FeOB treatments. In contrast, bacteria from the air and other such sources would probably not have had as big of an effect on the colonization of bacteria on coupons in the other treatments. These bacteria would have had to compete with bacteria already present in the water.

Sulfate-reducing bacteria were surprisingly abundant in the DSH and in the microcosm (Figure 9), especially given that sulfate concentrations in the harbor are relatively low when compared to environments where accelerated low-water corrosion

and other microbiologically influenced corrosion processes are known to involve SRB (i.e salt water environments). The sulfate concentration in seawater (~2 g L^{-1}) is roughly two orders of magnitude higher than the concentration in the Duluth-Superior harbor. Lee et al. (2004) found 5×10^6 SRB per cm^{-2} on coupons in stagnant, anaerobic seawater after 1 year of undisturbed exposure. Rao et al. (2000) measured SRB abundance of coupons in nuclear power cooling water (freshwater) after 30 days of exposure and found that coupons typically had ~10^3 CFU of SRB. SRB on microcosm coupons in my study reached abundances similar to those Rao observed after 5 to 7 months. Several lines of evidence suggest that SRB were present in a substantial quantity on steel coupons in this experiment. In addition to the *dsr*A gene data, the presence of microaerophilic, iron-oxidizing bacteria in the tubercles gives reason to believe that the area under the tubercle might be anaerobic, thus providing a niche for SRB. Emerson and Ghiorse (1992) discovered *Leptothrix discophora* iron-oxidizers in close proximity to sulfate-reducing zones. Further, the underside of tubercles from coupons in the DSH contained "elevated concentrations" of sulfur (Ray et al. 2009). Sulfur was consistently more abundant than all elements other than iron in Ray's energy dispersive x-ray spectroscopy scans (EDS). The corresponding elemental maps of the underside of tubercles showed a wide distribution of sulfur (Ray and Little 2009; Richard Ray, Pers. Comm, unreferenced; see Notes). Indeed, sulfur composed an average of around 10% of the elemental mass of the material under the tubercle in the EDS results (Ray and Little 2009; Richard Ray, Pers. Comm, unreferenced; see Notes). While these EDS data did not examine the valence of the elements in question, other elements that would indicate the deposition of salts (e.g. chlorine, magnesium, calcium, and potassium) are not present or are only found in

scattered spots on the underside of tubercles. Therefore, these data suggest that the undersides of tubercles contain a substantial presence of elemental sulfur and/or sulfide. These findings are consistent with the qPCR *dsr*A gene results from the microcosm and harbor coupons by providing evidence that sulfur is present in these corrosion tubercles and that sulfate-reducing bacteria are often associated with iron-oxidizing bacteria in these corrosion tubercles. Further, the odor of sulfide was detected on coupons and in corrosion products during the microcosm experiment. Personnel at the Stennis Space Center (doing pit depth analysis) also reported that the odor of sulfide was particularly pungent when the coupons were opened after being enclosed overnight shipping for pit depth analyses. This hydrogen sulfide odor was another indication that sulfate-reducing bacteria were present on the microcosm coupons. SRB may be enriched in industrial harbors (especially in sediments) due to the presence of polycyclic aromatic hydrocarbons (Coates et al. 1996).

There were differences in SRB abundance between treatments in the microcosm experiment. The Lake Superior water treatment had a lower abundance ($p<0.05$) than the harbor water and harbor water plus sulfate treatments through much of the experiment. A similar difference was observed in iron-oxidizing bacterial abundance in these treatments. While the method used to measure FeOB abundance is only accurate to an order-of-magnitude, some treatments had significantly different FeOB abundances. At the end of the experiment, steel coupons in the harbor water plus sulfate and unaltered harbor water treatments had the most iron-oxidizing bacteria ($p<0.05$), while coupons in the autoclaved and autoclaved plus FeOB treatments had intermediate FeOB abundances, and coupons in the lake water treatment had the lowest FeOB abundance.

The abundance of FeOB in the microcosm experiment was as high as 6×10^5 FeOB CFU cm^{-2} (in the sulfate treatment). Rao et al. (2000) also found that FeOB rapidly colonized their coupons that were placed in freshwater nuclear power cooling water, with abundances reaching 3×10^4 CFU cm^{-2} after 30 days of exposure. This was a more rapid increase in FeOB abundance than observed in our experimental microcosms. In work on corrosion in the Duluth-Superior harbor, Ray et al. (2009) showed scanning electron microscopy images that indicated a spatial relationship between tubercles, bacteria, and amorphous deposited iron corrosion products, suggesting FeOB might be present in the tubercles.

The differences in bacterial communities detected in different treatments were similar to the pattern of differences in FeOB and SRB abundances that were observed. The bacterial community on steel coupons in the Lake Superior water treatment was different from all other treatments and the steel coupons had fewer sulfate-reducing and iron-oxidizing bacteria. Similarly, the similarity of bacterial communities on steel coupons in the autoclaved harbor water treatments (with and without FeOB) and the live harbor water treatments were different, and there were fewer FeOB on coupons in these autoclaved harbor water treatments. Further, these differences correlated with the polarization resistance measurements. The harbor water supplemented with sulfate treatment had the highest R_p^{-1} values ($p<0.05$), while the unaltered harbor water treatment had the next highest ($p<0.05$), and the R_p^{-1} was lowest in steel coupons in the Lake Superior water treatment ($p<0.05$).

In contrast to this pattern, the accumulated measures of corrosion did not show the same differences between treatments. There was no difference between treatments when

the area of the coupons covered by tubercles was calculated for the different treatments. The area covered by tubercles only increased from the near the beginning (2 months) to the end of the experiment (12 months) in the sulfate treatment. There was little difference in the amount of mass lost from the steel coupons in different treatments. The only difference was that steel coupons in the Lake Superior water lost more mass ($p<0.05$) than coupons in the autoclaved harbor water treatment. There were some differences in the depths of corrosion pits in the steel coupons in the various treatments. However, these differences had a very different pattern than the microbiological and inverse polarization resistance measurements. The pit depth measurements were correlated with the mass lost data ($r^2=0.78$, $p=0.05$; Figure 12). Steel coupons in the lake water and unaltered harbor water treatments, those with the deepest pits, lost the most mass on average (although the differences in mass lost were not significant), while coupons in the autoclaved harbor water lost the least mass during the experiment and had the shallowest corrosion pits.

While R_p^{-1} is often used to estimate instantaneous corrosion rate, it did not provide an accurate prediction of the corrosion rate in this experiment. Polarization resistance was measured on a regular (semi-monthly) basis in the microcosm experiment, and although measurements varied, there was generally a 4-fold to 5-fold difference in R_p^{-1} between the lake water and harbor water plus sulfate treatments (Figure 11). There was not a corresponding pattern of mass lost from the coupons at the end of the experiment, with treatments showing little or no difference in mass lost. The disparity between these measurements is almost certainly not a statistical anomaly. The difference between R_p^{-1} and mass lost might be explained because corrosion rate is actually proportional to βR_p^{-1},

not simply Rp^{-1}. β, the Tafel coefficient, is a function of the Tafel slope of the corrosion reaction. The Tafel slope of a corrosion reaction can be measured, but measuring this value requires a much larger range of voltages to be applied, and these voltages can damage the steel being tested. Therefore, R_p^{-1} is frequently used to estimate the instantaneous corrosion rate under the assumption that β is similar between the corrosion reactions being compared. β may not have been the same in all treatments in this experiment because the R_p^{-1} measurements suggested different corrosion rates than the mass lost from the steel coupons. One possible explanation for these different patterns is the relatively large difference in chemical composition of Lake Superior water, Duluth-Superior harbor water, and Duluth-Superior harbor water with added sodium sulfate. The specific electrical conductivity of Lake Superior water is reported to be approximately 90 $\mu S\ cm^{-1}$, while the conductivity of the Duluth-Superior harbor and St Louis River is reported to be about 200 $\mu S\ cm^{-1}$ (Axler 2009; R. Axler, pers. comm., unreferenced; see Notes).

Total dissolved ions, conductivity, and other water quality characteristics may influence the polarization resistance of steel. For example, when Bushman (2006) measured the polarization resistance of steel structures in the Duluth-Superior harbor, his measurements showed that R_p^{-1} was closely related to water conductivity. Further, Bushman's work showed that some of the lowest polarization resistance values were found in the northeastern part of St. Louis Bay near the Midwest Energy and Hallett Dock 5 sites. Water chemistry measurements performed by Trace Analytical Labs (Muskegon, MI) during the same time period showed that sites having higher levels of dissolved chloride and sulfate were the same sites that had higher inverse polarization

resistance values (Hicks 2009). The extreme water quality differences between seawater and freshwater are likewise correlated with large differences in polarization resistance (compare Dubiel et al. 2002; Zuo et al. 2004 to Lee et al. 2004).

It is possible that some of the same water quality differences that influenced the composition of the microbiological communities on steel also may have influenced the polarization measurements of the steel coupons in the microcosm experiment. Both the bacterial community differences on steel shown by the T-RFLP (Figures 5–7) and the specific population differences shown by the SRB and FeOB measurements (Figures 9–10) could be influenced by water quality of the respective treatments.

One interesting phenomenon in this experiment may be attributed to differences in FeOB, though not necessarily to differences in FeOB abundance. The autoclaved harbor water treatments with and without FeOB inoculation did not show differences in subsequent abundance of total FeOB, mass lost, or coupon R_p^{-1} values. However, the FeOB treatment did have significantly deeper pits, suggesting that although final FeOB abundances were similar, the FeOB inoculums induced more pitting corrosion. Pure cultures of *Sideroxydans lithotrophicus* were sprayed onto coupons for the FeOB microcosm treatment, and this bacterium was isolated from scrapings of corrosion product in the Duluth-Superior harbor (Hicks 2009). The pit depth data indicate that this particular isolate may be responsible for more aggressive pitting than other iron-oxidizing bacteria that were seeded via deposition from other sources during the experiment.

Both FeOB and SRB abundances increased through the course of the experiment by several orders of magnitude (Figures 9–10). While these differences correlated with R_p^{-1} measurements, R_p^{-1} did not show an increasing or decreasing trend. Further, these

measurements did not correlate with pit depth measurements. The coupons in the Lake Superior water treatment had a lower abundance of SRBs than coupons in the harbor water treatments for much of the experiment and a lower FeOB abundance at the end of the experiment. However, there was no difference between the pit depths in steel between the harbor water and Lake Superior water treatments, and pit depths were 15-20% shallower in the sulfate amended harbor water treatment than those in the harbor and Lake Superior water treatments. Valencia-Cantero et al. (2003) showed that SRB could reduce the corrosion rate of steel in certain cases, although their experiment design was different (e.g. different steel composition, steel was incubated in culture medium with a monoculture of prokaryotes). Despite these differences, higher SRB abundance and other aspects of the bacterial communities may explain the patterns of pit depth and mass lost from coupons observed in the treatments of this experiment.

Scour Microcosm Treatment

Bacterial communities were not different ($p>0.05$) on steel coupons in the treatments that mimicked the effect of ice scouring and in unaltered harbor water with no scouring. Scour treatment coupons had fewer ($p<0.05$) total prokaryotic cells and sulfate-reducing bacteria, but there was not a significant difference ($p>0.05$) in iron-oxidizing bacterial cell abundance. There was no difference in mass lost or pit depth between these treatments. Physical disruption and the removal of tubercles did reduce the abundance of total bacteria and SRB each month, and this observation was consistent with the hypothesis that ice scouring would reset the populations of bacteria.

Harbor Coupons

Coupons in the Duluth-Superior harbor had a greater abundance of total prokaryotic cells than the coupons in our microcosm. This difference could be partially explained in that abundances of prokaryotic cells were taken after 24 months, while abundances for the microcosm were after 12 months. Further, the microcosm coupons were incubated in the dark while the harbor coupons were exposed to natural light levels. This difference permit the growth of photoautotrophic microbes on the coupon surfaces, and in turn these microbes might provide another source of organic matter for the growth of heterotrophic bacteria on the harbor coupons. In contrast, the microcosm coupon were incubated in the dark. The microcosm coupons in the unaltered harbor water treatment had a larger abundance of SRB than the harbor coupons. Only the coupon from the DSPA Berth 4 site (Figure 4) had an SRB abundance that was similar to the higher abundances observed on coupons in the harbor water microcosm. There was no difference in iron-oxidizing bacterial abundance ($p>0.05$) between the harbor coupons and the microcosm coupons. Tubercles on the harbor coupons were more rigid and difficult to scrape than those on the microcosm coupons.

All of the microcosm coupons lost more mass per year than the harbor coupons. This difference in the amount of mass lost could be due to the different temperatures of these environments. Microcosms were incubated at 13°C for the entire experiment, while the harbor coupons experienced many months of low water temperatures in the harbor (often below 5°C), as well as some months with water temperatures above 13°C. There were differences in the mass lost from steel coupons at different sites in the harbor, with more mass lost at sites in Superior Bay and less mass lost from coupons at sites farther

upstream in the Duluth-Superior harbor. In the microcosms, mass lost from steel coupons did not show a striking pattern. At the end of the experiment, a similar amount of mass was lost from steel coupons in all of the experimental treatments.

Conclusion

In this experiment, I examined whether some water chemistry and microbiological parameters might be responsible for the type corrosion observed in the Duluth-Superior harbor. The similarity of bacterial communities and the abundance of SRB and FeOB populations were correlated with the inverse polarization resistance measurements. Both water quality and the composition of bacterial communities attached to steel surfaces may be important factors in this correlation. Although water quality alone may not be the direct cause of the corrosion, water quality appears to influence the composition of bacterial communities on steel surfaces and the abundances of bacteria populations known to be associated with steel corrosion in other ecosystems.

Notes

1. A similar corrosion pattern has been observed in other harbors in western Lake Superior, but has not been documented in an official capacity or in published work to the author's knowledge. Personal communication with Gene Clark, Wisconsin Sea Grant, and Jim Sharrow, Duluth Seaway Port Authority, provided information regarding the presence of similar corrosion patterns in other harbors.

2. Approximately 90% of steel docks in the Duluth-Superior harbor are composed of ASTM A328 steel according to personal communication with Chad Scott, AMI Consulting Engineers (Superior, WI).

3. Personal communication with Richard Axler, University of Minnesota Duluth, provided information regarding the specific conductivity of Lake Superior water and the Duluth-Superior harbor water.

4. Personal communication with Richard Ray, Naval Research Lab, Stennis Space Center, provided quantitative estimates of the amount of sulfur in his EDS scans of corroded steel from the Duluth-Superior harbor.

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