

Salt marsh pore water geochemistry does not correlate with microbial community structure

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Abstract

Spatial and temporal trends in pore water geochemistry and sediment microbial community structure are compared at three intertidal sites of a saltmarsh on Sapelo Island, GA. The sites include a heavily bioturbated, unvegetated creek bank, a levee with dense growth of *Spartina alterniflora*, and a more sparsely vegetated ponded marsh site. The redox chemistry of the pore waters ranges from sulfide-dominated at the ponded marsh site to suboxic at the creek bank site. At the three sites, the vertical redox stratification of the pore waters is more compressed in summer than in winter. The trends in redox chemistry reflect opposing effects of sediment respiration and pore water irrigation. Intense and deep burrowing activity by fiddler crabs at the creek bank site results in the efficient oxidation of reduced byproducts of microbial metabolism and, hence, the persistence of suboxic conditions to depths of 50 cm below the sediment surface. Increased supply of labile organic substrates at the vegetated sites promotes microbial degradation processes, leading to sharper redox gradients. At the levee site, this is partly offset by the higher density and deeper penetration of roots and macrofaunal burrows. Surprisingly, the microbial community structure shows little correlation with the variable vertical redox zonation of the pore waters across the saltmarsh. At the three sites, the highest population densities of aerobic microorganisms, iron- plus manganese-reducing bacteria, and sulfate reducers coexist within the upper 10 cm of sediment. The absence of a clear vertical separation of these microorganisms is ascribed to the high supply of labile organic matter and intense mixing of the topmost sediment via bioturbation. © 2004 Elsevier Ltd. All rights reserved.

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1. Introduction

Saltmarsh ecosystems are among the most productive environments on earth, with estimates of net primary productivity ranging from 930 to greater than 7600 g m⁻² yr⁻¹ (Teal, 1962; Odum and Fanning, 1973; Kirby and Gosselink, 1976; Gallagher et al., 1980; Hardisky, 1980; Pomeroy and Wiegert, 1981; Good et al., 1982; Schubauer and Hopkinson, 1984; Dame and Kenny, 1986; Dai and Wiegert, 1996). This leads to very high rates of organic carbon oxidation in the upper

centimeters of the sediment column, which is generally attributed primarily to coupling with microbial sulfate reduction (Howarth and Teal, 1979; Skyring et al., 1979; Howarth and Hobbie, 1982; Howarth and Giblin, 1983; Howarth and Merkel, 1984; Howes et al., 1984; King, 1988; Kostka et al., 2002a). However, significant organic carbon degradation may also occur via other pathways, including aerobic respiration (e.g., Teal and Kanwisher, 1961; Howes et al., 1984), ferric iron reduction (e.g., Kostka et al., 2002a,b) or methanogenesis (e.g., King and Wiebe, 1978, 1980).

Saltmarsh sediments are redox-stratified, meaning that a series of horizontal layers (e.g., oxic, suboxic, sulfidic, methanic) can be delineated from pore water profiles, with more reducing conditions found in pore waters with increasing depth (e.g., Koretsky et al., 2003). Especially in relatively laterally-homogeneous environments with low rates of organic carbon oxidation, such pore water redox stratification has been attributed to a spatially-corresponding vertical succession of microbial respiratory pathways coupling organic matter oxidation to progressively less energetically-favorable terminal electron acceptors with depth (e.g., Froelich et al., 1979). However, this spatial correspondence is less likely to be maintained in highly productive, temporally and spatially heterogeneous saltmarsh sediments.

The presence of macrophyte roots and macrofaunal burrows in surficial saltmarsh sediments creates a three-dimensional mosaic of temporally and spatially fluctuating redox environments (e.g., Aller, 1978, 1980, 1994; Aller and Yingst, 1978; Canfield, 1993; Boudreau and Marinelli, 1994; Fenchel, 1996a,b; Rooney-Varga et al., 1997; Sundby et al., 1998; Hines et al., 1999; Lee et al., 1999; Koretsky et al., 2003). Labile organic substrates may be supplied at the sediment surface, for example by trapping of detrital organic matter in vegetated intertidal sediments, or at depth, via belowground *Spartina* biomass production (e.g., Dame and Kenny, 1986) and by rapid downward mixing of surficially-deposited organic matter via bioturbation. Furthermore, solute transport in the upper portion of the saltmarsh is dominated by intense bioirrigation, a non-local transport process that supplies oxygenated surface water (and removes water with more reduced aqueous species) to deeper sediment strata with bulk suboxic or anoxic pore waters (e.g., Meile et al., 2001; Koretsky et al., 2002), fueling rapid internal oxidation–reduction cycles. The geochemical microzones created by roots and burrows also promote competition between biological and chemical redox reactions (e.g., Koretsky et al., 2003). The combination of enhanced transport and sediment heterogeneity created by abundant macrophyte and macrofaunal populations may cause multiple organic carbon degradation pathways to co-exist within a particular horizontal interval of the

saltmarsh sediment. Such overlapping pathways have been reported in other redox-stratified environments (e.g., Canfield and Des Marais, 1991; Jørgensen and Bak, 1991; Elsgaard and Jørgensen, 1992; Canfield, 1993; Urban et al., 1994; Fenchel, 1996a).

In this study, geochemistry and microbial community structure and activity are assessed seasonally at three sites in a saltmarsh at Sapelo Island, GA. Pore water geochemistry, microbial culture enumerations for aerobic, manganese-reducing and iron-reducing bacteria and 16S rRNA analyses of prokaryotes, sulfate-reducing and iron-reducing bacteria measured at the same time at immediately adjacent sites are presented. Sulfate reduction rates and solid phase iron speciation data collected simultaneously at these same sites are the subject of another paper (Kostka et al., 2002b). The combination of geochemical and microbial data allows us to assess the relationship between pore water redox stratification and microbial community structure in the saltmarsh environment. Because data were measured at sites with significant differences in macrofaunal and macrophyte populations, we can also assess the influence of macrophytes and macrofauna on observed seasonal and spatial trends in sediment pore water geochemistry and microbiology in these saltmarsh sediments.

2. Site

The three sites form a transect through a saltmarsh located on the southern tip of Sapelo Island, GA (see also Kostka et al., 2002b; Koretsky et al., 2003). These sites include an exposed, unvegetated creek bank adjacent to an approximately 20 m wide tidal creek, a levee densely vegetated with *Spartina alterniflora* reaching 1.5–2 m in height, and a more sparsely vegetated ponded marsh with maximum ~0.5 m tall *S. alterniflora*. Stalk densities of 70 m⁻² and 45 m⁻² have been reported at levee and ponded marsh sites in a nearby marsh at Sapelo Island (Basan and Frey, 1977).

During April 1999 (the final full field-sampling trip; see below), *Spartina alterniflora* began to colonize the previously unvegetated creek bank site. In April 1999 and May 2000, peepers were deployed approximately ~1.5 m apart, at the same elevation and lateral distance from the creek bank, with one peeper in the remaining unvegetated portion of the initial creek bank site, and the second peeper located in the adjacent, newly colonized portion of the creek bank (stalk density in May ~100/m²). Because the full suite of geochemical and microbial data was not collected at the *Spartina*-colonized creek bank site, and because data were only collected during two spring sampling trips, these data are discussed separately in Section 5.4.

All sites host large populations of macrofauna, the most conspicuous of which are fiddler crabs (*Uca* sp.). Fiddler crab burrow densities are typically much higher at creek bank or levee sites, compared to higher, ponded marsh sites (e.g., McCraith et al., 2003 and references within). For example, fiddler crab burrow aperture densities of 1040, 745 and 415 per m² (Basan and Frey, 1977), and fiddler crab population densities of 65, 61 and 27 per m² (Teal, 1958) have been reported previously for Sapelo Island unvegetated creek bank, levee and ponded marsh sites. These relative densities are in qualitative agreement with densities observed visually at the sites during this study and at similar marshes. In addition, fiddler crab burrows are typically deeper at unvegetated creek bank or levee sites, averaging approximately 15–30 cm depth, compared to burrows at ponded marsh sites, which average only about 15–20 cm depth (Dembowski, 1926; Teal, 1958; Frey and Mayou, 1971; Allen and Curran, 1974; Basan and Frey, 1977; Christy, 1982; Koretsky et al., 2002). Population densities of polychaete worms and shrimp are also highest at the unvegetated creek bank (e.g., Koretsky et al., 2002 and references within).

3. Methods

A suite of complementary geochemical and microbial analyses was completed at all three primary sites during seven sampling trips (May and August, 1997; January, June, and November, 1998; January and April, 1999). Detailed descriptions of sampling and analysis methods are given in Kostka et al. (2002b) and Koretsky et al. (2003). Briefly, pore waters were sampled at 1–2 cm intervals using pore water diffusion equilibrators ('peepers'). Alkalinity, dissolved ferric and ferrous iron, total sulfide, total ammonium and total phosphate were measured immediately using colorimetric techniques. pH and conductivity were measured immediately using field portable meters. Filtered pore water samples were preserved in 0.05 N HCl for sulfate analyses or in concentrated sulfuric acid for manganese analyses.

Sediment cores collected near peepers (within 0.5 m) were used for microbial population enumerations, 16S rRNA hybridization experiments, sulfate reduction rate measurements and solid phase extractions. Sediment samples from depth intervals of 5 or 10 cm were collected, well mixed by hand and split into three portions. The first portion was used to measure sulfate reduction rates and to complete solid phase extractions for poorly crystalline iron oxide, acid volatile sulfide and chromium reducible sulfide fractions (see Kostka et al., 2002b).

The second portion of the sediment was used for enumeration of culturable populations of anaerobic Fe(III)-reducing bacteria (FeRB), anaerobic Mn(IV)-reducing bacteria (MnRB) and aerobic bacteria (AEB)

(Koretsky et al., 2003). For each sample, 1 g of wet-weight sediment was suspended in 9 mL of anaerobic, phosphate-buffered saline solution (DiChristina and DeLong, 1993) and serially-diluted in duplicate to 10⁻⁹ in an anaerobic glove box. For FeRB enumeration, sediment suspensions were plated on synthetic growth medium (Lowe et al., 2000) supplemented with Bacto agar (1.5% w/v), NaCl (4% w/v), lactate (15 mM) and Fe(III)-citrate (50 mM), placed into anaerobic canisters (BBL Gas Pak, Becton Dickinson Co., Cockeysville, MD) and incubated at room temperature for one month before enumerating colony-forming units (CFU). Aliquots for MnRB enumeration were treated similarly, except that 5 mM δ -MnO₂ (synthesized as described by Burnes et al., 1998) was supplied as sole terminal electron acceptor (DiChristina and DeLong, 1993). Anaerobic control plates lacking Fe(III)-citrate and δ -MnO₂ were included in each canister. Aliquots for AEB enumeration were treated similarly, except that plates were incubated aerobically at room temperature for 2–4 days prior to enumeration of CFU. All enumerations were completed in duplicate.

The third portion of each sediment was used in nucleic acid hybridization experiments with RNA extracted from each sediment layer and a set of 16S rRNA-targeted oligonucleotide probes, including SRB385 (targets a range of SRB within the δ -proteobacteria; Amann et al., 1990), DSV687 (targets the SRB genera *Desulfovibrio*, Devereaux et al., 1992), DSB804 (targets the SRB genera *Desulfobacter*, Devereaux et al., 1992), EUB338 (targets the domain *Bacteria*, Amann et al., 1990) and ARC915 (targets the domain *Archaea*, Amann et al., 1990). Sediment samples were frozen at -20 °C and processed immediately upon thawing. RNA was extracted directly from thawed sediment samples by following previously described procedures (Devereaux et al., 1992) and further purified via passage through Sephadex G-75 columns (Moran et al., 1993). RNA was denatured by adding 3 volumes of 2% (v/v) glutaraldehyde in 50 mM NaPO₄ (pH 7.0) and incubating at room temperature *T* for 10 min (Stahl and Amann, 1991). Serial dilutions of denatured RNA were loading onto nylon filters, immobilized by UV-cross-linking and utilized in subsequent nucleic acid hybridization experiments. Radiolabeling of oligonucleotide probes and RNA hybridization experiments followed previously described procedures (Stahl and Amann, 1991). Filters were washed at predetermined wash temperatures (Pace et al., 1986; Amann et al., 1990) to remove non-specifically bound probe and hybridization signals quantified by using a gas-proportional radioisotope detection system (Ambis Model 4000, San Diego, CA). The slope of the binding curve (counts per minute (cpm) of probe bound RNA) for each sediment sample was determined from results of four to eight points in the serial dilution. Estimates of percent *Archaea*, *Bacteria*

and the genus-specific SRB were calculated by dividing the group-specific slope of the sum of the *Archaea*- and *Bacteria*-specific (designated prokaryotic) slopes.

4. Results

4.1. Pore water profiles

To illustrate general trends observed at the sites, the full set of pore water profiles measured in winter (January 1999) and summer (August 1997) are shown in Fig. 1. Duplicate pore water profiles were collected at the levee site in January using peepers placed approximately 1 m apart. Although, the profiles exhibit similar trends with depth, the inherent lateral heterogeneity of saltmarsh sediments leads to significant differences in the magnitude of the measured concentrations.

Especially at the levee site, a rapid drop in pH followed by essentially constant pH values is evident (Fig. 1A–C). Values of pH below the subsurface minima are similar among the sites, with slightly more acidic conditions at the vegetated sites, compared to the unvegetated creek bank. Alkalinity increases with depth at all sites, with the lowest levels present in the creek bank and the highest levels at the ponded marsh site (Fig. 1D–F). Salinity is lowest at the creek bank and increases somewhat moving outward to the levee and ponded marsh sites (Figs. 1G–I). There is very little change in salinity with depth at either of the vegetated sites, whereas salinity decreases somewhat below 25 cm at the creek bank. Seasonal changes in salinity are quite small at the creek bank, whereas salinities at the ponded marsh are slightly higher in summer compared to winter. Salinity was not measured at the levee in August 1997.

The concentration of dissolved orthophosphate at the three sites typically increases from less than 10 μM at the sediment surface to 150–300 μM at a depth of 50 cm (Fig. 1J–L). In the upper 15 and 40 cm of the ponded marsh and levee, respectively, phosphate concentrations are higher in summer than in winter. In contrast, phosphate concentrations at all depths in the creek bank are higher in winter compared to summer. Like phosphate, ammonia concentrations increase with depth at all sites, but much larger concentrations of ammonia (nearly 1000 μM) are always present at the creek bank site, compared to the two vegetated sites (Fig. 1M–O). During August 1997, concentrations of ammonia at the ponded marsh are nearly as high as those measured at the creek bank (Fig. 1M and O), but this is not always the case in summer. In June 1998, for example, ammonia concentrations at the ponded marsh reach a maximum of just 170 μM , compared to ammonia concentrations of 1180 μM at the creek bank (data not shown).

Pore water sulfate concentrations decrease with depth at all sites; more rapid depletion occurs farther from the

creek bank and during summer (Fig. 1P–R). Similarly, a clear spatial trend in sulfide concentration is apparent, with sulfide concentrations near or below detection limit (a few μM) at all depths at the creek bank site, and with the highest sulfide concentrations occurring at the ponded marsh site (Fig. 1S–U). At the vegetated sites, pore water sulfide concentrations increase with depth and generally reach higher concentrations in summer compared to winter. In addition, sulfide concentrations at these sites increase rapidly at much shallower depths in summer than in winter (e.g., 1 mM sulfide at 3.5 cm depth in summer at the ponded marsh, as compared to 7 cm depth in the winter).

At the levee and creek bank sites, a much larger peak in the dissolved iron profiles is typically found in summer compared to winter (Fig. 1V and W). Dissolved iron concentrations at the ponded marsh remain low in winter and in summer, although a small peak in dissolved total iron is apparent at approximately 2 cm depth in summer (Fig. 1X). Iron concentrations at the levee typically form a well-defined peak between 5 and 25 cm and then decrease to very low levels. Relatively high concentrations of dissolved iron (100–200 μM) persist to depths of 45 cm at the creek bank site in both winter and summer. Pore water manganese concentrations are generally higher in summer than in winter, and are higher at the creek bank and levee sites as compared to the ponded marsh site (Fig. 1Y–AA). As observed for pore water iron concentrations, high concentrations of manganese begin to accumulate at shallower depths in summer compared to winter.

4.2. Microbial community structure profiles

The largest number of culturable aerobic bacteria (AEB) are always found in the upper 5–10 cm of the sediment column (Fig. 2A–C), although large numbers of AEB are sometimes found deeper in the sediment (see also Lowe et al., 2000). Colony-forming units (CFU) of AEB are typically higher in summer than in winter, particularly in the upper portion of the vegetated sediments. The highest populations of AEB are found in the upper 5–10 cm of sediment of the levee site.

Enumerations of anaerobic manganese-reducing bacteria (MnRB) suggest that manganese reducers are much more abundant in summer than in winter at all sites. As for the AEB, at all sites the largest culturable populations of MnRB are found in the upper 10 cm of sediment. These populations are much larger at the levee site than at the creek bank or ponded marsh (note difference in scale in Fig. 2E compared to Fig. 2D and F). In contrast to the MnRB, populations of culturable iron-reducing bacteria (FeRB) are considerably lower in summer than in winter (Fig. 2G–I; see also Koretsky et al., 2003). At all three sites, the highest populations of culturable FeRB are measured in the same upper 10 cm

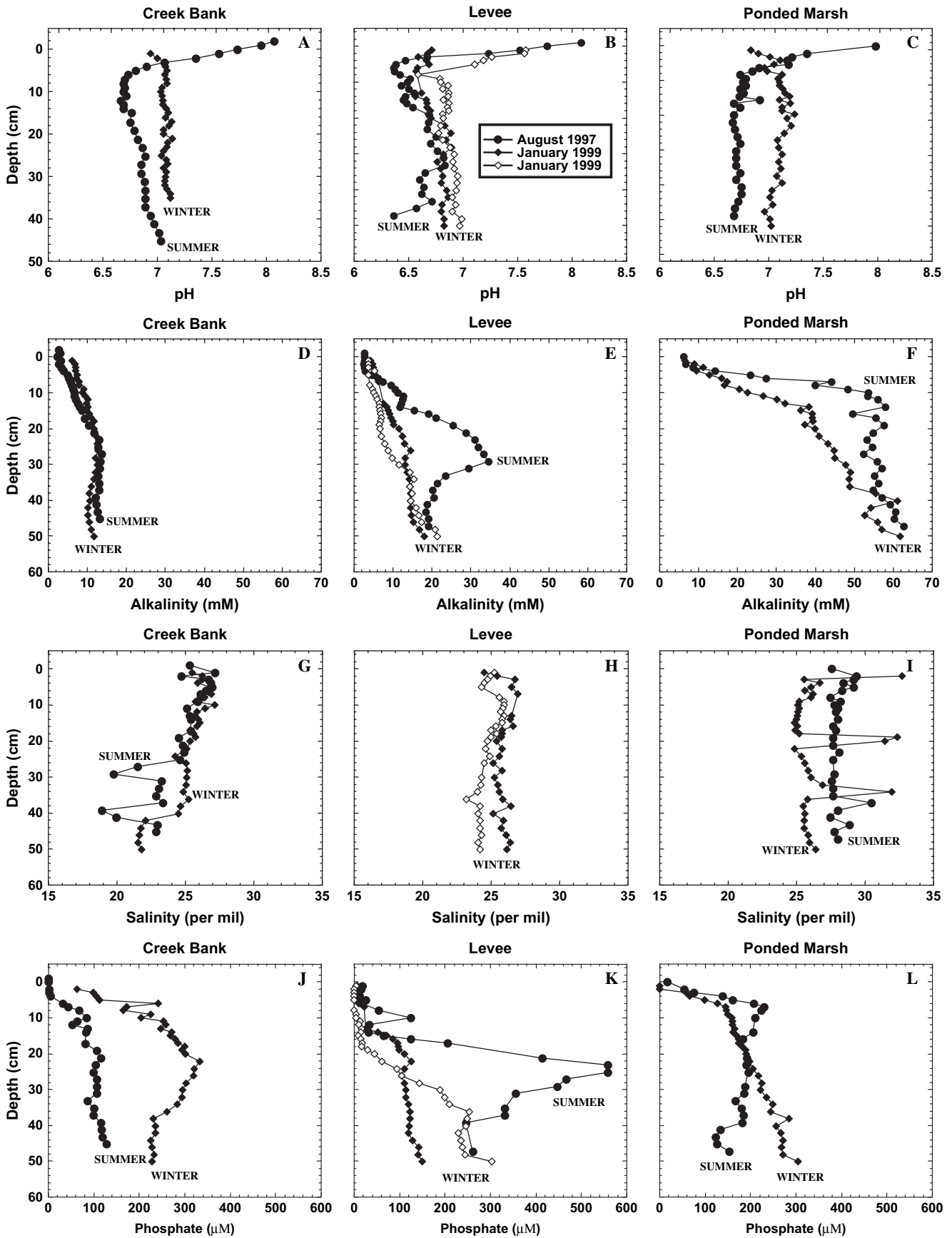


Fig. 1. Pore water data measured at the creek bank, levee and ponded marsh sites in summer (August 1997) shown as filled circles and in winter (January 1999) shown as diamonds. The two sets of data (open and filled diamonds) shown for the levee site were measured in January 1999 using duplicate peepers placed approximately 1 m apart. Summer iron data at the ponded marsh are for total dissolved iron; all other iron data are dissolved ferrous iron.

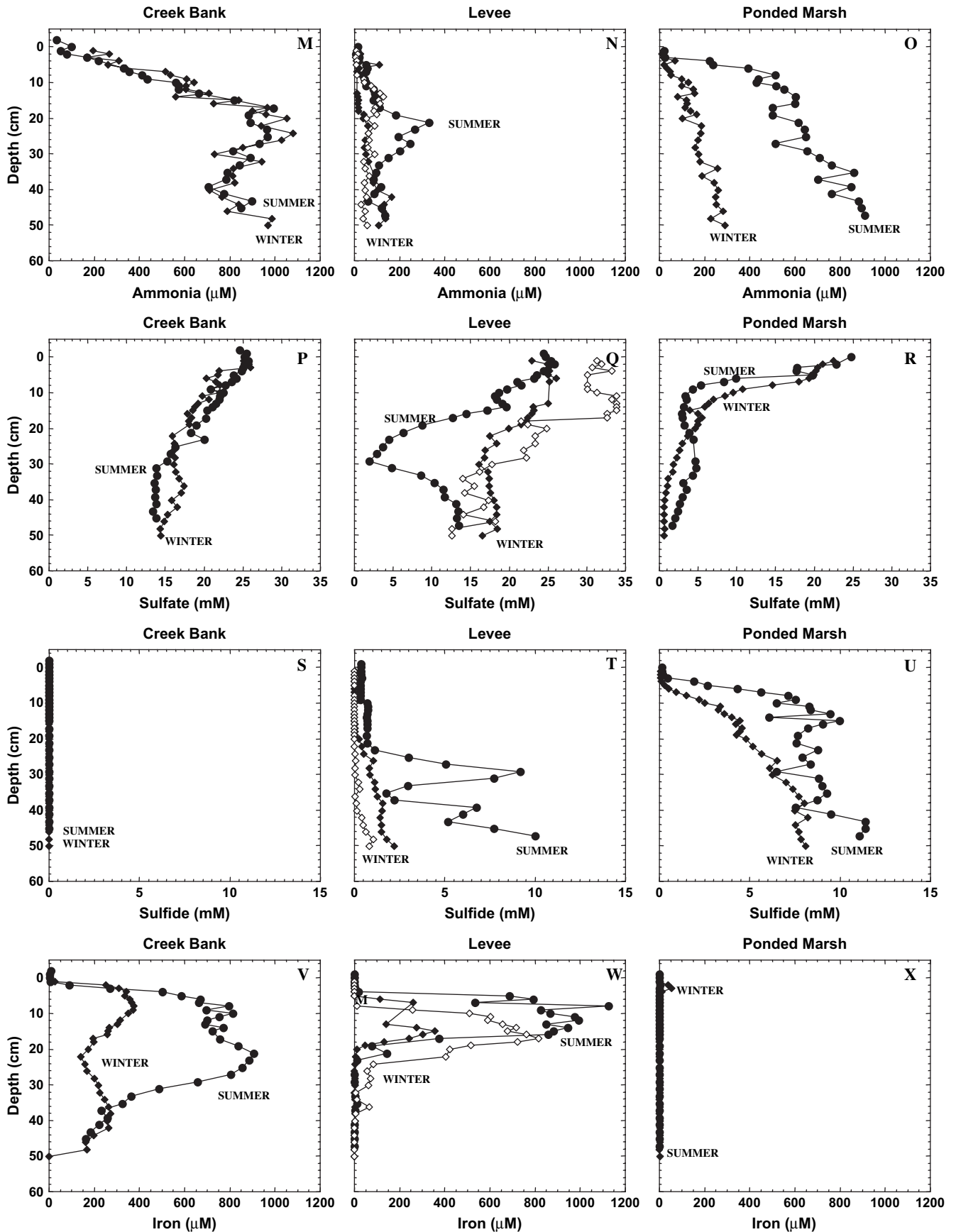


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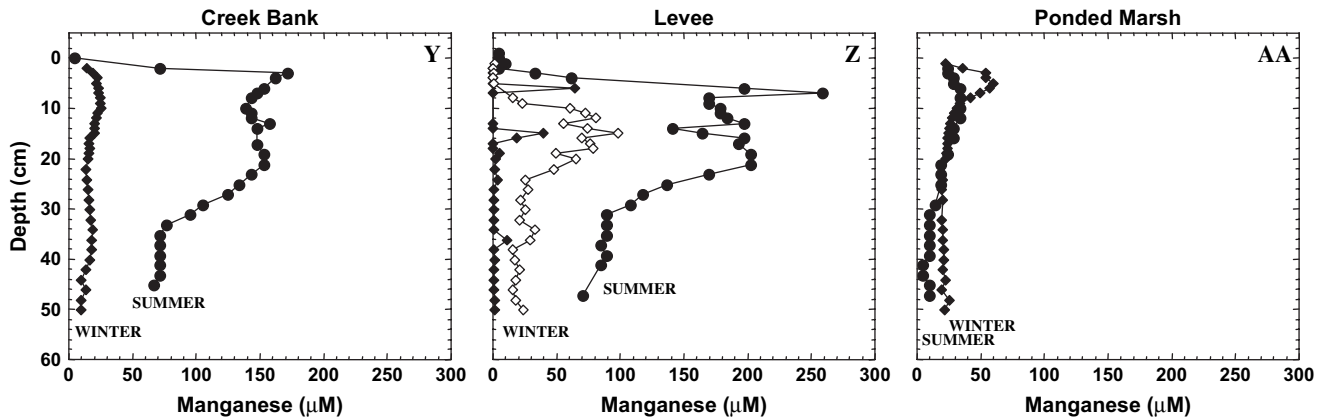


Fig. 1 (continued)

of sediment where the peak MnRB and AEB populations are found. As for the MnRB and AEB, the highest numbers of FeRB are present at the vegetated levee site.

The percentage of prokaryotic 16S rRNA represented by SRB385 (an oligonucleotide specific for sulfate-reducing bacteria) is quite large at all three sites. The highest percentages typically occur in the upper 10 cm of the sediment column, although there is an increase in SRB385 at ~50 cm depth in the levee and at the creek bank in summer (Fig. 3A–C). Because these data are presented as percentages of the total pool of prokaryotic 16S rRNA (sum of *Archaea*- and *Bacteria*-specific rRNA signals), this increase may reflect a decrease in the pool of 16S rRNA from other prokaryotes, rather than an increase in the contribution from SRB-specific 16S rRNA. The high percentages of SRB385 in the upper 10 cm are in good agreement with sulfate reduction rates (SRR) measured using the same cores, which indicate that SRR are also highest in the upper 10 cm of the sediment column at all three sites (Fig. 3J–L; Kostka et al., 2002b).

Although SRR increase considerably from winter to summer (Fig. 3J–L; Kostka et al., 2002b), the percentage of prokaryotes represented by SRB385 does not change significantly with season at any of the sites (Fig. 3A–C). The more specific oligonucleotide probes point to larger populations of *Desulfovibrio* (DSV387) and *Desulfobacter* (DSB804) in the summer at all three sites, particularly at depths greater than 20 cm or so (Fig. 3D–I). In the upper 20 cm of the sediment column, *Desulfovibrio* is most abundant at the ponded marsh and levee, whereas *Desulfobacter* is more abundant in the upper 20 cm of the creek bank.

4.3. Seasonal trends in pore water pools across the transect

To illustrate seasonal trends in pore water pools at the three sites, pore water concentrations are integrated from the sediment–water interface to a depth of 35 cm

(Fig. 4). Over this depth interval, integrated alkalinity is always highest at the ponded marsh (Fig. 4A). From late spring to fall, alkalinity is intermediate at the levee and lowest at the creek bank. At the vegetated levee and creek bank sites, alkalinity exhibits a clear seasonal oscillation, with peak values in late spring to fall and lower values in winter and early spring. Seasonal changes in integrated alkalinity are much less pronounced at the unvegetated creek bank site.

Integrated phosphate concentrations at the creek bank are highest in winter and lowest in late spring and summer (Fig. 4B). As phosphate concentrations at the unvegetated creek bank decrease from winter to spring, the reverse trend is observed at both the vegetated sites. In late spring, phosphate concentrations are highest at the levee and in summer they are highest at the ponded marsh. Ammonium concentrations are typically much higher at the creek bank than at the vegetated sites (data not shown). Ammonium was not measured at all sites during all seasons. However, as shown above (Fig. 1M–O), the summer–winter data suggest that ammonium levels do not fluctuate greatly with season at the creek bank, but increase during summer at the vegetated sites.

Integrated sulfate concentrations are typically highest at the creek bank and lowest at the ponded marsh (Fig. 4C). Except for a decrease in sulfate during June 1998, there is relatively little seasonal change in sulfate pools in the upper 35 cm of the creek bank. In contrast, sulfate levels at the levee site show a strong seasonal dependence, decreasing from winter to summer and then increasing from summer to winter. A similar, although less pronounced, trend is apparent at the ponded marsh site (Fig. 4C). Sulfide concentrations are always highest at the ponded marsh and are always lowest at the creek bank (Fig. 4D). At both the vegetated sites, sulfide concentrations are highest in summer.

Dissolved ferrous iron concentrations at the ponded marsh are always low (Fig. 4E). Integrated ferrous iron concentrations at the creek bank are greatest in May 1997 and August 1997. At the levee, ferrous iron

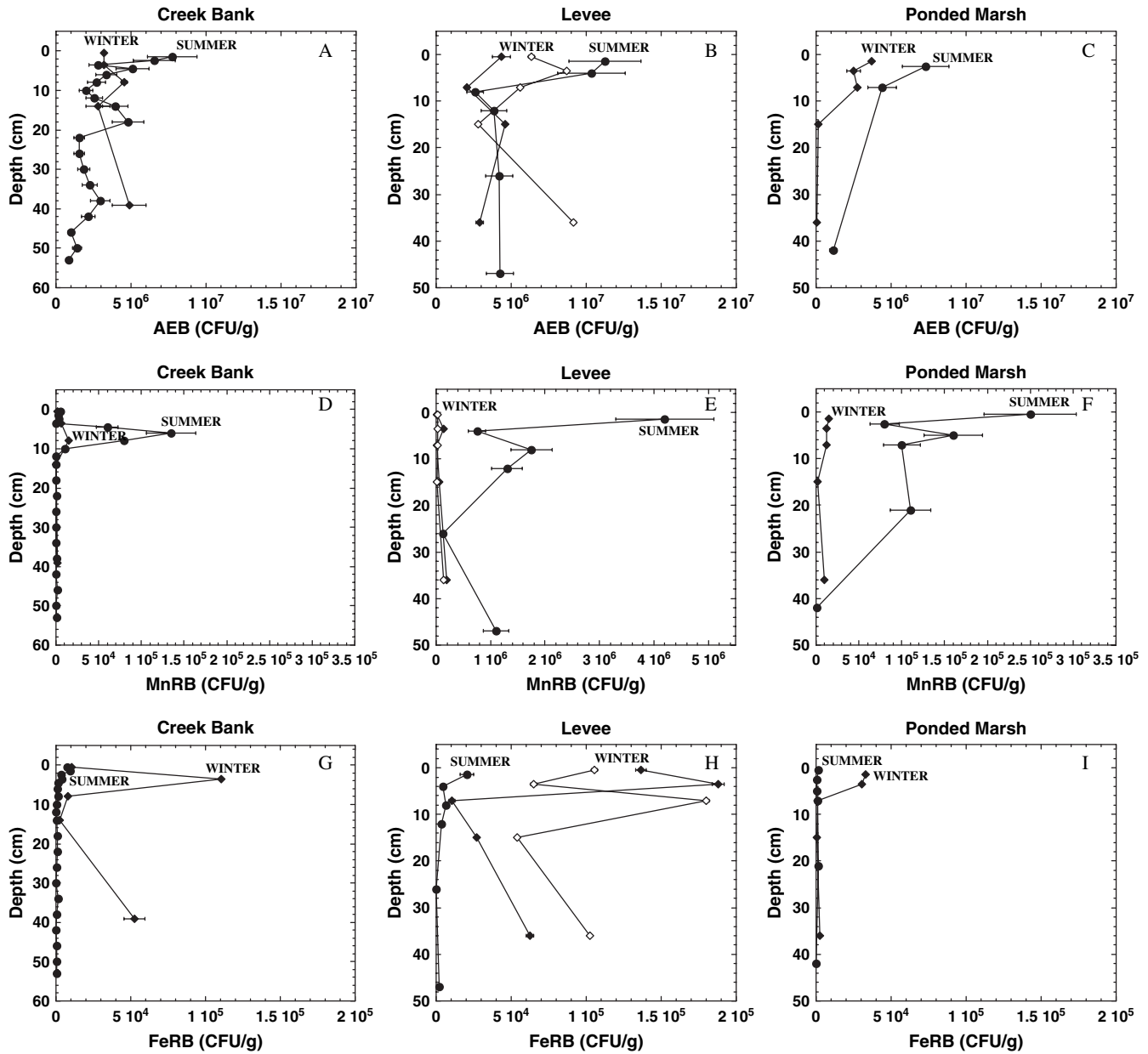


Fig. 2. Microbial culture enumeration data for (A–C) AEB using Marine 2216 media, (D–F) MnRB using lactate as the sole carbon source and (G–I) FeRB using lactate as the sole carbon source for sediments collected in January 1999 (diamonds) and August 1997 (circles).

concentrations are greatest during August 1997. Dissolved manganese trends are similar to those observed for ferrous iron. Integrated manganese concentrations are always lowest at the pondered marsh and vary little with season (Fig. 4F). At both the creek bank and levee sites, dissolved manganese concentrations are lowest in winter and highest in summer.

4.4. Seasonal trends in microbial community structure across the transect

Integrated AEB levels are typically greatest at the levee site, where they do not show much seasonal

variation (Fig. 5A). Similarly, there is little variation in integrated AEB in the upper 35 cm of the sediment at the creek bank site. At the pondered marsh, AEB levels decrease somewhat during winter.

In contrast to the culturable AEB populations, integrated populations of culturable MnRB and FeRB do show significant seasonal changes. The MnRB populations are lowest in winter at all three sites (Fig. 5B). The seasonal change in MnRB is most pronounced at the levee site, where the highest culturable MnRB populations are typically found. MnRB in the upper 35 cm fluctuate least with season at the unvegetated creek bank. Integrated FeRB populations also vary

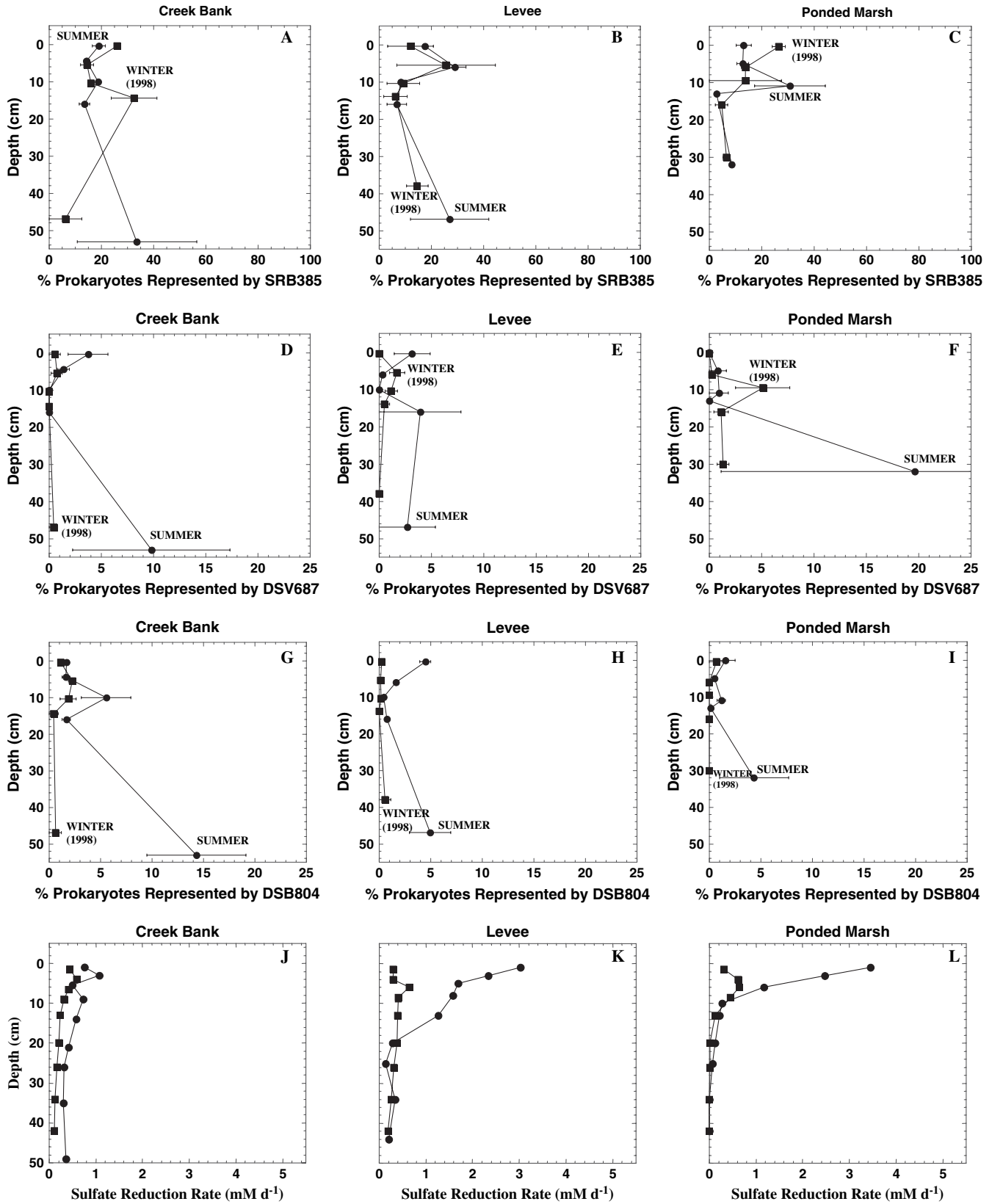


Fig. 3. 16S rRNA data indicating the percentage of prokaryotes represented by (A–C) sulfate-reducing bacteria, using probe SRB385, (D–F) *Desulfovibrio*, using probe DSV387, and (G–I) *Desulfobacter*, using probe DSB804, in January 1998 (squares) and August 1997 (circles). Sulfate reduction rates (J–L) are reproduced from Kostka et al. (2002b).

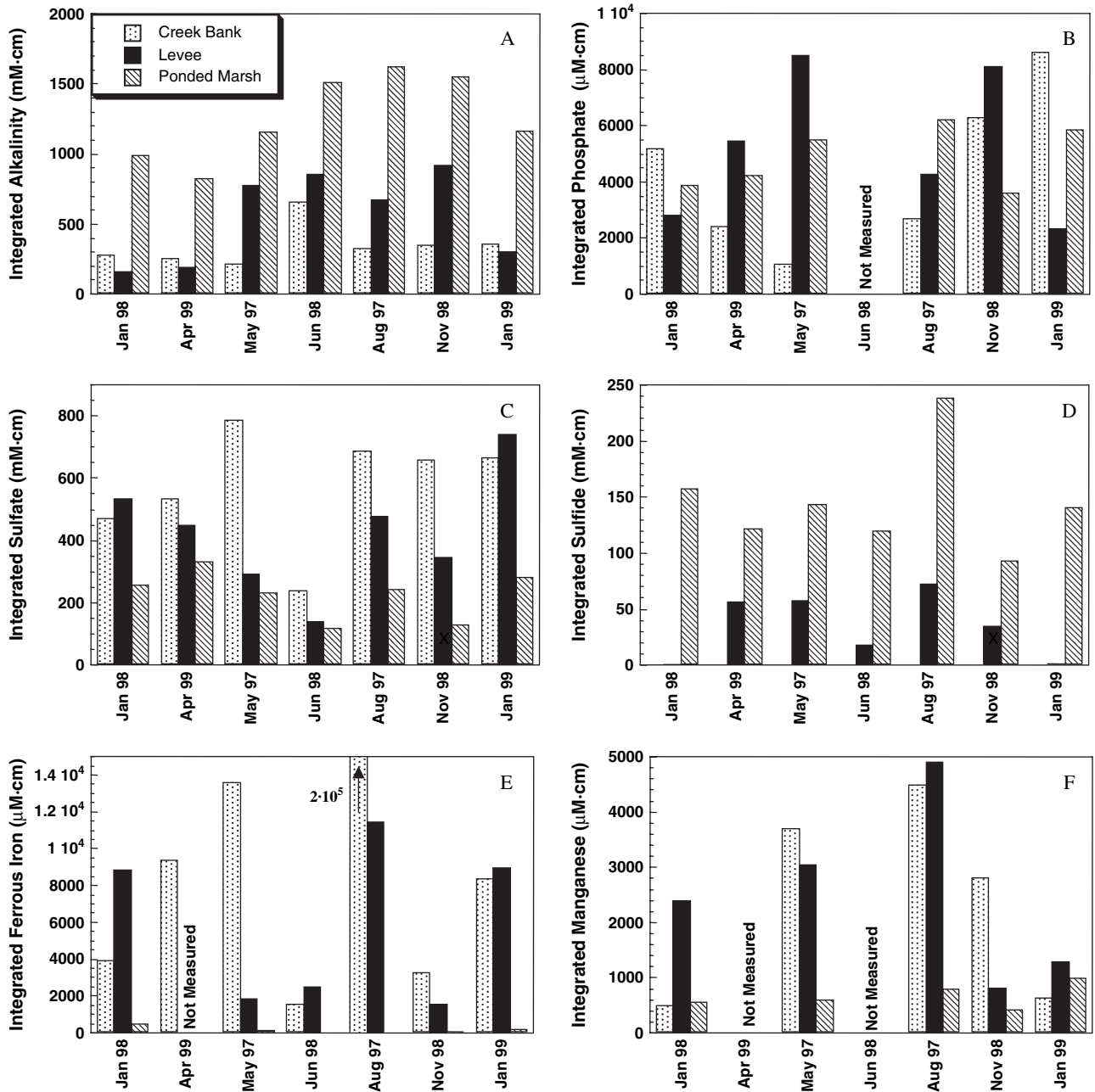


Fig. 4. Seasonal pore water concentrations at the creek bank, levee and ponded marsh sites integrated from the sediment surface to a depth of 35 cm.

with season, but the seasonal oscillation is in strong contrast to that observed for MnRB. Culturable FeRB populations are highest in winter and early spring and decrease dramatically in summer at all three sites (Fig. 4C; see also Koretsky et al., 2003).

Measured SRR (see also Kostka et al., 2002b) show a clear dependence on season (Fig. 4D). At all sites, the highest SRR occur in summer; in the upper 35 cm of the sediment column, integrated SRR are always greatest at the levee and least at the ponded marsh. Oligonucleotide

probing for DSV387 and DSB804 was only completed during January 1998 and August 1997. These data indicate that *Desulfovibrio* populations as a percentage of prokaryotes increase moving from the creek bank towards the ponded marsh, while *Desulfobacter* populations show the opposite trend, decreasing from the creek bank toward the interior of the marsh. At all sites, both the *Desulfovibrio* and the *Desulfobacter* percentages of the prokaryotic populations are considerably higher in summer than in winter.

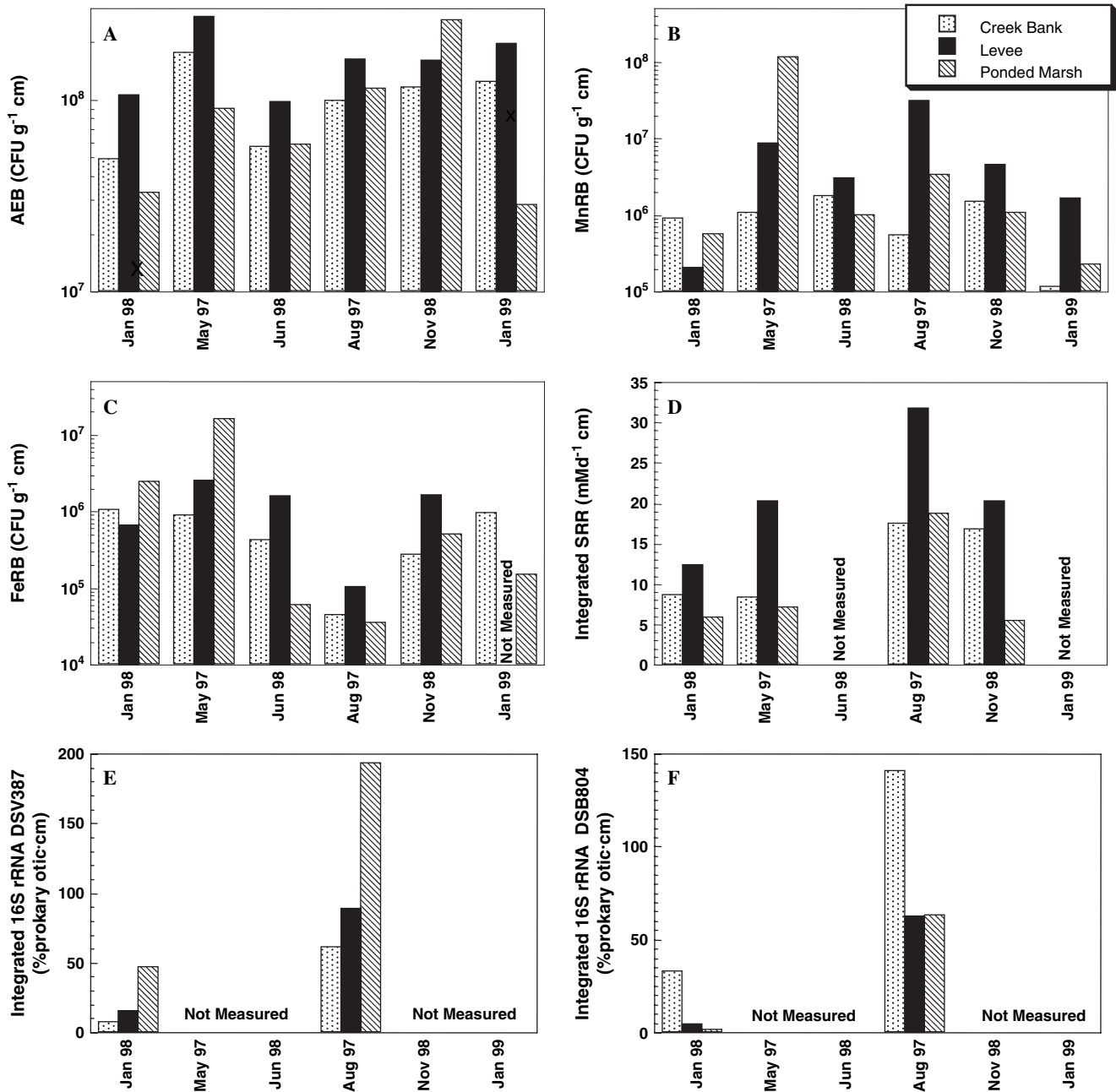


Fig. 5. Microbial culture enumeration data, measured sulfate reduction rates (from Kostka et al., 2002b), and 16S rRNA data at the creek bank, levee and ponded marsh sites integrated from the sediment surface to a depth of 35 cm.

5. Discussion

5.1. Pore water redox zonation and microbial respiratory pathways

Pore water profiles collected at the ponded marsh, levee and creek bank sites exhibit classical vertical redox stratification, with progressive build-up of dissolved manganese, ferrous iron and sulfide with depth in the sediments (Fig. 1). There is also a clear trend in redox

stratification across the transect: pore waters are more reduced at a given depth moving away from the creek bank and towards the ponded marsh. For example, in August 1997 dissolved sulfide concentrations at the ponded marsh reach 1 mM at just 3.5 cm depth, but do not reach 1 mM at the levee until ~22 cm depth. Sulfide remains below detection limits at all depths (to 50 cm) at the creek bank.

In more organic-poor, laterally homogenous sediments it has been postulated that vertical redox

stratification of sediment pore waters indicates vertical stratification of dominant microbial respiratory pathways (e.g., Froelich et al., 1979). However, enrichment culture and nucleic acid hybridization collected in this study, as well as measured SRR (Kostka et al., 2002b), all demonstrate the absence of a clear vertical separation of microbial respiratory pathways at the three sites during any of the seasons studied (Figs. 2 and 3).

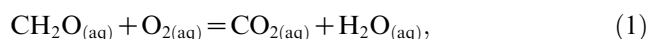
A comparison of measured total organic carbon oxidation rates with sulfate reduction rates (Kostka et al., 2002b) has shown that bacterial sulfate reduction is the primary organic matter degradation pathway in the upper 5 cm at all three sites, including the creek bank site, where pore waters remain oxic or suboxic over the entire upper 50 cm (Fig. 1). The highest sulfate reduction rates and the highest percentages of SRB-targeted 16S rRNA occur not in the sulfidic zone of the pore waters, but within the upper 10 cm of sediment (Fig. 3). The highest populations of culturable AEB and anaerobic FeRB and MnRB are found within the same surface sediment layers (Fig. 2). In addition, comparable population densities of culturable AEB and anaerobic FeRB and MnRB are found in the upper 10 cm of sediment at all three of the sites, and the highest sulfate reduction rates are typically found at the levee, rather than at the ponded marsh site. Pronounced differences in microbial community structure and activity are apparent mostly at depths below 20 cm, where, in contrast to the creek bank and levee sites, AEB population levels and microbial SRR at the ponded marsh site approach zero (Figs. 2 and 3). Thus, vertical redox stratification of the pore water profiles and differences in pore water profiles at the three sites cannot simply be explained by differences in the microbial community structures of the sediments.

Pore water stratification is observed in the measured profiles, in spite of the lack of microbial stratification, because the pore water structure reflects not only the local production and consumption of solute species through microbial organic carbon oxidation, but also reflects chemical (abiotic) reactions as well as physical and biological solute transport processes. By providing a continuous influx of O₂ in suboxic or anoxic sediments, roots and burrows create a variety of distinct geochemical microzones that can host a diverse population of organisms in sediment strata with bulk (i.e., laterally-averaged) conditions that would not support their presence. AEB, MnRB and FeRB are thus likely to function within sequential *horizontal* zones surrounding roots or burrows in sediment strata that are dominated, in a “bulk” sense, by very high SRR. It has also been postulated that the wide variety of labile organic compounds in the surface sediments may favor certain microbial populations, allowing them to outcompete or at least persist in the same strata as microorganisms using more energetically-favorable terminal electron acceptors (e.g., Lovley and Phillips, 1987).

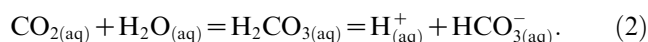
5.2. Spatial trends in pore water geochemistry and microbial community structure: effects of bioirrigation and bioturbation

Unlike many subtidal sediments, in which solute transport occurs primarily via molecular diffusion, previous studies indicate that solute transport at the intertidal sites in this study, with their high macrofaunal population densities, is dominated by bioirrigation (Meile et al., 2001; Koretsky et al., 2002). For example, an inverse approach has been applied to sulfate reduction rate and concentration profiles at these sites to calculate depth-dependent irrigation intensities (Meile et al., 2001). Such calculations indicate that irrigation intensities are always greatest in the upper 15 cm of the sediment and that irrigation is most intense at the creek bank and levee sites and least intense at the ponded marsh. Results of a stochastic modeling study, using ecological rather than chemical data, are in agreement with the inverse model results, and also suggest that irrigation is deepest, as well as most intense, at the levee and creek bank sites (Koretsky et al., 2002).

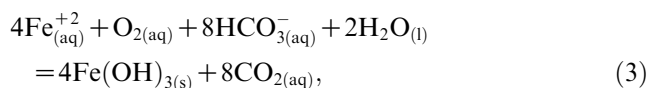
At all three sites, a rapid decline of subsurface pH is observed in the upper 5–10 cm, particularly during summer (Fig. 1A–C). This coincides with the depth interval that has the highest macrofaunal population densities (Koretsky et al., 2002) and lends further evidence that there is a significant bioirrigation flux in the upper portion of these sediments. Decreasing pH occurs as flushing of oxygenated water into burrows promotes aerobic respiration, which produces acidity via reactions such as



and



Additionally, O₂-promoted reoxidation of reduced solutes produces acidity, for example, via



(e.g., Van Cappellen and Wang, 1996).

Because less oxygen is introduced through the sparser, shallower burrows at the ponded marsh, reoxidation is expected to occur with less intensity and confined to shallower depths compared to the levee or creek bank sites. This less intense bioirrigation results in the accumulation of much higher levels of dissolved sulfide near the sediment surface at the ponded marsh compared to the other sites (Fig. 1). Furthermore, because more dissolved sulfide is available to react with dissolved ferrous iron to form amorphous or crystalline

oxides (e.g., Berner, 1970; Giblin and Howarth, 1984; Howarth and Merkel, 1984; Luther et al., 1992), there is much less accumulation of dissolved iron in the pore waters of the ponded marsh compared to the other sites (Fig. 1).

Such differences in bioirrigation are also consistent with accumulation of chromium reducible sulfides (thought to represent pyrite) and ascorbate extractable iron (thought to represent readily reducible Fe(III) oxides) at the three sites. Higher concentrations of pyrite have been reported to accumulate in the solid phase of the ponded marsh compared to the creek bank or levee (Kostka et al., 2002b). This is because the less intense irrigation at this site allows plentiful dissolved sulfide to react with dissolved ferrous iron or solid phase ferric iron. This in turn leads to the much lower concentrations of ascorbate extractable iron reported at the ponded marsh site (Kostka et al., 2002b).

Particle mixing via bioturbation also influences pore water and solid sediment profiles. Enhanced particle mixing at the creek bank and levee sites rapidly transports Fe(III) oxides produced near the surface to greater depths than at the ponded marsh sites. These Fe(III) oxides react with dissolved sulfide, which keeps sulfide concentrations low at the creek bank and levee sites in spite of high sulfate reduction rates (Fig. 1), and produce solid phase acid volatile sulfide and pyrite (Kostka et al., 2002b).

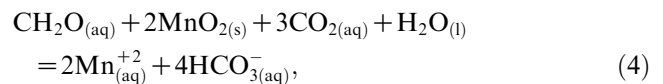
5.3. Spatial and seasonal trends in pore water geochemistry and microbial community structure: influence of macrophytes

Pore water redox stratification is also influenced by the presence or absence of *Spartina alterniflora* at the three saltmarsh sites. Pumping of dissolved oxygen into the sediments by *Spartina* roots (e.g., Mendelsohn et al., 1981; Howes and Teal, 1994) leads to reoxidation of reduced solutes in the rhizosphere (e.g., Mendelsohn and Postek, 1982; Sundby et al., 1998; Lee et al., 1999). Thus, at the vegetated levee and ponded marsh sites, dissolved manganese, ferrous iron, sulfide and other reduced aqueous species may be produced and then rapidly reoxidized, preventing their accumulation in the shallow pore waters, in spite of the presence of actively respiring anaerobic bacteria. Furthermore, reoxidation of ferrous iron produces ferric (hydr)oxide minerals (e.g., Mendelsohn and Postek, 1982; Sundby et al., 1998), which may in turn reoxidize dissolved sulfide (e.g., Yao and Millero, 1996; Koretsky et al., 2003).

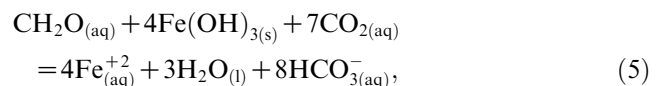
The *Spartina* roots are, however, not only a source of dissolved oxygen but also provide an increased supply of labile organic carbon within the root zone (e.g., Howarth and Teal, 1979; Mendelsohn et al., 1981; Hines et al., 1989, 1999; Hines, 1991). This is likely responsible for the elevated SRR that have been

measured within the rhizosphere of *Spartina* and other macrophytes relative to rates in surrounding bulk sediments (e.g., Isaksen and Finster, 1996; Holmer and Nielsen, 1997; Hines et al., 1989, 1999; Nielsen et al., 2001). 16S rRNA analyses also point to the presence of large SRB populations within the *Spartina* rhizosphere (Rooney-Varga et al., 1997, 1998; Hines et al., 1999). Hines et al. (1989, 1999) have demonstrated that seasonal variations in SRR measured in the bulk sediments of a vegetated saltmarsh correlate more closely with the *Spartina* growth cycle than with temperature. Similar variations in SRR with season occur at the vegetated sites in this study (Fig. 5D; Kostka et al., 2002b) with the highest SRR found at the levee site. This is expected: the dense vegetation at the levee is a source of abundant labile organic carbon, while dissolved oxygen supplied by roots and intense irrigation allows the sulfate supply to be constantly replenished. Thus, sulfate pools in the upper 35 cm of the sediment remain large (Fig. 4), in spite of the very high SRR (Fig. 5).

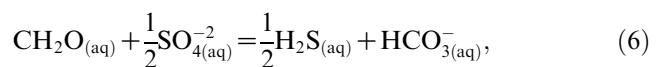
The presence of *Spartina* also influences pore water alkalinity: concentrations are typically greater at the vegetated sites, and seasonal variations in alkalinity pools are much more pronounced at the levee and ponded marsh compared to the unvegetated creek bank (Figs. 1D–F and 4A). Alkalinity is produced by the anaerobic respiration of organic matter via dissimilatory manganese reduction,



producing 4 moles alkalinity per mol organic C, via dissimilatory iron reduction,



producing 8 moles alkalinity per mol organic C, and via microbial sulfate reduction,



producing 1 mole of alkalinity per mole of organic C (e.g., Van Cappellen and Wang, 1996). In contrast, aerobic respiration does not produce (or consume) carbonate alkalinity, although it does produce acidity, as can be seen from reactions (1) and (2). Therefore, if carbonate dissolution and precipitation reactions do not contribute significantly to the measured total alkalinity, then alkalinity can be regarded as a measure of anaerobic respiration. This suggests that microbial activity is significantly enhanced at the vegetated sites,

particularly during the growth season, in agreement with the measured SRR.

Enhanced microbial activity in saltmarsh sediments in summer is also evident from previously reported seasonal measurements of adenosine triphosphate (ATP) at Sapelo Island (Christian et al., 1975). Because ATP degrades fairly rapidly in nonliving cells, it serves as an indicator of seasonal and spatial changes in active microbial biomass of the sediments. Christian et al. (1975) made seasonal measurements of ATP concentrations in the upper 25 cm of the sediment column at two sites ('tall *Spartina*' and 'short *Spartina*') on Sapelo Island, GA. The depth-integrated ATP data from their study correlate reasonably well with depth-integrated alkalinities measured at the levee and ponded marsh sites in this study (Fig. 6), supporting the idea that alkalinity measurements are a useful proxy for seasonal trends in anaerobic microbial biomass.

Other trends in pore water composition across the transect are also likely due to the presence of the *Spartina*. For example, the more acidic pore water pH measured at the vegetated sites (Fig. 1A–C) is a likely indicative of enhanced dissolved oxygen release within the root zone, fueling aerobic respiration and reoxidation reactions. The limited ammonia accumulation in pore waters at the ponded marsh and levee is most likely due to uptake by the *Spartina*, the growth of which has

been postulated to be nitrogen-limited in many salt-marshes (e.g., Sullivan and Daiber, 1974; Linthurst, 1980; Dai and Wiegert, 1997).

5.4. Pore water profiles from vegetated vs. unvegetated creek bank

Naturally-occurring colonization of *Spartina* at the previously unvegetated creek bank site (see Section 2) is used to further test our hypotheses concerning the influence of *Spartina* on pore water redox stratification. During spring, significant differences in pore water composition are apparent at the unvegetated compared to the adjacent, newly vegetated creek bank site. In April 1999, pH is slightly lower in the upper 35 cm of the vegetated site (Fig. 7A), which might reflect enhanced aerobic respiration and reoxidation reactions promoted by O₂ pumping through the *Spartina* roots, although in May 2000, pH in the upper 20 cm is somewhat lower in the unvegetated sediments (Fig. 7B). Thus, protons generated by enhanced aerobic respiration or reoxidation reactions during May 2000 must be more than balanced by proton removal due to enhanced anaerobic respiration.

The pore water data from these sites provide further evidence that an overwhelming result of *Spartina* colonization, at least during spring, is a large stimulation of microbial activity, particularly anaerobic respiration. Alkalinity accumulation is considerably greater in the vegetated sediments during both April and May than in the adjacent unvegetated sediments (Fig. 7C, D). Similarly, phosphate accumulation, which results from organic matter degradation, is much larger at the vegetated sites, particularly below 40 cm (Fig. 7E, F). Ammonium was not measured in April 1999, however, in May 2000, it is clear that ammonium accumulation is much greater in the unvegetated portion of the creek bank sediments, which probably reflects uptake of ammonium by the *Spartina* (Fig. 7G). During May, peak concentrations of dissolved manganese and ferrous iron are considerably larger in the vegetated sediments (Fig. 7I, K). During both April and May, dissolved sulfide in the unvegetated sediments is near or below the detection limit at all depths, however, there is significant accumulation of dissolved sulfide in the pore waters of the vegetated sediments (Fig. 7L, M).

5.5. Spatial trends in pore water redox structure: combined effects of reaction and transport

The spatial trends in pore water redox stratification at these three sites result from the combined effects of enhanced transport via bioirrigation, root pumping and bioturbation (macrofaunal particle mixing) and enhanced reaction due to increased labile organic matter availability at vegetated sites. At the ponded marsh site,

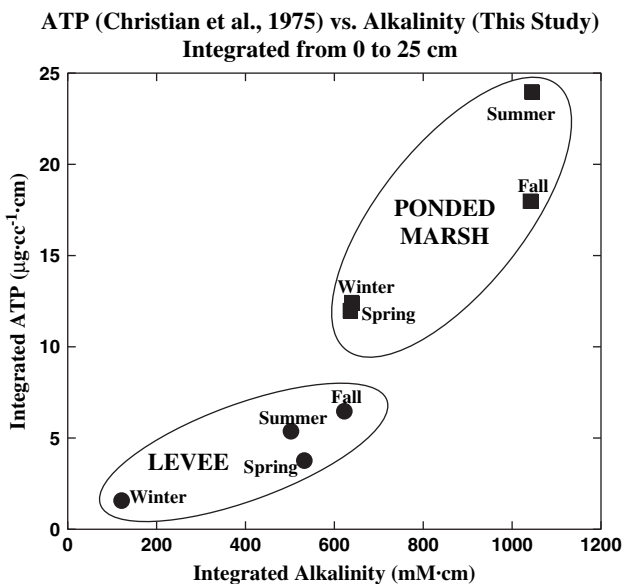


Fig. 6. Measured concentrations of adenosine triphosphate (in $\mu\text{g cc}^{-1}\text{ cm}$) integrated from 0 to 25 cm from Christian et al. (1975) vs. measured alkalinities (in mM cm) integrated from 0 to 25 cm. Circles represent data measured at levee site ('tall *Spartina*' site in Christian et al., 1975) and squares indicate data from ponded marsh site ('short *Spartina*' site in Christian et al., 1975). Winter data are from December, January, and February, spring data are from April and May, summer data are from June, July and August and fall data are from October and November.

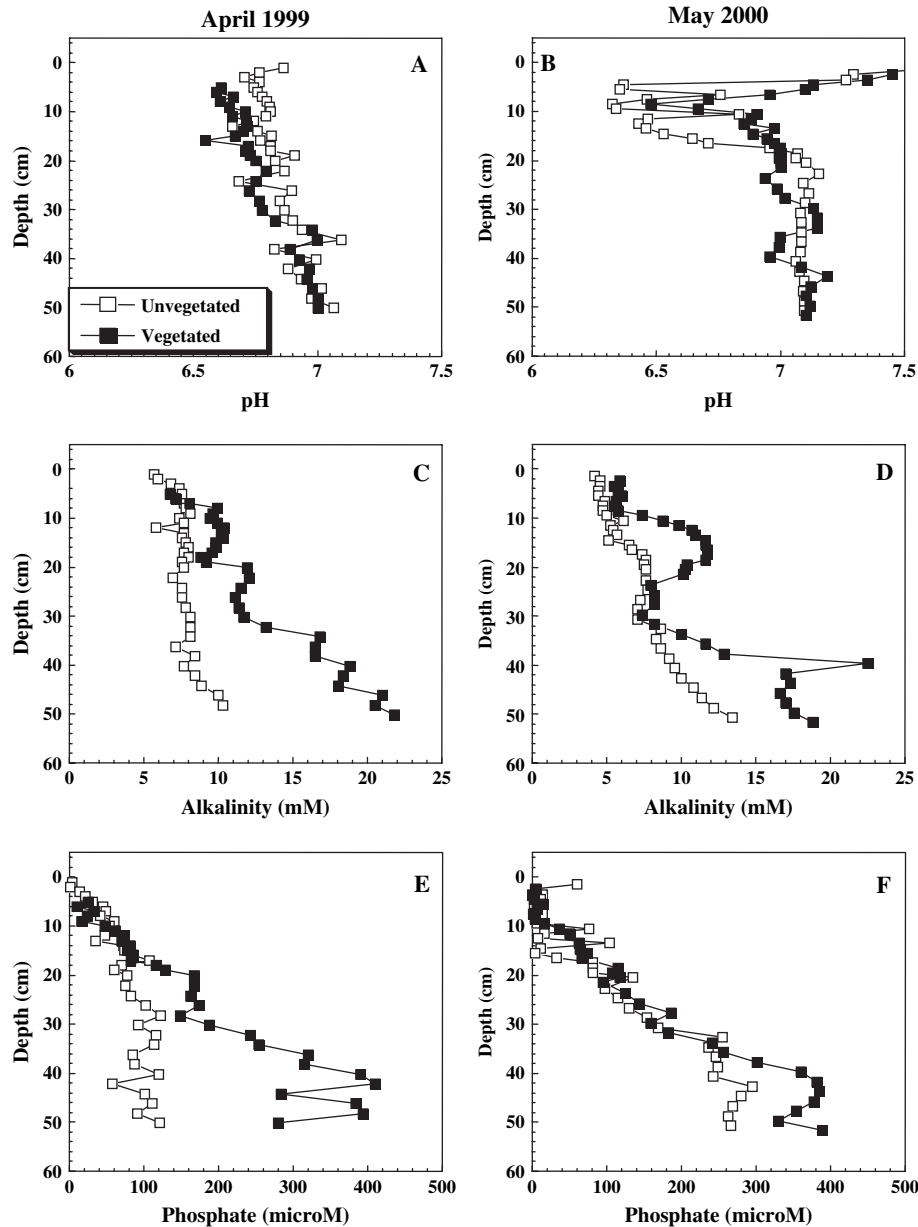


Fig. 7. Pore water data measured at the vegetated (filled squares) and unvegetated (open squares) creek bank in April 1999 and May 2000. Dissolved ammonium at both sites, Fe^{+2} at the vegetated site and Mn^{+2} at the unvegetated site were not measured during April 1999.

bioirrigation and bioturbation activity is shallowest and least intense. Combined aboveground and belowground primary productivity at the ponded marsh is of the same order of magnitude as at the levee site (Dame and Kenny, 1986). Thus, reduced solutes are produced at relatively rapid rates during microbial organic matter oxidation, but are reoxidized relatively slowly, except very near the sediment surface, leading to highly compressed redox stratification with large accumulation of dissolved sulfide. At the creek bank site, bioirrigation and bioturbation is deep and intense, but there is no vegetation. Organic matter introduced at the sediment surface is efficiently redistributed throughout the sediment column by the intense bioturbation. Thus, sulfate

reduction rates are relatively low, but do not decrease significantly with depth. No accumulation of sulfide in the pore waters occurs at the creek bank, because of the rapid sulfide reoxidation fueled by the intense bioirrigation and the persistence of Fe(III) oxides with depth due to rapid bioturbation. Both the low organic matter availability (relative to the vegetated sites) and the high irrigation intensity maintain the relatively oxidized pore water conditions at the creek bank site.

Competition between high irrigation intensity and high organic matter availability occurs at the levee site. Although the dense *Spartina* fuels organic matter oxidation and reduced solute production, intense irrigation and O_2 supply by roots lead to efficient

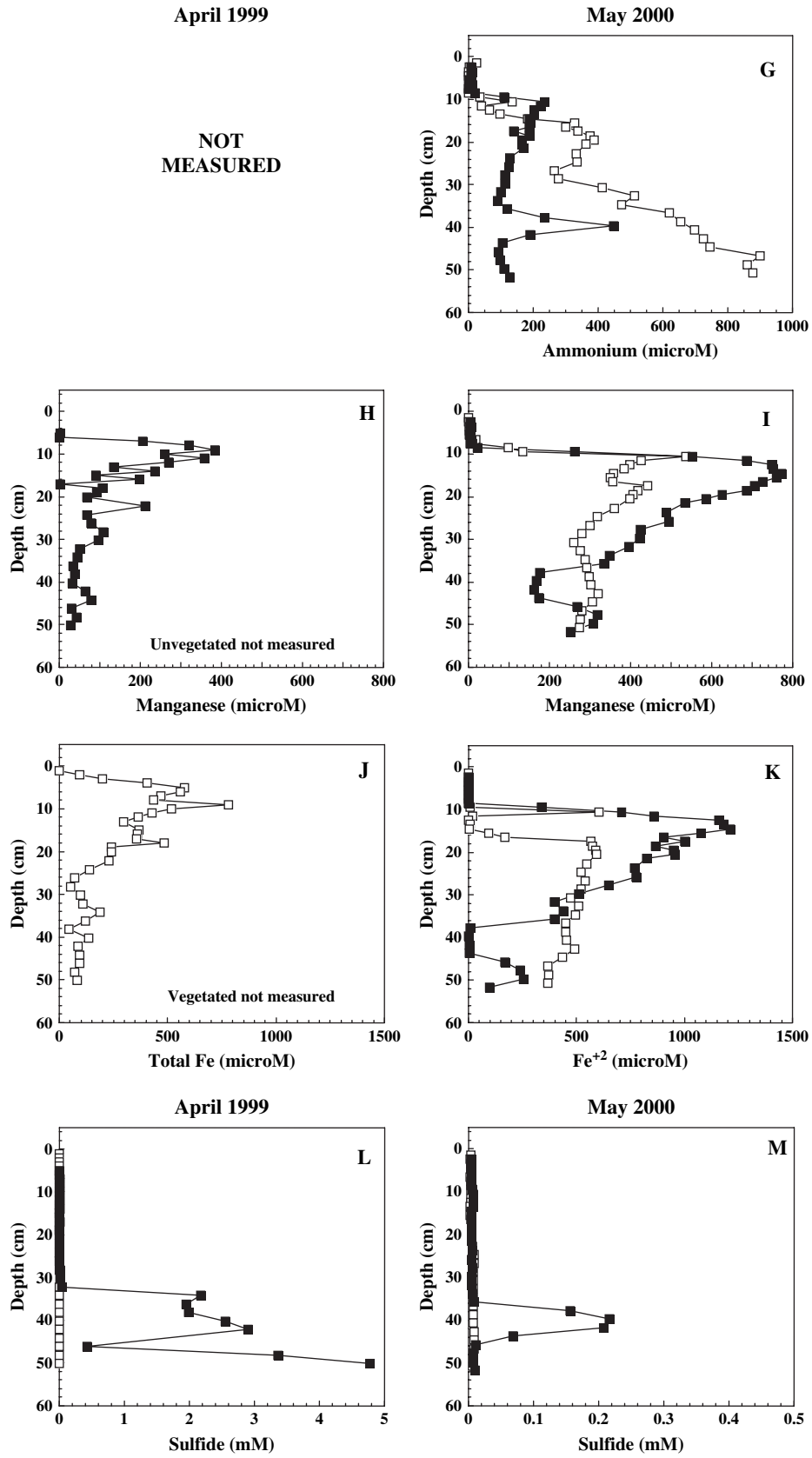


Fig. 7 (continued)

reoxidation of reduced solutes. Thus, the pore water redox structure at the levee site is more reduced than at the creek bank, but is less reduced than at the ponded marsh. Very high sulfate reduction rates are sustained by the availability of both sulfate and reduced organic matter, but sulfide accumulates in the pore waters only seasonally, when sulfate reduction rates are highest and reactive Fe(III) oxides are depleted.

6. Conclusions

High labile organic matter input, bioirrigation and bioturbation intensities in saltmarsh sediments lead to intense internal redox cycles. This prevents a simple one-to-one correlation between pore water and microbial community structure as has been proposed to explain pore water redox stratification in deep-sea sediments. Instead, macrofaunal and rhizospheric activity at these sites leads to diverse, co-existing heterotrophic populations in the upper 10 cm of the saltmarsh sediments. Nonetheless, distinct spatial and temporal trends in microbial activity and pore water redox stratification are apparent in these saltmarsh sediments, and these trends are clearly related to seasonal and spatial changes in macrofaunal activity and labile organic carbon availability.

Seasonal changes in pore water structure are primarily the result of changes in the rate of microbial organic matter oxidation, driven by seasonal oscillations of temperature and labile organic matter availability. Hence, more compressed redox stratification occurs in summer compared to winter. Differences in pore water structure across the saltmarsh transect reflect spatial trends in bioirrigation, bioturbation, and vegetation. Intense and deep bioirrigation at the levee and creek bank sites leads to rapid reoxidation of reduced solutes, enhances the supply of oxidants from the overlying water, and regenerates Fe(III) (hydr)oxides. Intense bioturbation at these sites leads to the downward transport of solid phase Fe(III) as well as organic matter. In contrast, enhanced labile organic carbon availability at the vegetated sites fuels microbial respiration and less intense bioturbation and bioirrigation allows reduced phases to accumulate at much shallower depths. Thus, pore water redox stratification is most compressed at the ponded marsh and is least compressed at the unvegetated creek bank.

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