

Relationships between microbial communities and environmental parameters at sites impacted by mining of volcanogenic massive sulfide deposits, Prince William Sound, Alaska

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Abstract

The relations among geochemical parameters and sediment microbial communities were examined at three shoreline sites in the Prince William Sound, Alaska, which display varying degrees of impact by acid-rock drainage (ARD) associated with historic mining of volcanogenic massive sulfide deposits. Microbial communities were examined using total fatty acid methyl esters (FAMES), a class of compounds derived from lipids produced by eukaryotes and prokaryotes (bacteria and Archaea); standard extraction techniques detect FAMES from both living (viable) and dead (non-viable) biomass, but do *not* detect Archaeal FAMES. Biomass and diversity (as estimated by FAMES) varied strongly as a function of position in the tidal zone, not by study site; subtidal muds, Fe oxyhydroxide undergoing biogenic reductive dissolution, and peat-rich intertidal sediment had the highest values. These estimates were lowest in acid-generating, intertidal zone sediment; if valid, the estimates suggest that only one or two bacterial species predominate in these communities, and/or that Archeal species are important members of the microbial community in this sediment.

All samples were dominated by bacterial FAMES (median value >90%). Samples with the highest absolute abundance of eukaryotic FAMES were biogenic Fe oxyhydroxides from shallow freshwater pools (fungi) and subtidal muds (diatoms). Eukaryotic FAMES were practically absent from low-pH, sulfide-rich intertidal zone sediments. The relative abundance of general microbial functional groups such as aerobes/anaerobes and gram(+)/gram(–) was not estimated due to severe inconsistency among the results obtained using several metrics reported in the literature.

Principal component analyses (PCAs) were performed to investigate the relationship among samples as separate functions of water, sediment, and FAMES data. PCAs based on water chemistry and FAMES data resulted in similar relations among samples, whereas the PCA based on sediment chemistry produced a very different sample arrangement. Specifically, the sediment parameter PCA grouped samples with high bulk trace metal concentration regardless of whether the metals were incorporated into secondary precipitates or primary sulfides. The water chemistry PCA and FAMES PCA appear to

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be less prone to this type of artifact. Signature lipids in sulfide-rich sediments could indicate the presence of acid-tolerant and/or acidophilic members of the genus *Thiobacillus* or they could indicate the presence of SO_4 -reducing bacteria. The microbial community documented in subtidal and offshore sediments is rich in SRB and/or facultative anaerobes of the *Cytophaga-Flavobacterium* group; both could reasonably be expected in PWS coastal environments. The results of this study provide evidence for substantial feedback between local (meter to centimeter-scale) geochemical variations, and sediment microbial community composition, and show that microbial community signatures in the intertidal zone are significantly altered at sites where ARD drainage is present relative to sites where it is not, even if the sediment geochemistry indicates net accumulation of ARD-generated trace metals in the intertidal zone.

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1. Introduction

Between 1897 and 1930, Cu, Au and Ag were mined from volcanogenic massive sulfide (VMS)

deposits located near the shorelines of several islands in the Prince William Sound (PWS), Alaska (Fig. 1a; Crowe et al., 1992; Koski et al., 2008). Low-pH, metal-rich plumes of water (typically

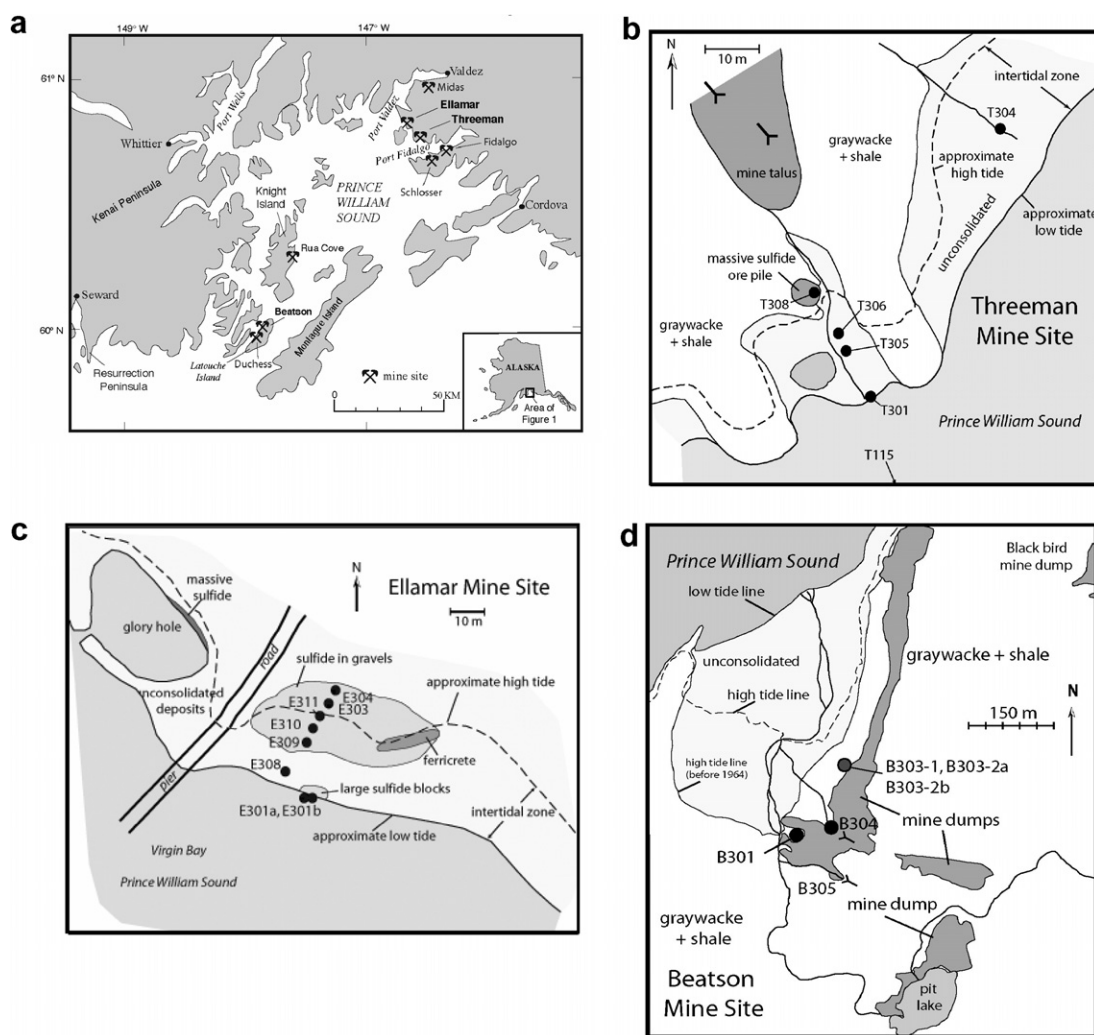


Fig. 1. (a) Landform map of Prince William Sound, AK showing study sites; generalized maps of the (b) Threeman, (c) Ellamar, and (d) Beatson study sites show major features and sampling locations.

called “acid-rock drainage”, or ARD) generated by the oxidative breakdown of sulfide minerals emanate from several of the abandoned mine workings (Goldfarb et al., 1996), reaching the tidal zone at many locations; at others, sulfide-rich mining debris is already present in the tidal zone sediments (Koski et al., 2003; Shanks et al., 2003). Where either or both occur, dissolved trace metals derived from sulfides accumulate in several types of fine-grained phases that precipitate in tidal sediments (Koski et al., 2008).

The feeding habits of shellfish and other invertebrates common to tidal zones of the PWS (including mussels, clams, gunnelfish, barnacles, worms, chitons, shrimps, crabs, and snails; O’Clair and O’Clair, 1998) brings them into close contact with sediment and pore water. Recent work has shown that the concentrations of Cu in blue mussels (*Mytilus edulis*) collected from 3 VMS mine sites in the PWS are about 7 times higher than Cu concentrations in blue mussels from background PWS sites, from other sites in Alaska, and from other sites in the lower 48 states (Koski et al., 2008). A 2- to 3-fold enrichment in Zn and Pb relative to other mussels was also observed at one of the three sites in that study. These data suggest that biota inhabiting shorelines where mining occurred are at risk for accumulating a greater body burden of selected trace metals, with potentially deleterious effects to themselves and the food webs they support. This food web also includes an unknown number of humans that consume local shellfish.

Microorganisms are also an important part of shoreline food webs, but tidal zone microbial communities have not been studied in great detail (Novitsky and MacSween, 1989). Microorganisms include microscopic eukaryotes (hereafter called “microeukaryotes”), bacteria, and Archaea. Diatoms, fungi, green algae, and the protists are examples of microeukaryotes (Musselwhite et al., 2003). Microbes (especially prokaryotes and fungi) play a critical role in the cycling of several major elements (C, N, P, S, Fe) essential for life functions in higher plants and animals (Brock et al., 1994). They also influence the speciation and distribution of trace elements via active metabolic pathways, active metal resistance pathways (many of which are regulated by the bioavailable amount of one or more trace elements in the microbe’s local environment), and passive mechanisms such as metal ion sorption on microbial

cell walls (Lovley, 2000) and Hg methylation (Macalady et al., 2000).

The presence of several abandoned mining operations in VMS deposits of similar mineralogy and history along PWS shorelines (Fig. 1a) presents a unique opportunity to investigate the variability among tidal zone sediment microbial communities at the meter scale, which is approximately the same scale over which environmental parameters that might be expected to force the development of different microbial community assemblages have been observed to change. In this study, microbial lipids (total fatty acid methyl esters, or FAMES) from viable and non-viable populations of bacteria and microeukaryotes are used to gain insight into the biomass, diversity, domain-level composition, and presence/absence of microorganisms performing specific processes of geochemical interest in 19 sediment microbial communities collected from 3 mining-impacted shoreline study sites in the PWS. Similarities and differences among three principal component analyses (PCAs) describing the variance (relationships) within and among study sites as a function of water, sediment, and fatty acid methyl ester datasets are discussed, and the importance of specific parameters in each analysis is summarized.

1.1. Overview of lipid-based microbial community analyses

Microbial biomass carbon (C) is usually a small fraction (1–3%) of total carbon soil or sediment, but is thought to be the most biologically active portion (Acosta-Martinez et al., 2004). A direct relationship between microbial biomass and ecosystem health has been a paradigm in soil science for some time (Atlas et al., 1991). In a typical study, more microbial biomass was documented in rotated crop systems than continuous monoculture systems; crop biomass also correlated positively with total organic C and with an increase in protozoan and fungal populations, leading the authors to conclude that the rotated crop system resulted in superior “soil function” and “sustainability” (Acosta-Martinez et al., 2004). Similarly, higher microbial biomass in sediments upstream of an abandoned pyrite mine and lower biomass in downstream sediment was taken as evidence for measurable impacts on the microbial community due to the low-pH, metalliferous water being released from the mine to the stream (Ben-David et al., 2003). However, it does not follow that higher diversity is always directly

proportional to biomass; examples are algal blooms, in which a low-diversity consortium achieves high biomass (at least temporarily; Atlas, 1984) and microbial slimes from ARD sites that are dominated by one or two species (Bond et al., 2000). At least one study found no changes in microbial biomass in hyporheic riverine sediments downgradient from an ARD-impacted watershed (Feris et al., 2003). Clearly, if microbial biomass is but a small fraction of the total, its variation is likely to be swamped by that of the total biomass, if the metric used is common to all biomass.

Microbial lipid analysis is one of only a few techniques that can be used to quantify microbial biomass and determine its composition (Vestal and White, 1989; Zelles, 1999). Lipid analysis has been widely used to assess changes in natural microbial communities across gradients (e.g., heavy metal or hydrocarbon contamination) as a function of time, space, or various treatments (Pennanen et al., 1996; Zelles, 1999; Macalady et al., 2000; Chang et al., 2001; Pinkart et al., 2002; Ben-David et al., 2003; Feris et al., 2003; Piotrowska-Seget and Mroziak, 2003). The term “lipid” describes a class of linear organic molecules having a lipophilic end and a polar end. They are synthesized by living organisms primarily for energy storage, construction of cell membranes, and biochemical signaling (Zelles, 1999; Green and Scow, 2000). Lipids are described in standard “A:B ω C” format (IUPAC-IUB, 1977), where “A” indicates the total number of C atoms, “B” the number of unsaturated bonds, and “ ω C” indicates the number of C atoms between the aliphatic end of the molecule and the first unsaturated bond. *Cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. Other standard abbreviations are “Me” for methyl groups, “cy” for cyclopropyl groups, “XOH” where *X* = the position of the OH substitution, counting from the carboxyl group (α) end of the fatty acid molecule, and the prefixes “*i*” and “*a*” for *iso* and *anteiso* branching, respectively.

Cellular lipids [specifically, the polar phospholipid fatty acids (PLFAs)] are the lipids of choice for studies of microbial biomass and diversity because they are: (1) universally distributed among bacteria and eukaryotes; (2) rapidly degraded upon cell death (exogenous and endogenous phospholipase enzymes cleave the polar headgroups, converting the PLFAs to diglycerides); (3) expressed at a relatively constant level throughout growth and among different microorganisms; (4) occur in

characteristic assemblages for each type of microorganism, resulting in a pattern, or fingerprint, that can be used for identification (Pinkart et al., 2002). Among microorganisms, PLFA assemblage similarity increases with phylogenetic similarity; because many functionally similar organisms are also phylogenetically related, analysis of cellular lipids allows resolution of microbial communities at the kingdom, group, and species levels, depending on the system in question (Table 1; Zelles, 1999; Green and Scow, 2000; Pinkart et al., 2002). For example, the eukaryotes (diatoms, protists, green algae, fungi and plants) are identified by their high abundances of polyunsaturated fatty acids (PUFAs; rare in bacteria), long chain saturated fatty acids (SFAs), and alcohols (Table 1; Green and Scow, 2000). PLFA unique to bacteria are 10–20 carbons in length and usually are mono-unsaturated, branched, hydroxyl-substituted, or contain a cyclopropyl ring (Table 1). The least ambiguous identification of anaerobic bacteria may be the abundance of cyclopropyl FAMES, although they are also relatively common among gram-negative bacteria (Table 1). Many non-pathogenic anaerobes also display a negative sign in the gram stain test, which relates bacteria on the basis of cellular structure (Brock et al., 1994); these organisms have been quantified in the past by the relative abundance of iso-branched SFA and by ω 7-substituted, monounsaturated fatty acids (MUFAs), but several different metrics exist for quantifying the relative abundance of some groups. The procedure of identifying microbial groups on the basis of one or more PLFA (or FAME) assemblages has been named “signature lipid biomarker” (SLB) analysis (Pinkart et al., 2002).

Extraction of PLFAs from environmental samples is a laborious, expensive procedure. It nevertheless ensures that only the lipid component of *viable* cells is examined; this is a critical distinction when some aspect of the physiological status of the microbial community is the parameter under study (e.g., response to toxic substances, starvation, or nutritional status (Green and Scow, 2000)). A significantly less expensive procedure (MIDI) extracts all classes of lipids that can be methylated (the total fatty acid methyl esters, or FAMES), not just the PLFAs. Additional lipids extracted during the total FAMES procedure include PLFAs derived from non-living organic matter (such as humus), storage lipids, and lipids from higher plants (Table 1; Kaufman et al., 1999; Zelles, 1999; Schutter and Dick, 2000). Due to consid-

Table 1

FAMES groups used to infer microbial community composition and presence/absence of taxonomic and functional groups

Group composition ^a	Inferred community	Reference	Notes
$\Sigma[16:1\omega13t, 20:5\omega5, 20:5\omega3]$	Diatoms	Salomonova et al. (2003)	
$\Sigma[\text{all PUFA and all } > C_{19} \text{ SFA}]$ (total of 14 FAMES in this study)	Microeukaryotes	Modified from groupings in Stoeck et al. (2002) and Zelles (1999)	Protists + algae; some overlap with diatom, fungal groups
$\Sigma[18:3\omega6c, 18:2\omega6c, 18:1\omega9c]$	Fungi	Acosta-Martinez et al. (2004)	Widely used, but might overpredict because 18:2 $\omega6c$ is also present in higher plants
$\Sigma[\text{primary alcohols} + \text{dicarboxylic acid}]$ (7 FAMES, this study)	Higher plant matter	Kaufman et al. (1999) and Zelles (1999)	
$\Sigma[C_{12}\text{--}C_{19} \text{ BrSFA}, C_{12}\text{--}C_{19} \text{ CyFA}, C_{12}\text{--}C_{19} \text{ 3OH-SFA, and non-}\omega7 \text{ or } \omega9 C_{12}\text{--}C_{19} \text{ MUFA}]$ (total of 39 FAMES in this study)	Bacteria	Modified from groupings in Stoeck et al. (2002) and Zelles (1999)	$\omega7$ and $\omega9 C_{12}\text{--}C_{19}$ MUFA are also common in eukaryotes
$\Sigma\text{Iso-branched SFA}$ (7, this study)	Gram(–) + other anaerobes	Ben-David et al. (2003) and references therein	Significant overlap between indicator FAMES for Gram sign and oxygen tolerance has been noted several times in the literature
$\Sigma\text{Anteiso-branched SFA}$ (5, this study)	Gram(+), widespread	Ben-David et al. (2003) and references therein	See above
$\Sigma\omega7$ MUFA	Gram(–) aerobes + strict anaerobes	Zelles (1999) and references therein	See above
$\Sigma\omega9$ MUFA	Gram(+), widespread	Zelles (1999) and references therein	See above
$\Sigma\text{Cyclopropyl FAMES}$ ($n = 3$, this study)	Anaerobic bacteria	Zelles (1999) and references therein	Indicator of physiological stress only in PLFA studies
10Me:16:0, 10Me:18:0	Sulfate reducing bacteria and/or actinomycetes	Sundh et al. (1997)	Known sulfate reducers with high 10Me:16:0 have low 10Me:18:0; known actinomycetes have opposite proportions
11:7:1 $\omega7$, 11:5:0	<i>Desulfovibrio</i>	Sundh et al. (1997)	
16:1 $\omega8c$, 16:1 $\omega6c$ (type I); 18:1 $\omega8c$, 18:1 $\omega8t$, 18:1 $\omega6c$ (type II)	Type I and II methanotrophs	Salomonova et al. (2003)	Present in peats
16:1 $\omega7c$, 16:0	β -Proteobacteria	Nakatsu et al. (2006)	May indicate <i>Leptothrix</i> if abundant
11:7:1 $\omega5$, 10Me18:1 $\omega6$, 11Me18:1 $\omega6$	<i>Thiobacillus</i>	Salomonova et al. (2003)	Methyl FAMES significant only for <i>T. intermedius</i> (Kerger et al., 1987)
16:0 (40%), cy17:0(9–10) (15%), 16:1 $\omega9t$ (9%)	<i>T. denitrificans</i>	Kerger et al. (1987)	Listed in order of abundance in mol%; only facultative anaerobe currently identified in the genus
(2,3)-OH 16:0 (54%), cy19:0($\omega8,9$) (25%), unidentified (21%)	<i>T. intermedius</i>	Kerger et al. (1987)	Listed in order of abundance in mol%
2-OH cy18:0 (38%), 18:1 $\omega7(c+t)$ (37%), 16:0 (28%)	<i>T. neapolitanus</i>	Kerger et al. (1987)	Listed in order of abundance in mol%
2-OH cy19:0 (42%), 18:1 $\omega7(c+t)$ (40%), 16:0 (36%)	<i>T. thiooxidans</i>	Kerger et al. (1987)	Listed in order of abundance in mol%

^a FAMES group abbreviations are as follows: SFA = saturated fatty acid; EC and OC = even and odd C chain length, respectively; Br = branched, MIX = multiple characteristics (e.g., branched SFA with hydroxyl substitution on third carbon); ECSFA = saturated; MUFA = monounsaturated FA, PUFA = polyunsaturated FA; MCMUFA = mid-chain branched monounsaturated FA. OH-MUFA = monounsaturated FA with hydroxyl substitution; CYFA = cyclopropyl FA; BRMUFA = branched monounsaturated FA; MCMUFA = mid-chain branched monounsaturated FA.

erations of cost, sample handling and storage, anticipation of low biomass samples, and the focus of this study on monitoring variation in microbial communities rather than identification of specific community members, total FAMES were analyzed in this study.

The use of total FAMES for SLB analysis has been criticized on the grounds that non-PLFAs and non-viable PLFAs (free fatty acids) are also extracted during the procedures (Zelles, 1999). In this study, non-PLFA FAMES were removed from

the FAMES assemblage prior to SLB or other analyses, in recognition of the fact that the fraction of non-viable PLFA liberated with viable PLFA during the MIDI extraction procedure cannot be quantified or removed as easily, and represents a major limitation of the technique.

There are several additional limitations on the resolving power of total FAME patterns derived from environmental samples: there is clearly a limit to the information contained in cellular lipids with respect to identifying members of high-diversity natural microbial communities down to the genus and species level (an estimated 7000 prokaryotic species exist in a single gram of soil, compared to only 72 lipids identified in a PLFA analysis of a peat sample (Zelles, 1999; Curtis and Sloan, 2004)). The gas chromatographic technique employed was not very sensitive, nor was it diagnostic of low abundance peaks; confirmation by mass spectrometry is needed for the unambiguous identification of conformational isomers (*cis*, *trans* orientation). Unless one or more lipids known from previous studies to be strong indicators of a certain microbial group or taxon is present in the lipid assemblage, the community analysis is restricted to the highest taxonomic levels (i.e., clade or kingdom), and perhaps to analysis of bacterial populations at a generic functional level (e.g., gram sign, or anaerobe/aerobe).

1.2. Interplay between environmental parameters and microbial community composition in the tidal zone

Salinity and moisture exert fundamental controls on microbial communities (Atlas et al., 1991); within a site, both change regularly as a function of shoreline position (decreasing from subtidal to supratidal). The following section presents an overview of the effects of tidal forces on environmental characteristics in the three zones just described, the general characteristics of microbial communities expected in each zone, and the FAMES used to identify members of those communities. A brief introduction to the geochemical characteristics of each study site, a discussion of site-specific environmental variables (where present), and their anticipated effect on the composition of microbial communities as determined from total FAMES is discussed in Section 2.1.

Subtidal mudflats are highly diverse, shallow water environments in which primary producers (algae, benthic diatoms and higher plants such as seagrasses) support microeukaryotic, fungal and heterotrophic bacterial populations. As light is required

for photosynthesis, the uppermost portion of the mudflat should be enriched in these lifeforms. In the field investigations, O_2 was observed to be exhausted or become limiting within a few centimeters of the sediment–water interface, as indicated by darkening of the sediment. Sub- to anoxic environments are usually dominated by bacteria or Archaea rather than by eukaryotes, so a fundamental shift in community composition might be expected with depth for this sediment type. Bacteria living in the lower portion of subtidal mudflat sediment will likely have adapted to tolerate O_2 -low or O_2 -free conditions, and therefore be facultative or strict anaerobes (although the former would be more likely in the dynamic tidal environment). Organic matter available as a substrate for microbial activity is likely to be high and of diverse composition in this zone.

The intertidal zone is inundated twice daily by seawater, so microorganisms inhabiting this environment are likely to be adapted to tolerate dramatic changes in salinity and moisture. Gram-positive bacteria have thicker cell walls (characterized by a high abundance of branched fatty acids; Table 1) that may provide them with a competitive advantage in the dynamic intertidal environment, so they may be more abundant than gram-negative bacteria in this zone. Although periods without water saturation would be expected to increase O_2 levels in intertidal zone sediment relative to that of subtidal zone sediment, temporary microreducing zones can persist in the intertidal zone (within a piece of decaying organic matter, for example). Therefore, sub- or anoxic environments (micro- to macro-) probably exist in shallow intertidal zone sediments. These zones would likely support a community of facultatively anaerobic bacteria, which have a competitive advantage over strict aerobes and anaerobes under conditions of changing O_2 concentration. The overall biomass in this zone would be expected to be lower than in the subtidal muds, due to the lack of safe anchorage for primary producers in this dynamic environment.

The supratidal zone is only rarely wetted by seawater, therefore microbial communities in this zone may be limited by moisture, dissolved organic matter, or other nutrients. Due to limitations on community growth, biomass is likely to be lower than that measured in intertidal and subtidal samples. Gram-positive bacteria again might be expected to have a competitive advantage over Gram-negative bacteria in this zone. On the other hand, since the subsurface is not flushed with oxygenated water

regularly, sub- to anoxic microzones may persist within the sediments for longer time periods.

2. Materials and methods

2.1. Site characterization

2.1.1. Threeman

At this site, about 6000 tons of ore were removed from underground deposits utilizing several mining adits; each presently has a pile of sulfide-bearing waste rock at its base. The lowest elevation adit is about 20 m above the approximate high tide line (Fig. 1b), and there is also a small (5 m-wide, roughly hemispherical) mound of deeply weathered, sulfide-rich ore (thought to be a remnant of a much larger one; Koski et al., 2003). Threeman ore contains, in order of abundance, pyrrhotite (Fe_{1-x}S ; $x = 0\text{--}0.2$), chalcopyrite (CuFeS_2), sphalerite (ZnS) and minor pyrite (FeS_2) (Koski et al., 2008, and references therein). The abundance of sulfide minerals in Threeman beach gravels is low. Samples were collected from pits approximately 1 m deep through the tidal zone along a transect intersecting a natural drainage that receives water from the lowermost mine adit (Fig. 1b). A background sample was also collected from a pit dug in intertidal sediment away from the mine discharge (T304). Two of the pits (T304 and T306) dug in Threeman beach gravels intersected peat/muskeg deposits (Fig. 2a), and two others (T303 and T305) contained copious amounts of ferric oxyhydroxide (Fig. 2b; T303 not shown). The abundance of particulate and dissolved C in Threeman beach sediments is expected to exert a strong control on the microbial communities developed therein. Previous studies have identified anaerobic SO_4 -reducing bacteria and CH_4 oxidizing (methanotrophic) bacteria in peats from Sweden using a variety of signature lipids (Sundh et al., 1997; Table 1). The occurrence of both Fe oxide and elevated concentrations of organic matter might spur the activity of Fe-reducing bacteria, a phylogenetically diverse group of organisms for which no group-level SLB are known, to the authors' knowledge. Other samples collected at Threeman were a sample of offshore sediment collected from about 45 m depth (T115), and a sample of intertidal mud (T301; Fig. 1b).

2.1.2. Ellamar

About 536,000 tons of ore were mined from the Ellamar site: most of that material came from work-

ings situated below sea level, utilizing a cofferdam to hold back the water of Virgin Bay (Koski et al., 2008; Fig. 1c). Much of the mine waste was back-filled into the main shaft (now flooded with seawater), but a large amount of ore was apparently left on the shoreline when mining ceased, and has since become incorporated into the beach sediments. In contrast to Threeman, sulfide-rich sediment from Ellamar contain more pyrite (up to 95% modal abundance) with minor amounts of chalcopyrite, sphalerite, and pyrrhotite (Koski et al., 2008). Samples were collected from subtidal muds (E301a, E301b), intertidal zone sediment (E308, E309, E310, Fig. 2c) and supratidal zone sediment (E311, E303, E304; Fig. 2d) along a transect intersecting the sulfide-rich portion of beach sediment (Fig. 1c). Formation of yellow-green, fine-grained K-jarosite ($\text{KFe}_3(\text{SO}_4)_2 \cdot \text{OH}_6$) in beach sediments is pervasive (Fig. 2c and d). Ferric oxyhydroxides are much less abundant at Ellamar, and no organic-matter-rich lenses were observed in beach sediments.

The abundance of sulfide minerals in tidal zone sediment at Ellamar is anticipated to exert a profound impact on the microbial community. Numerous studies conducted in freshwater environments have indicated that sediment and mine wastes rich in sulfide minerals can support the growth of Fe and S-oxidizing bacteria of the gram-negative *Thiobacillus* and *Leptospirillum* genera (Ben-David et al., 2003), gram-positive *Acidithiobacillus* bacteria (Kerger et al., 1987), and several types of moderately thermophilic archaea (Bond et al., 2000; Edwards et al., 2000; Johnson, 1998). Previous work has shown that pore water pH values in Ellamar intertidal zone sediment vary over four orders of magnitude (between 3 and 7) during an incoming tide, suggesting that ARD conditions predominate in this zone and are only briefly perturbed by tidal water movement (Koski et al., 2003). The geochemistry of Ellamar sediments might be selective for acidophilic or acid-tolerant bacteria that are also adapted to fluctuating pH conditions and perhaps also to colder temperatures. Signature lipids of *Thiobacillus* and *Acidithiobacillus* bacterial species have been described in the literature.

2.1.3. Beatson

Approximately 6 million tons of sulfide-rich ore was mined from the Beatson site on Latouche Island (Fig. 1a), making it the single largest operation in the region. Beatson ores apparently



Fig. 2. Representative photographs of (a) peat-rich Threeman sediment at (T306); (b) Fe oxyhydroxide-rich Threeman sediment (T303; similar to T305); (c) intertidal zone, pyrite-rich sediment from Ellamar (E310) containing moderate amounts of yellow-green jarosite; (d) supertidal zone, clay-rich, and jarosite-rich clayey sediment from Ellamar, (E303/E304); (e) biogenic ferric oxyhydroxide coating sticks and plant debris in a shallow, seep-fed freshwater pool fed by surface drainage from a tailings waste pile Beatson (B301); (f) overbank deposits of ferric oxyhydroxide formed from freshwater, subsurface seep-fed drainage, Beatson mine (B303-2). The upper portion of this sediment was collected as sample B303-2a, and the lower, darker portion (presumably undergoing reductive dissolution of the ferric oxyhydroxide) was collected as sample B303-2b.

contained both pyrrhotite- and pyrite-rich zones (Koski et al., 2008). Most of the mining took place in subsurface workings; the remains of mining include an open pit located about 600 m inland and several large mine dumps rich in Fe scrap as well as mineralized waste rock closer to, but still

well above, the intertidal zone (Koski et al., 2008; Fig. 1d). Water from the open pit flows rapidly (approximately $0.3 \text{ m}^3 \text{ s}^{-1}$) through the subsurface mine workings and surface mine dumps before it becomes a braided stream as it reaches the flat beach, which contains very little sulfide material.

Several slow-running slightly acidic (pH 5–6) seeps also drain the mine dumps, and at their point of issue, abundant ferric oxyhydroxides accumulate in shallow, stagnant ponds filled with fluffy orange ferric oxyhydroxide reminiscent of the habit of *Leptothrix*, an oligotrophic, aerobic, gram-negative β -Proteobacterium known to possess proteins for Mn oxidation (van Veen et al., 1978; Fig. 2e). On steeper slopes, the seeps form rills with zones of active precipitation of bright red ferric oxyhydroxide (B303-1, B304). Thick overbank deposits of the latter material were sampled along the side of one of the rills (B303-2a, B303-2b; Fig. 2f).

2.2. Sediment and water chemistry

All field activities were supported by the research vessel *Auklet*. Measurements of pH, conductivity, temperature, and alkalinity were made in the field using standard techniques. All sample sites were located by handheld GPS. Submarine samples of unconsolidated sediment were collected by gravity core and dredge. A remotely operated vehicle (ROV) was deployed from the *Auklet* to sample bottom waters, precipitates and sediment in shallow subtidal areas (Koski et al., 2003).

Surface water, pore water, and seawater samples were stored in HDPE bottles until analysis. Total metals were determined on unfiltered samples acidified with ultra-pure HNO_3 and total dissolved metals on acidified samples filtered to 0.45 μm . ICP-MS and ICP-AES were used to determine dissolved concentrations of a total of 55 elements; due to mass and emission interferences generally and salinity interferences specifically, only a subset of these data are presented (Briggs and Meier, 2002; Lamothe et al., 2002; Table 2). Samples for total Hg determination were collected into glass ICHM 300 series bottles and stored on ice without further preservation until analysis by continuous flow-cold vapor-atomic absorption spectroscopy (CV-AAS). Anions (SO_4 , Cl , NO_3 and F) were analyzed by ion chromatography (IC) on filtered samples collected in HDPE bottles and stored without preservation (Theodorakos et al., 2002). Sediments and precipitates were digested using a multi-acid procedure and analyzed by ICP-MS in the same manner as the waters; selected data are presented in Table 3 (additional data can be found in Koski et al., 2008). Data validation was performed by the analysis of internal concentration standards, reagent blanks, and reference samples after every 20 samples and

by re-analysis of samples collected from similar environments in 2003 (Koski et al., 2008). The quality control criterion for accuracy was $\pm 15\%$ of the expected value at five times the lower limit of detection and for precision the acceptable calculated relative standard deviation of replicates was $\leq 15\%$ of the replicate mean. Values for the lower limit of detection (defined as five times the value of the method blank) are given for each parameter measured by ICP-MS in Tables 2 and 3.

Dissolved organic C (DOC) was measured from 16 sites in the 3 study areas (Table 2). Water samples were collected into pre-cleaned bottles and acidified with ultra-pure H_2SO_4 in the field. Samples were stored on ice for the duration of the research cruise (up to 8 days), then shipped on ice to Huffman Laboratories (Golden, CO) for organic C analyses by standard EPA method 5310-C (2005). The instrumental detection limit was 0.05 mg/L. Dilution was necessary for high-Cl samples, with proportional increases in the base precision of ± 0.05 mg/L. A blank, duplicate, and calibration check were performed for every 10 samples; calibration checks fell within 10% of the theoretical value. Due to the large amount of particulates in some samples, the samples were filtered at 0.45 μm (nylon) prior to analysis. Acidification prior to filtration (not the standard technique) may have enhanced the precipitation of humic acids, thereby lowering the humic contribution to DOC, but conversely may have also dissolved some types of particulate-bound organic C. The extent to which these possible artifacts occurred in samples was not evaluated.

2.3. Sampling and quantification of total fatty acid methyl esters (FAMES) and their use in estimates of biomass and diversity

Sediments and precipitates were collected into sterile containers using sterilized implements. Samples were frozen (-20°C) within approximately 4 h of collection, shipped to the laboratory on dry ice, and stored at -20°C until freeze-drying (within 2 weeks). Freeze-dried samples were sent to Microbial ID, Inc (125 Sandy Drive, Newark, DE; www.microbialid.com) for analysis. Use of trade names is for informative purposes only and does not constitute endorsement by the US Geological Survey. FAMES were extracted from 0.6 to 3.07 g of sample using the standard four-step method of saponification, methylation, extraction in organic solvent, and cleanup (Sasser, 2001); extracts were

Table 2
Field pH, dissolved organic C (DOC) and concentrations of selected elements (determined by ICP-MS) in 0.45 µm-filtered water samples from the study sites

Site name ^a	Sample type, Location	Field pH ^b ±0.5	DOC ^b ±0.1 (mg/L)	Ca ±0.3 (mg/L)	Mg ±0.01 (mg/L)	Fe ±48 (µg/L)	Al ±2 (µg/L)	Mn ±0.3 (µg/L)	Cd ±0.02 (µg/L)	Co ±0.03 (µg/L)	Cr ±1.9 (µg/L)	Cu ±0.74 (µg/L)	Ni ±0.5 (µg/L)	Pb ±0.1 (µg/L)	Zn ±0.9 (µg/L)	Cl ±0.08 (mg/L)	SO ₄ ±0.05 (mg/L)	Hg ±20 (ng/L)
<i>Threeman</i>																		
T301	Subtidal pore water, pit 1	6.8	1.6	133	314	1910	14	53.6	bd	0.37	3.6	6.2	4.6	bd	7.0	5427	1111	bd
T305	Intertidal pore water, pit 3	7.2	5.1	266	692	108	32	23.9	0.33	2.58	3.2	3.3	2.6	bd	4.9	3858	626	bd
T304	Intertidal pore water, background pit 2	7.7	4.3	14	30	bd ^c	19	1.0	bd	0.41	1.1	28.7	1.0	0.3	8.2	10,481	1490	bd
T306	Intertidal pore water, pit 4	8.3	27.3	126	297	575	40	192	0.3	8.62	4.0	23.7	6.8	bd	84	4000	787	bd
<i>Ellamar</i>																		
E301a/E301b	Seawater, low tide offshore	8.4	1.6	526	1660	431	17	3.6	0.07	0.81	6.9	13	13	0.1	18	16,552	2157	bd
E308	Intertidal pore water, pit 6	6.8	0.5	494	1500	11,600	25	100	0.05	1.18	6.7	13	12	0.3	23	17,446	2260	bd
E309	Intertidal pore water, pit 8	3.8	0.2	383	1090	21,700	14,900	460	10.6	61.9	8.6	3570	29	186	4050	11,673	1892	46
E310	Intertidal pore water, pit 9	3.4	0.5	335	1020	14,000	15,400	393	6.22	64.2	7.9	2950	36	47	2590	10,495	1816	74
E311	Intertidal pore water, pit 10	2.9	bd	371	1020	28,300	7850	439	8.19	101	9.1	8480	27	2020	3290	11341	2298	3700
<i>Beatson</i>																		
B301	Seep below main waste dump	5.8	0.3	5.6	1.85	bd	25	43	0.89	1.82	bd	388	2.4	1.4	393	bd	289	bd
B303-1	Seep below house	6.6	0.4	3.2	1.35	15,000	15	325	0.11	2.82	bd	138	2.6	8.7	67	bd	285	bd
B304	Seep near foundation	6.1	bd	4.8	0.65	251	20	36	0.25	0.30	bd	37	0.9	2.4	132	bd	283	bd

Analytical methods are described in the text. Lower limits of detection 5σ method blank values are given for each analyte except pH and DOC (standard error).

^a Field sample numbers have the prefix “05PWS” in Koski et al. (2008).

^b Operational detection limit.

^c bd = below detection.

Table 3

Sediment pH and selected element concentrations in sediment samples from PWS sites determined by ICP-MS on acid-digests

Site name ^a	Sample description, location	pH ^b 0.1	Al 0.005 (%)	Ca 0.013 (%)	Na 0.003 (%)	Mg 0.0006 (%)	P 0.0005 (%)	Fe 0.004 (%)	Cu 1.4 (ppm)	Zn 2.3 (ppm)	Cd 0.0069 (ppm)	Co 0.025 (ppm)	Cr 0.48 (ppm)	Hg ^c 0.02 (ppm)	Mn 0.71 (ppm)	Mo 0.053 (ppm)	Ni 0.26 (ppm)	Pb 0.37 (ppm)
<i>Threeman</i>																		
T115	Olive-brown mud, 45' water depth offshore	8.1	8.52	1.44	2.93	1.78	0.130	4.00	88.6	113	0.55	10.4	89.3	0.05	830	4	28.9	14.6
T301	Black mud, pit 1	7.6	7.87	1.63	1.77	2.83	0.130	6.70	1180	232	0.52	17.3	153	0.09	1260	12.4	39.8	37.0
T304	Beach sand, (background)	8.1	10.4	1.11	2.33	2.15	0.100	4.80	377	139	0.05	14.1	105	0.03	951	0.36	31.0	23.5
T305	Sand with orange precipitate, pit 3	8.1	10.0	3.44	1.43	4.66	0.072	9.90	870	289	0.16	26.7	280	0.02	2010	1.2	65.0	22.2
T306	Dark gray sand + peat, pit 4	6.6	7.14	1.24	1.98	3.50	0.160	7.60	4690	938	1.4	27.5	192	0.11	1650	5.8	39.5	7.80
T308	Ore pile remnant (dry, sulfide-rich)	4.2	1.20	0.21	0.107	0.060	0.021	40	2.67	0.071	1.8	97.3	48.6	0.04	380	18.6	18.5	4.70
<i>Ellamar</i>																		
E301a	Top layer of subtidal mud, exposed only during minus tide	7.2	5.63	0.377	0.985	0.704	0.095	15.0	3220	909	bd	16.9	63.3	1.5	234	23.5	10.8	385
E301b	Bottom layer of subtidal mud, exposed only during minus tide	n.d.	7.01	1.10	1.58	1.31	0.120	4.70	809	462	0.59	9.4	92.4	0.38	463	5.5	23.2	192
E303	Sediment with abundant jarosite supertidal (dry)	4.1	2.56	1.35	0.577	0.808	0.2	37.0	989	389	0.12	11.1	43.3	0.86	463	11.6	15.8	13.3
E304	Sediment with abundant jarosite supertidal (dry)	3.0	5.90	0.042	1.47	0.483	0.067	16.0	1160	49.8	0.12	2.9	64.9	1.6	134	2.8	4.7	69.9
E308	Coarse sand, pit 6	6.2	4.94	0.089	0.74	0.598	0.041	18.0	2660	1250	1.6	29	56	2.2	228	20.8	15.0	235
E309	Brown sand, pit 8	4.5	1.71	0.030	0.249	0.197	0.018	34.0	3090	820	4.4	187	24	3.2	115	19.6	7.9	116
E310	Pit 9 sediment with minor jarosite supertidal (dry) no water until 1.5 m	3.6	0.914	0.015	0.191	0.046	0.06	28	1290	126	0.05	4.2	11.2	2.6	12.7	4.7	1.1	1160
E311	Pyritic sand, pit 10	4.0	0.290	0.007	0.078	0.015	bd ^d	41	0.14	0.068	2.7	13.9	2.8	7.2	6.3	15.8	4.6	258
<i>Beatson</i>																		
B301	Brown precipitate, seep below dump	6.4	6.67	0.247	0.646	1.27	0.077	16.0	10,700	704	2.1	29.6	75.8	1.0	2140	2.7	28.4	917
B303-1	Red-brown precipitate below house, floc in stream	5.7	3.09	0.133	0.265	1.84	0.053	20.0	8000	332	0.46	5	42	0.56	365	1.2	9.1	909
B304	Brown ppt in seep at foundation of old mill	n.d.	5.66	0.200	0.789	1.83	0.055	8.90	7240	762	1.3	9	75.5	0.67	583	1.5	12.1	365

Lower limits of detection (5 σ method blank) values are given for each analyte. Error in pH is estimated for a laboratory instrument.^a Field sample numbers have the prefix "05PWS" in Koski et al. (2008).^b pH of reagent-grade deionized water incubated with sediment at a ratio of 2.5:1 (solid:liquid) for 2 h.^c Determined by CV-AAS; see text for details.^d bd = below detection.

Table 4
Fatty acid methyl ester (FAME) composition of samples by structural group

Sample name	∑SFA ^b	∑MUFA	∑PUFA	EC SFA	Br SFA	OC SFA	MIX SFA	2OH-SFA	3OH-SFA	OH-MUFA	CYFA	Br MUFA	MC MUFA
<i>Subtidal/offshore mud near ARD^a</i>													
T115	47	45	0	19	17	1	4	1	5	0	0	2	0
T301	44	44	1	8	28	1	1	4	4	0	0	2	1
E301a	63	18	7	44	5	2	5	2	4	1	2	1	3
E301b	41	43	3	21	5	3	3	3	5	1	1	9	5
<i>Intertidal zone, peat/muskeg-rich sediment with some sulfides</i>													
T306	22	14	0	8	3	1	1	1	8	0	30	2	0
T305	49	19	1	11	29	1	4	1	3	0	0	2	1
T308	61	26	0	25	16	2	6	1	11	1	2	4	3
T304 ^c	51	36	0	21	20	1	3	1	5	0	0	3	0
<i>Intertidal zone, organic matter-poor sediment with abundant sulfides</i>													
E309	73	14	1	22	6	2	16	0	26	1	2	7	0
E310	84	6	0	50	12	3	10	0	9	6	10	0	0
E311	90	10	0	3	14	3	58	0	11	0	0	10	0
E308	54	33	0	24	8	1	6	6	8	0	0	22	0
<i>Supratidal zone, organic matter-poor sediment with abundant sulfides</i>													
E303	67	19	0	46	0	11	0	0	11	0	9	19	0
E304	77	11	0	57	0	10	0	0	9	11	12	0	0
<i>Freshwater, seep-fed drainages and slow-running, shallow pools with abundant Fe oxyhydroxide</i>													
B301	34	54	1	10	15	2	2	5	3	0	0	2	16
B304	42	37	2	7	25	1	1	5	3	3	1	3	0
B303	24	66	0	12	7	2	0	0	3	0	0	0	0
B303-2a	20	64	2	6	3	4	1	3	2	0	0	2	1
B303-2b	33	56	2	5	20	1	1	4	3	0	0	1	0

Data are percent of the total named peak areas, and have been rounded to the nearest digit.

^a ARD = acid-rock drainage, a general term describing low-pH, high dissolved metal waters formed from the weathering of sulfide minerals in mined or unmined, mineralized areas.

^b FAMES group abbreviations are as follows: SFA = saturated fatty acid; EC and OC = even and odd C chain length, respectively; Br = branched, MIX = multiple characteristics (e.g., branched SFA with hydroxyl substitution on third C); ECSFA = saturated; MUFA = monounsaturated FA, PUFA = polyunsaturated FA; MCMUFA = mid-chain branched monounsaturated FA. OH-MUFA = monounsaturated FA with hydroxyl substitution; CYFA = cyclopropyl FA; BRMUFA = branched monounsaturated FA; MCMUFA = mid-chain branched monounsaturated FA.

^c From a background site in the intertidal zone, but believed to be outside the zone of ARD.

then 8-fold concentrated (K. Dorman, MIDI, pers. comm.). An external standard consisting of a quantitative mixture of straight chained fatty acids from 9 to 20 carbons in length was run between samples to calibrate retention times and accurately name peaks; internal concentration standards (needed to convert peak areas to molar concentrations) were not run along with samples. Fatty acids were identified and peak areas determined using the Sherlock Microbial Identification System (MIS) software (version 4.5) and the EUKARY peak naming table. Peaks with area-to-height ratios less than 0.017 (typically electronic noise spikes) or greater than 0.070 (typically sterols and other non-fatty acid compounds) were excluded from analysis. The fatty acids were typically grouped by structure to facilitate abundance comparisons among samples (Table 4). Acronyms associated with each group are as follows: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ECSFA = SFA with even-numbered C chain; BrSFA = *iso* or *anteiso* branched SFA; OCSFA = SFA with odd-numbered C chain; MIX-SFA = SFA with both branching and hydroxylation at the second or third C from the acidic end; 2OH- and 3OH-SFA = SFA with hydroxyl group at the second or third C from the acidic end of the molecule; OHMUFA and BrMUFA = MUFA with or hydroxylation or *iso/anteiso* branching, respectively; CYFA = cyclopropane fatty acids; BRMUFA = MUFA with *iso/anteiso* branching, MCMUFA = MUFA with unsaturation in the middle of the C chain.

Microbial biomass was estimated as the summed area of all identified peaks divided by the weight (mg) of sample used in the MIDI extraction. Lacking a value for the mass of total FAMES extracted from each sample (not quantified by Microbial ID), the biomass estimate used in this study has no physical meaning. It should be emphasized that the biomass estimate thus obtained is a sum of viable and non-viable biomass fractions, and has a component from macroscopic (“higher”) plants (e.g., mosses, liverworts, and vascular plants) as well. The biomass estimate is anticipated to be biased in favor of finer-grained samples (particularly for the Ellamar samples) because bulk lyophilized sediment was analyzed in this study.

Microbial diversity was estimated by a simple sum of the number of uniquely identified FAME peaks in each sample. Diversity estimates based on this assumption may be heavily suppressed relative

to true values in high-diversity samples due to the aforementioned limitations on the absolute number of FAMES relative to microbial species, and the limitations on the detection and quantification of FAMES.

2.4. Principal component analyses (PCA)

Data for use in PCA were transformed to reduce non-normality, positive skew, unit differences, and other characteristics of environmental datasets that are undesirable for PCA. With the exception of pH, water and sediment chemical data were log transformed $\{\ln(x + 1)\}$ to correct for anticipated posi-

Table 5
Individual FAMES used in principal component analysis

FAME ^a	% Presence ^b	FAME	% Presence
9:0	100	12:1w8c	47
10:0	89	14:1w7c	32
11:0	37	15:1w8c	32
12:0	100	16:1w7c	68
13:0	37	16:1w6c	26
14:0	89	16:1w3c	32
16:0	95	17:1w7c	68
18:00	32	17:1w3c	42
i11:0	53	18:1w3c	26
i13:0	42	18:1w9t	32
a13:0	58	19:1w8t	42
i14:0	47	22:1w3c	37
i15:0	84	i17:1w5c	21
a15:0	74	i17:1	37
i21:0	58	i16:1	74
2OH12:0	26	3OH12:1	42
2OH15:0	58	14:2w6c	32
2OH18:0	42	18:3w6c	37
3OH10:0	100	20:5w3c	26
3OH12:0	79	cy17:0	42
3OH16:0	74	C14NA1ch	26
i3OH11:0	21	C16NA1ch	68
i3OH13:0	68	C18NA1ch	21
i3OH14:0	37	2Me14:0	26
i3OH15:0	63		
i3OH17:0	42		

^a Lipids are described in standard “A:BωC” format (IUPAC-IUB, 1977), where “A” indicates the total number of C atoms, “B” the number of unsaturated bonds, and “ωC” indicates the number of C atoms between the aliphatic end of the molecule and the first unsaturated bond. *Cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. Other standard abbreviations are “Me” for methyl groups, “cy” for cyclopropyl groups, “XOH” where X = the position of the OH substitution, counting from the carboxyl group (a) end of the fatty acid molecule, and the prefixes “i” and “a” for *iso* and *anteiso* branching, respectively.

^b “% presence” refers to the percentage of samples containing that particular FAME.

tive skew in frequency distribution (Ben-David et al., 2003). FAMES data from each sample were expressed as a fraction of the total area of named peaks and arcsine square root transformed to reduce the non-normality of the dataset (Schutter and Dick, 2000). Two samples were missing DOC values (B304 and E311), so relevant estimates were made from the median value of all samples from that site. Other samples having parameters below the lower limit of detection (LLD) were assigned non-negative values 10 times lower than the LLD, but missing data were rare for the environmental parameters. Following the procedures of several previous studies (Kaufman et al., 1999; Schutter and Dick, 2000; Ben-David et al., 2003), FAMES most likely to be exogenous to the sampling site and/or derived from higher plants (primary alcohols and dicarboxylic acids; Kaufman et al., 1999) and C₂₄ and greater FAMES (known to be derived from either eukaryotic or non-living biomass; Kaufman et al., 1999) were removed from the total set of 127 identified FAMES prior to PCA. The remaining 50 FAMES were used for PCA (Table 5). The analyses were performed using PC-ORD v. 5.0 (McCune and Mefford, 2006). Pearson correlation matrices of the data used for PCA had row by column dimensions of 12 × 17 (water) 15 × 17 (sediment) and 19 × 51 (FAMES), where samples are rows and environmental parameters (sediment, water, or FAMES data) are columns; use of correlation matrices removes unit differences among variables (variables with high values are not preferentially weighted relative to those with low values), while preserving the relative abundance differences for each variable among sample types (McCune and Mefford, 1997, 2006). The results of PCA are usually visualized in 2-d plots of sample scores for the first two principal component ('PC') axes, which usually account for much of the variance within a dataset (Herrmann and Shann, 1997); the proximity of sample scores is an indicator of their relatedness relative to the variables under consideration. In addition to samples, the environmental variables' scores (in this case, calculated from a weighted average of the sample scores; McCune and Mefford, 1997) can be plotted along with the samples. In this case, the smaller the angle made between the PC axis and a line through the origin intersecting a variable's score coordinates, the stronger the correlation with that axis. Furthermore, samples lying in the path of the radial line are enriched in that particular variable relative to other samples.

The mathematical operations of PCA always yield a number of components equal to that of the number of samples, but the percent of dataset variance explained by each component decreases with each additional component. The determination of significant (i.e., principal) components is subjective, but several criteria, indicator functions, and significance estimators exist to assist the analyst in the identification of principal components and the assignment of a level of confidence in the identification. The reader is referred to the PC-ORD manuals (McCune and Mefford, 1997, 2006) and references therein for further details. One selection rule not discussed in the PC-ORD manuals but employed in this study is that the number of samples (independent observations) in a robust PCA should be $2n + 1$ the number of significant components (n) (Ressler, 1998); this cannot be known *a priori*, but it can be used to evaluate the quality of the PCA after it has been run.

3. Results

3.1. Sediment and pore water chemistry

The DOC analyses in Table 2 show that the Threeman intertidal zone samples (T304, T305, and T306) are elevated in DOC relative to samples from other sites: all of the tidal zone water samples from this site have DOC values in excess of 4 mg/L, but Ellamar and Beatson water samples have DOC values below 1 mg/L. Offshore water samples collected near the Threeman and Beatson sites have identical values (within error) of 1.6 ± 0.1 mg/L. The highest DOC measured in this study was in pore water extracted from peat-rich intertidal sediment at Threeman (T306; 27 ± 0.1 mg/L).

Sample pH was strongly related to the dissolved metal load of the sediment within the set of water samples examined. The Ellamar beach has the highest volume of sulfide-rich waste in the intertidal zone, correspondingly low-pH values in intertidal zone pore water (range of 3.9 ± 0.5 – 6.8 ± 0.5), and high dissolved metal concentrations (Table 2). Beatson seep and surface drainage waters have only slightly acidic values (5.8 ± 0.5 – 6.6 ± 0.5) and low dissolved metal concentrations. Threeman pore water pHs range from 6.8 to 8.3 (Table 2), and are characterized by dissolved metal loads that were on average comparable to those measured at Beatson, but both sites have approximately one order of magnitude lower total dissolved metals in

intertidal zone pore water than Ellamar (Table 2). High SO_4 and Cl in Threeman and Ellamar pore water suggest a substantial seawater component to the water at the time of collection.

Although water pH is clearly a factor in dissolved metal loads, it is not necessarily a predictor of the metal load of bulk sediments. The total trace metals in Threeman sediment averages about 3000 mg/L, which is within the same range of the Ellamar bulk sediment (Table 3). However, there is one outlier: peat-rich beach sediment (T306) contains 2–4 times more trace metals than other Threeman sediment samples. Trace metal concentrations are even higher in Beatson ferric oxyhydroxide precipitates sampled from slightly acidic (pH 5–6) environments (Table 3). The Beatson precipitates are about 10 times more enriched in trace metals than the other bulk sediments (except T306), but their trace metal loads are comparable to fine-grained precipitates from Threeman or Ellamar that were analyzed separately from bulk sediment (Koski et al., 2008).

The only sediment capable of generating low-pH water upon equilibration with DI water (ratio 1:2.5 sediment to water) for 2 h were debris from the ore pile at Threeman (T308; pH 4.2) and 5 of the 7 intertidal and supratidal zone sediments at Ellamar (pH 3.0–4.5; Table 3). Ellamar sediments that did *not* generate low-pH water were E301-a, a subtidal mud, and E308, which is situated outside the zone of sulfide-rich beach sediment (Fig. 1c).

3.2. Biomass and diversity estimates using total FAMES

Fig. 3a–c summarizes the estimated total biomass (viable + non-viable) and FAMES diversity both by site and by sample type within each site. The estimated biomass of subtidal mud samples is similar at Ellamar and Threeman, and the biomass of most of the Threeman and Ellamar intertidal/supratidal sediments is substantially lower. The exceptions to this generalization are T306 (the peat-rich sediment), and B303-2b, a ferric oxyhydroxide-rich Beatson precipitate collected from a subsurface zone of Fe reduction: these samples had roughly twice the biomass of subtidal mud samples. Most of the other Beatson samples have similar estimated biomass (within the standard error of the mean; Fig. 3c).

Diverse FAME profiles (50–69 identified peaks) were identified in subtidal muds, peat deposits, and ferric oxyhydroxide precipitates; Fig. 3a–c.

The number of individual FAMES identified in the most diverse samples in this study was within the range of those found in other FAMES and PLFA analyses of soils (20–72 peaks; Zelles, 1999). Intermediate diversity (20–40 identified FAMES) was found in Threeman sediments from a variety of environments (including the background site T304), in the Ellamar beach sediments located outside or on the periphery of sulfide-rich deposits, and in Beatson ferric oxyhydroxide precipitate collected from a shallow pool (B301). Low-diversity profiles (7–13 identified FAMES) were found in Ellamar sediments from sulfide-rich beach deposits in supratidal (E303, E304) and intertidal (E310, E311) zones.

The biomass and diversity estimates used in this study are clearly related, because both are dependent on peak quantification. A plot of FAMES diversity as a function of estimated biomass for these samples shows a strong, pseudo-linear relationship at low biomass, which asymptotically approaches a constant value at high biomass (see Fig. A.1a in Appendix). The graph suggests that the operational limits of resolution/peak identification may have been reached for the highest biomass samples, and therefore, that the microbial diversity of these samples is likely to be underestimated by this technique.

3.3. Microbial community composition at the domain and kingdom level using total FAMES

Bacterial FAMES constitute greater than 80% of the total (viable + non-viable) microbial biomass in 11 of the 19 samples examined (Fig. 4a). They are lowest in T305 (32%), and B304 (43%). Six samples have intermediate abundances in the range of 65–76% bacteria, and 5 of the 6 sulfide-rich sediment samples from Ellamar contain very high relative abundances of bacteria (3 are 100%); as previously mentioned, however, the composition of Ellamar sediment microbial communities may not be well represented by total FAMES analysis.

The FAMES used to assess total eukaryotic abundance were subdivided to quantify eukaryotic subgroups such as diatoms, fungi, microeukaryotes and higher plants (Table 1; Fig. 4b). Although their absolute abundance is low, <20% of the total FAMES, higher plant FAMES predominated the eukaryotic FAME, signature in most of the samples examined. They were >90% of the total eukaryotic FAMES in T304, E303, E308 and B304; between

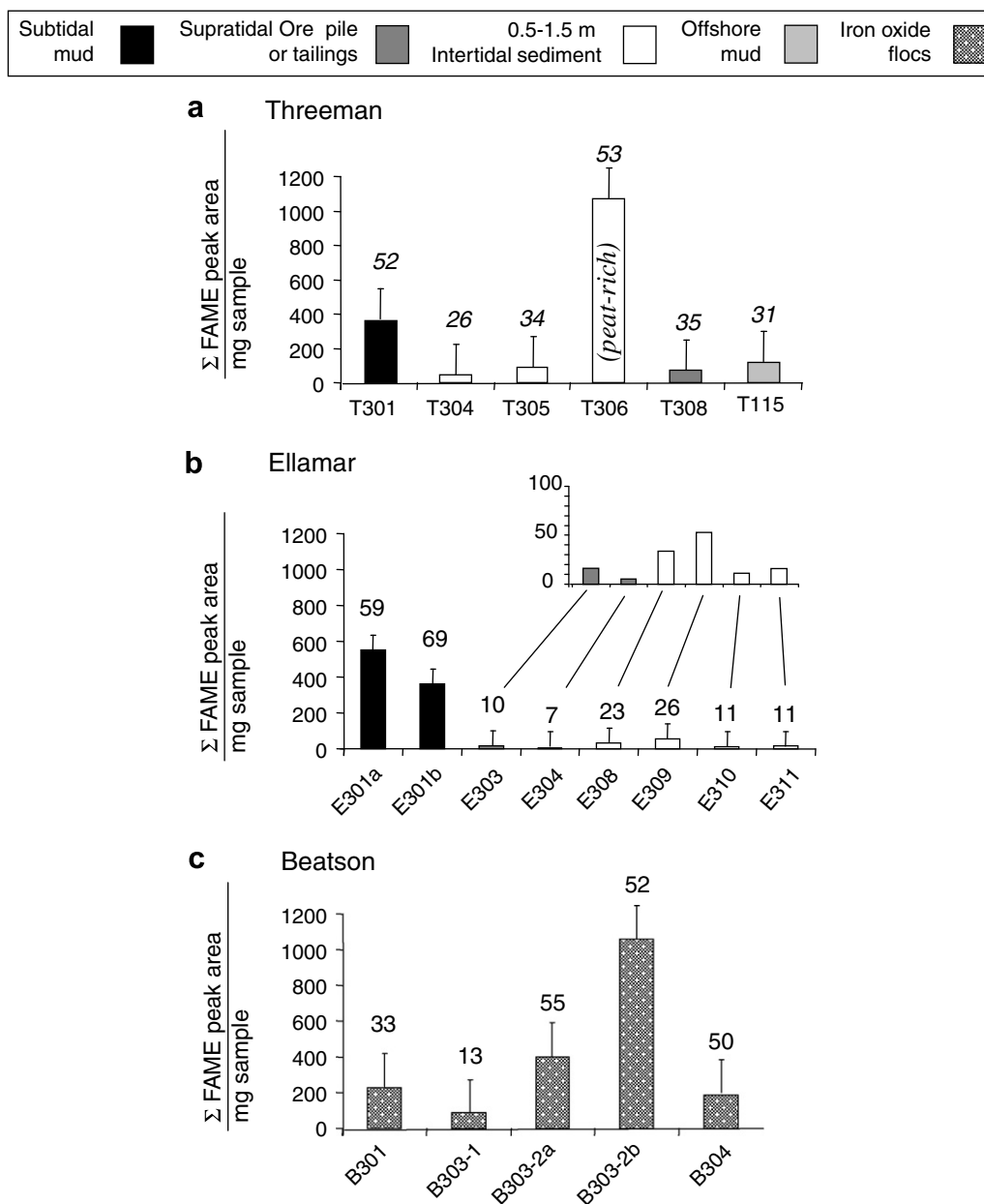


Fig. 3. Comparison of estimated total (living + dead) biomass at Threeman (a), Ellamar (b) and Beatson (c) sites. The estimate was made by normalizing the sum of all identified FAMES (as % peak area) present in a sample by the mass (mg) of freeze-dried sample used in the FAMES extraction; it does not correct for anticipated bias in favor of samples with small average particle size (i.e., subtidal/offshore muds and Fe oxyhydroxide flocs). Positive estimates of the standard error are given for each site-specific set of samples; the number immediately above the error bars in (a), (b), and (c) is an estimate of microbial community diversity as measured by a sum of the number of unambiguously identified FAMES present in each sample. There is a strong positive relationship between the biomass and diversity estimates in most samples (B304, and E301b, are notable exceptions).

50% and 80% in samples T301, T305, T306, T308, T115; and between 16% and 32% of total eukaryotes in samples E301a, E301b, E309 and B301. Of these groups, the abundance of indicator FAMES

was approximately (from most to least abundant): higher plants $\gg$ microeukaryotes $>$ fungi = diatoms. Fungal FAMES were identified in 8 of the 19 samples. They were most abundant in samples B301

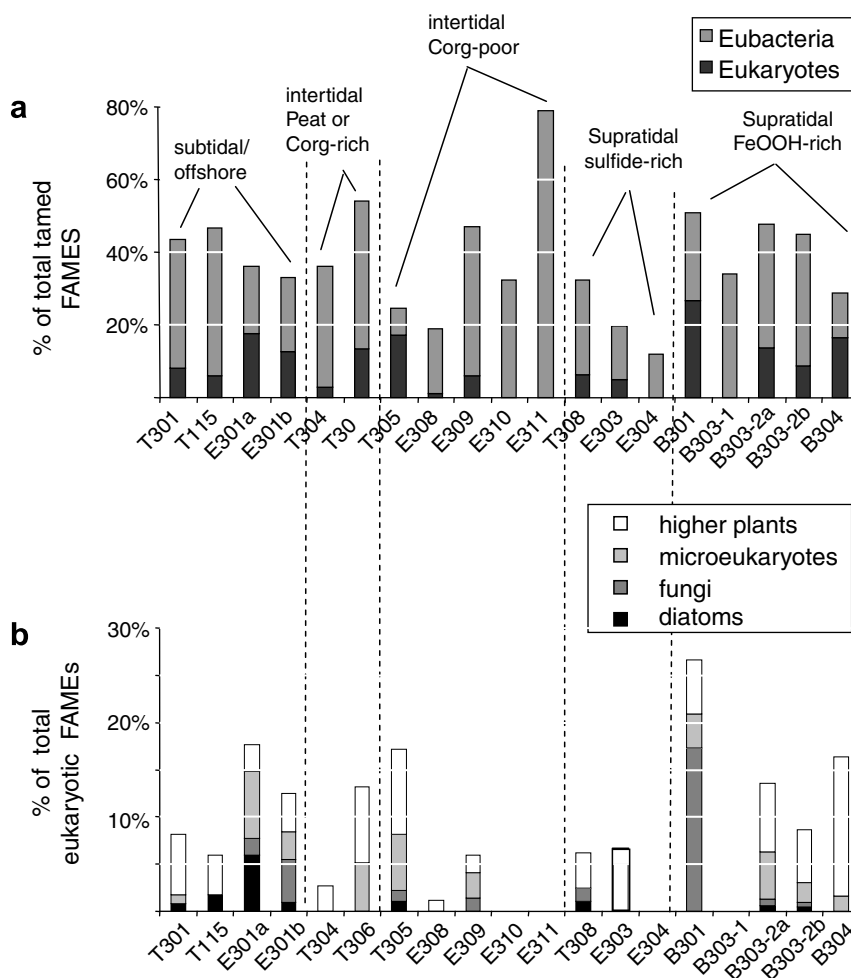


Fig. 4. Percent of total named FAMES characteristic for (a) eubacteria and eukaryotes and (b) eukaryotic subgroups. The composition of FAMES comprising each group used for quantification are described in Table 1.

(65%), E301b (37%), T308 (23%) and E307 (24%), and of intermediate abundance (5–10%) in samples T305, E301a, B303-2a and B303-2b. Diatom FAMES were detected in 8 of the 19 samples (Fig. 4b); samples with the highest proportion of diatom-indicator FAMES are E301a (34%), T115 (29%) and T308 (16%). Diatom FAMES were a substantially lower proportion (5–10%) of eukaryotic FAMES in the five remaining samples in which they were detected (Fig. 4b). Microeukaryotic FAMES were above 40% (of the total eukaryotic FAMES) in samples E301a and E309, between 20% and 40% in samples T305, T306, E301b, B303-2a and B303-2b, and were less than 15% in samples B304, B301 and T301 (Fig. 4b).

Several groups of FAMES have been used to quantify the abundance of aerobes/anaerobes and

gram-negative/gram-positive bacteria (Pennanen et al., 1996; Kaufman et al., 1999; Zelles, 1999; Stoek et al., 2002; Ben-David et al., 2003); some of these are listed in Table 1. The application of several of these metrics to the sample set gave inconsistent (for some samples, directly conflicting) results (see discussion in the Appendix). This conflict is due to the known overlap between those FAMES characteristic of Gram sign and those characteristic of O₂ tolerance, but also arises because the metrics differ in the degree of overlap between the characteristic FAMES. A high variability in the metrics with respect to the Ellamar intertidal zone sediments might be expected, due to the low diversity of the FAMES profiles obtained. However, the variability was seen across all sample types and levels of diversity. For this reason, Gram sign and aerobe/

anaerobe relative abundance data based on FAMES is not reported here. Instead, we rely on the presence/absence and absolute abundance of individual FAMES to identify probable key members of the bacterial communities in PWS samples.

3.4. Independent assessments of variance in water, sediment, and FAMES data: principal component analyses (PCAs)

One beneficial attribute of PCA is that the technique permits the visualization of sample variance in a two-dimensional space using the two most significant eigenvectors (principal components); in this space, distances among samples are proportional to their variance (McCune and Mefford, 1997). The significance of specific parameters (i.e., water, sediment, or FAMES) to the PCA can also be visualized by plotting their scores: in this study, scores for environmental parameters were calculated by weighted averaging. As with samples, proximity of parameter scores (plotted as points) indicates strong correlation (small variance) among parameters. Additionally, lines radiating from the origin (the center of the cloud of parameter scores) to the parameter points provide information about: (1) the significance of the parameter in the ordination (line length), (2) parameter enrichment in specific sample groups (lines point in the direction of samples enriched in that parameter), and (3) strength of correlation of environmental parameters with the two ordination axes.

Samples were separated into three clear groups by the PCA on water data (“water-PCA”; Fig. 5a). Only 2 of the 12 PCs generated were deemed significant out of a maximum of five allowable (see Section 2.4 for more details). Principal component (PC) axis 1 accounts for a total of 58% of the variance and PC axis 2 accounts for 25% (total of 83%) in the dataset. Samples E301(a,b), E308, T301, T304, T305 and T306 plot in the NE quadrant of the 2-d ordination space made from the first two PCs; this group of samples is enriched in DOC and pH as indicated by the variable loadings. Samples from Beatson (B301, B303-1, and B304) plot in the SE quadrant. Rather than enrichment in specific elements, these water samples are depleted in the 17 dissolved parameters considered. Ellamar intertidal zone pore water within sulfide-rich sediment (E309, E310, and E311) is enriched in H^+ (low-pH), Cd, Co, and Pb relative to other samples. The variable scores for Al, Hg, Fe and pH plot closest to or nearly

on top of PC axis 1, indicating a strong correlation with that axis. Together, these variables have the greatest effect on the separation of samples along PC axis 1. DOC and the strongly correlated variables Ca, Mg, Cl and SO_4 are primarily responsible for the separation of variables along PC axis 2.

PCA on the sediment data (“sediment-PCA”; Table 3) resulted in the separation of samples into new groups, some of which overlap with the groupings in the water-PCA (Fig. 5b). Between 1 and 5 of 7 allowable components were estimated to be significant, and the first two PC axes accounted for 69% of the dataset variance (PC axis 1 = 54% and PC axis 2 = 15%). Sediments exhibiting H^+ -generating capacity (E311, E309, T308, E310 and E304) are segregated from intertidal zone sediments lacking that capacity along PC axis 1, which shows strong correlations with Al, Ca, Cr, Fe, Hg, Mg, Mn, Na, P and pH. Of these, the variables with the strongest loadings on PC axis 1 are Ca, Mg and Hg. The primary discrimination along PC axis 2 is between two sets of H^+ -generating intertidal zone sediments from Ellamar: those in the NW quadrant of the graph (E310, E304 and B303-1) are enriched in Hg and Pb, and those in the SW quadrant (E311, E309 and T308) are enriched in Cd, Co and Mo relative to other samples. Threeman samples (T115, T301, T304, T305, and T306) cluster near the border of the NE and SE quadrants, indicating that they are little controlled by the variables influencing separation along axis 2; these samples are primarily characterized by enrichments in Ca and Mg relative to other samples. Several of the samples plot near the origin (E301, B301, E303, E308), an indication that the variables primarily responsible for the separation in both dimensions have relatively low abundances in these samples. Hydrogen ion concentration (pH) clearly plays a role in the offset of these samples from those of Threeman samples along PC axis 1. Sediment parameter scores indicate that E301 and B301 are enriched in Cu and Zn, and E308 and E303 are enriched in Pb relative to other samples.

PCA on FAMES using those present in $\geq 10\%$ of samples ($n = 79$) produced ordinations which were unstable according to significance tests (i.e., the number of significant components was determined to be greater than the 9 allowed for the set of 19 samples). PCA on FAMES (“FAMES-PCA”) present in $\geq 21\%$ of samples ($n = 51$) had between 3 and 9 significant PCs and is presented in Fig. 5c. The first two PCs accounted for 41% of the data

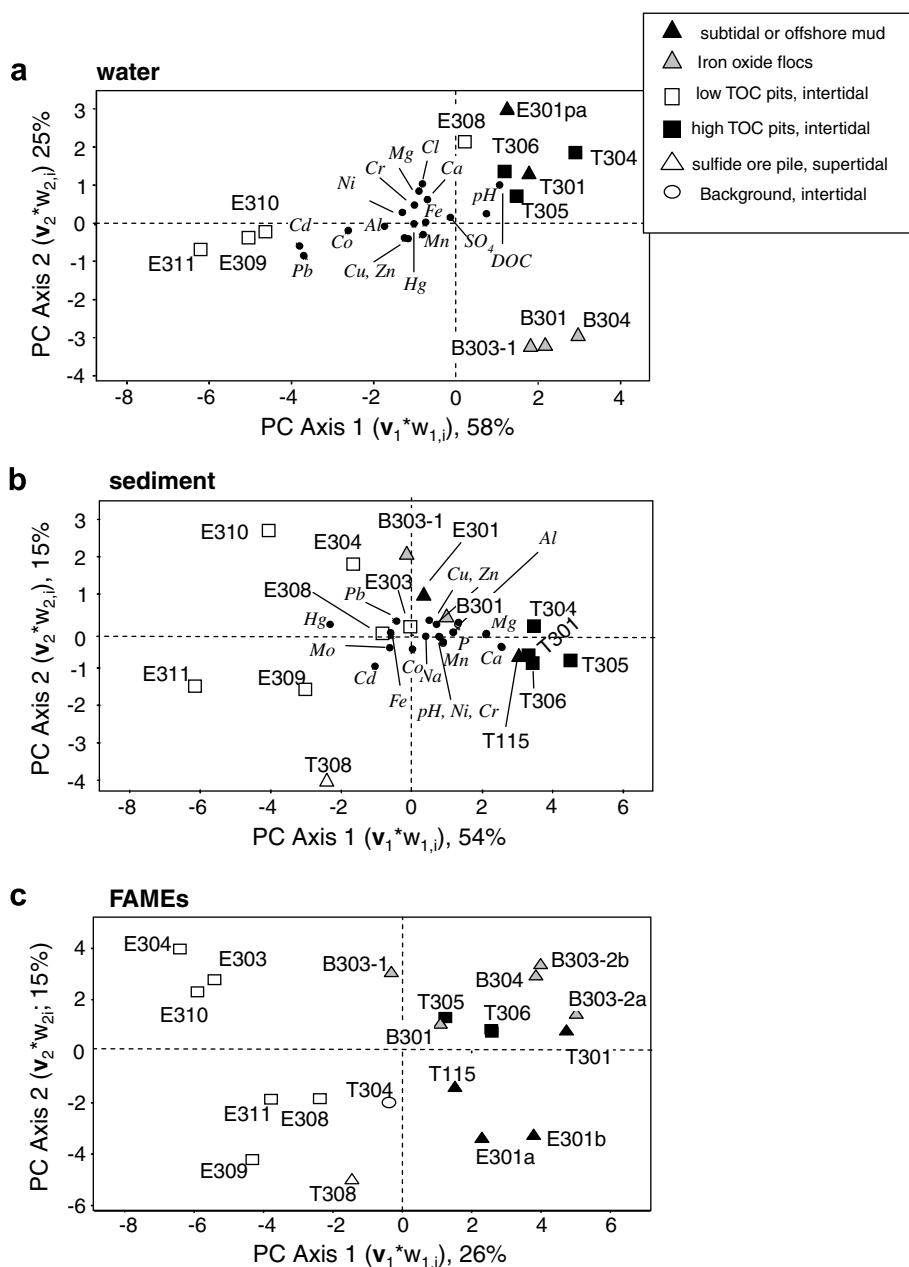


Fig. 5. Graphical representation of PCAs using (a) water, (b) sediment, and (c) total fatty acid methyl ester (FAMES) data. Plot axes represent the two components explaining most of the variance in the sample set; compositionally similar samples show close proximity. Sample type is dictated by symbol, sample names are the same as those used in all data tables, with the prefix indicating the site: B = Beatson, E = Ellamar, and T = Threeman. Note that not all samples were used in all ordinations due to missing data.

variance (PC1 = 26%, PC2 = 15%). The FAMES-PCA separated samples into four clear groups (with a few outliers) that follow the quadrants of the graph in Fig. 5c. The loadings of individual FAMES are plotted in Fig. 6; because of the sheer number of FAMES, they could not be plotted on the same

graph with the labeled samples. The position of the sample scores has been re-plotted in Fig. 6 to facilitate comparison.

The NE quadrant contains almost all of the Beatson samples (B303-1 is the distantly-related outlier) and all of the Threeman samples; this quadrant is

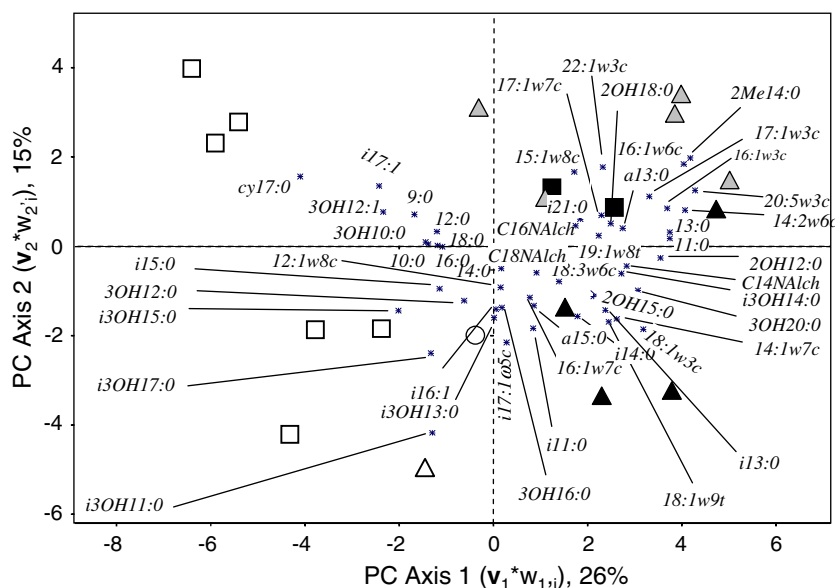


Fig. 6. Graphical representation of PCAs using FAMES data. Sample naming and graphing conventions are the same as described for Fig. 5. The scores (calculated by weighted averaging; see text), of the 51 FAMES used in the PCA are plotted (italicized words are the names of the FAMES); the proximity of these scores to each other, to the ordination axes, and to the sample points indicates the relative importance of the FAME to the ordination.

characterized by a high relative abundance of MUFAs generally, and is specifically enriched in 2Me14:0, 16:1 ω 6c, 22:1 ω 3c and 20:5 ω 3c relative to other quadrants (Fig. 6). It is important to emphasize that high relative enrichment is not the same as absolute enrichment. Although none of these FAMES are the most abundant ones among the samples in the NE quadrant, they are nevertheless important variables discriminating the NE quadrant samples from others. Subtidal and offshore muds (T115, E301b, and E301a) plot together in the SE quadrant (Fig. 5c); these samples show enrichment in 2OH-SFA relative to other quadrants (Fig. 6). The SE quadrant contains just three samples: T115 (offshore sediment), E301a and E301b (upper and lower portions of subtidal mud). The highest FAMES loadings in this quadrant are on 18:1 ω 9t, 18:1 ω 3c, a15:0, i14:0 and i17:1 ω 5c. The SW quadrant contains samples T304, T308, E308, E311 and E309, which have the strongest enrichment in i3OH11:0, i3OH13:0, i3OH17:0 and i3OH17:0.

The NW quadrant contains a tight cluster of samples from Ellamar (E303, E304 and E310) and one outlier (B303-1), which technically plots in this quadrant but it is more closely related (plots closer to) to NE quadrant samples B303-1 and T305 than to those in the NW quadrant. Ellamar samples are enriched in i17:1 and cy17:0 (the latter especially

for Ellamar samples). FAMES displaying the highest correlations to the PC axes (not necessarily the highest enrichments) are 11:0, 13:0, 19:1 ω 8t, 18:0, 16:0, 10:0 and 3OH10:0 on PC axis 1, and 14:0, 12:1 ω 8c, 3OH16:0, i16:1 and i3OH13:0 on PC axis 2. Saturated fatty acids (SFAs) are abundant in this quadrant; although several SFAs (10:0, 16:0 and 18:0) are highly correlated with PC axis 1, their low loadings indicate little control on the ordination. There is more correspondence between strongly covarying FAMES (9:0, 10:0, 12:0, 3OH10:0, 3OH12:1) and FAMES with the highest loadings (9:0, 12:0, 3OH12:1, i17:1 and cy17:0) in this quadrant than was observed in the other two quadrants, but this may simply be a function of the extremely low number of FAMES present in these samples.

4. Discussion

4.1. Relationships among samples as controlled by water and sediment parameters

Analysis of the water-PCA results suggests that much of the variance among samples is accounted for by pH, DOC, and 'salinity' (defined operationally by the strongly covarying parameters Ca, Mg, SO₄ and Cl) rather than by metal concentrations. When metal concentrations are extremely high, such

as in Ellamar pore water, they also play a role in the ordination. The water-PCA grouped samples by local geochemical environment (i.e., position within the tidal zone), not by site. This effect is most clearly observed for the Ellamar samples: low-pH pore water samples collected within the sulfide-rich intertidal zone (E310, E309 and E311) are closely related, but this group is distantly related to Ellamar pore water samples from outside the sulfide-rich zone (E308, and E301a,b, representing the intertidal and subtidal zones, respectively). However, this effect is not observed for the Beatson and Threeman samples, which each form their own cluster of sample points. The reasons for this are several: at Beatson, samples were not collected across gradients in pH, DOC, and salinity, but were collected from freshwater seeps having similar chemistry (especially if only pH, DOC, and ‘salinity’ are considered). Although samples from subtidal, intertidal and supratidal zones were collected at Threeman, the chemical gradients encountered were less steep because of the lack of ARD associated with weathering sulfides. In addition, the presence of peat deposits in the intertidal zone clearly produced elevated DOC concentrations in all the samples from this site (Table 1); because DOC is one of the major ordination parameters in this PCA, it exerted a large influence on the relatedness of Threeman samples.

Variation in trace element abundances influenced the sediment-PCA to a greater extent than the water-PCA; the resulting ordination is superficially similar to that based on water data, but contains fundamental differences. Just as in the water-parameter PCA, PC axis 1 segregates Ellamar sediments on the left from Threeman/Beatson sediments on the right, and samples from the Threeman site are more closely related to each other than to samples from other sites. However, there is a much closer relationship among the Ellamar sediment samples than there was among the Ellamar pore water samples. In addition, samples E309, E310, and E311 plot much closer in ordination space to (1) intertidal zone sediment from outside the sulfide-rich zone (E308); (2) subtidal zone sediment (E301a); and (3) secondary precipitates from freshwater Beatson seeps (B301, B303-1). There is an increased relatedness between these samples because both E301a and B303-1 contain secondary phases (jarosite in the case of E308/E301 and Fe oxyhydroxide in the case of Beatson) that are similarly enriched in trace elements. Sediment-PCA finds these samples to be closely related despite their obvious genetic differences

due to the fact that they contain similar concentrations of elements.

4.2. Biomass/diversity estimates

When compared within and among sites, the biomass/diversity estimates show clear discrimination among sediments as a function of the local (meter to centimeter-scale) geochemistry within a site, rather than between sites. They also suggest that the locations of highest microbial diversity and productivity are: (1) suboxic-to-anoxic, organic matter-rich sediment from sub- and intertidal zones and (2) zones of Fe and/or Mn reduction. Sites visibly enriched in organic matter or with elevated DOC (T301, T306, E301a, and E301b) have significantly higher biomass and diversity than samples collected just meters away in a different part of the tidal zone (Figs. 2 and 3). The site with the highest biomass (B303-2b) does not fit this generalization, however: it is a subsurface sample of a thick accumulation of Fe oxyhydroxide (Fig. 2f). As DOC and TOC were not measured at this site, no comparison can be made to the organic matter-rich samples. However, B303-2b differs from the other Beatson samples in that it was collected from the subsurface (approximately 25 cm below ground surface), whereas the others were surface grab samples. Its darker color relative to the overlying material (sampled as B303-2a; see Fig. 2f) suggests that it was collected from a zone of active reductive dissolution of Fe oxyhydroxide. The possibility that Fe reduction (a process known to be biogenically mediated; Lovley, 2000) could support biomass on the order of that found in organic-rich subtidal muds and peat-rich intertidal sediments is intriguing, but cannot be addressed with this dataset.

The conclusion that subtidal muds and peat-rich intertidal sediment support highly diverse, high biomass communities is consistent with previous studies linking biomass and diversity at so-called “receptive” sites (whose primary characteristic is abundant labile organic matter; Hurst, 2002). By the definitions in Hurst (2002), non-receptive sites are characterized by low labile organic matter and one or more of the following: increased salinity, extreme pH, low nutrients, and high light intensity. Sediments from the sulfide-rich inter- and supertidal zones at Ellamar have many of the characteristics of “non-receptive” sites, and were the locations of the lowest measured biomass and diversity in this study.

Three possibilities are advanced to explain the low-diversity data. The first is that the low FAMES diversity indicates the predominance of a few specialized microbes in the sulfide-oxidizing environments measured in this study. Evidence in favor of this scenario comes from the fact that pure strains of bacteria contain only a few FAMES (between 7 and 11 for SRB isolates; Knoblauch et al., 1999). However, unless they were very closely related, the FAME profile of even just two bacteria would probably have more than 7–11 unique FAMES (the same range encountered in 4 of the 6 Ellamar beach sediments). Another possibility is that the low-pH environment enhances FAME degradation, so that the measured low diversity does not accurately reflect the true diversity of the sediments. Although not much information on the stability of FAMES in low-pH solutions could be found in the literature, indirect evidence suggests that chemical degradation is not likely to be the cause of the low-diversity profiles observed in many of the Ellamar sediments. Despite evidence that degradation of cyclopropyl FAMES is enhanced in acidic solution (Zelles, 1999), the Ellamar sediments in question have the highest levels of the cyclopropyl FAME cy17:0 of all the samples in this study.

Another possibility is that Archaea constitute a substantial portion of the microbial community within these sediments, and were not detected by this analysis. Evidence for Archaea-dominated microbial communities in low-pH environments associated with the oxidation of sulfide minerals exists, but in every case identified in the literature, the Archaeal strains associated with AMD sites are moderately thermophilic (Bond et al., 2000). Temperatures in Ellamar sediments would not be expected to encourage the growth of even moderate thermophiles, but it is quite possible that any Archaea adapted to this environment will show genetic or morphological properties distinct from their ARD relatives in non-polar regions. Evidence for or against the presence of Archaea in these samples awaits the results of DNA based studies currently underway.

4.3. Analysis of sample relationships by FAMES-PCA, lipid structural groups, and signature lipids

PCA on a subset of the total FAMES produced a sample distribution that was more similar to that of the sediment-PCA than the water-PCA, but also differed from both in significant aspects. The discus-

sion to follow uses PCA results, FAMES abundances, and signature lipids of pure bacterial strains to relate sample distribution on an ordination diagram with changes in microbial community composition. FAMES data are discussed in two forms: as structurally-related groups (rather than individual FAMES) and individually as signature lipids. Presence or absence, and abundance of the latter are compared to organisms speculated to be of importance to geochemical processes occurring at the PWS study sites.

Intertidal sediment (with or without peat) from Threeman is more closely related to Beatson seep sediment by the FAMES-PCA than by water and chemistry PCAs (Figs. 5 and 6). However, sample T306 is still distantly related to all other samples in the NE quadrant. This quadrant contains more monounsaturated fatty acids (MUFAs) than any other. MUFAs are diagnostic of Gram-negative organisms (Zelles, 1999; Piotrowska-Seget and Mrozek, 2003; Table 1), but there is overlap with other groups: some Gram-positive bacteria have been reported to have ω 9 MUFA and some anaerobes have been reported to have ω 7 MUFAs (Zelles, 1999).

Sample T306 is uniquely enriched in particulate and dissolved organic matter derived from peat. FAMES specific for SO_4 -reducing bacteria and methanotrophs (identified in previous lipid-based studies of pure peats; Table 1; Sundh et al., 1997) were not detected in this sample. T306 is also unique in its enrichment in the cyclopropane FAME cy19:0c11-12, which constitutes 30% of the summed FAMES peak area in this sample, and which was not detected at significant ($> 1\%$) levels in other samples. Due to its rarity, this FAME was not included in the FAMES-PCA. CYFA have been used along with other FAMES to assess the abundance of anaerobic bacteria of gram-positive and gram-negative bacteria (Zelles, 1999). CYFA cy19:0c11-12 is abundant in pure cultures of gram-negative *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*, but is absent in other gram-negative acidophiles such as *Thiobacillus denitrificans*, *Thiobacillus intermedius*, *Thiobacillus neapolitanus*, *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans* (Kerger et al., 1987; Knief et al., 2003). These data strongly suggest the presence of organisms of the *Acidithiobacillus* genus, despite the near-neutral pH of pore water. Sulfur-oxidizing bacteria could reasonably be expected in peat-rich samples due to the high abundance of reduced S

present in this material; authigenic sulfides present in the peat and sulfides from mine wastes could also serve as sources of reduced S.

Members of the genus *Leptothrix* (Gram(–), β -Proteobacterium), were postulated to occur in shallow, stagnant pools draining mine waste piles at Beatson, and both of the two reported signature lipids for β -Proteobacteria (Table 1) were detected in sample B303-1 at relatively high abundance (13%), leaving open the possibility that *Leptothrix* species are present. However, as these FAMES were not detected in B301, the sample whose morphological characteristics were reminiscent of the colony habit of *Leptothrix* (Fig. 2e) it is doubtful that the Fe oxyhydroxide material sampled at Beatson represents near monocultures of *Leptothrix*, as has been observed at other sites (Foster and Ashley, 2002). Sample B303-1 contains a high abundance of 18:1 ω 9, a signature lipid for fungi (Table 1). The two most abundant FAMES in all the Beatson samples were 19:1 ω 8t and 17:1 ω 7c: these rank first or second in abundance in all of the samples, and constitute between 13% and 26% of the total FAMES peak area. These FAMES could not be definitively assigned to any group or organism.

The SE quadrant is characterized by samples containing high levels of eukaryotic FAMES reported to be specific for diatoms (Table 1). Diatoms are photosynthetic eukaryotes plentiful in fresh and seawater, and due to settling of pelagic material, would be expected to be present in sediments underlying waterbodies exposed to sunlight. The highest diatom FAME abundances were found in offshore sediment (T115) and in the uppermost layer of subtidal sediment from Ellamar (E301a). They were detected, but not nearly as abundant, in subtidal sediment from Threeman (T301), and in the lowermost layer of Ellamar subtidal sediment (E301b). Diatom FAMES were also abundant (16% of total eukaryotic FAMES) in sulfide-rich sediment collected from the surface of the heavily-weathered Threeman ore pile (T3008). According to the literature, diatoms can live as films at the sediment-water interfaces, and even on damp soils and on damp surfaces in addition to living in water (Wikipedia, 2007); such a lifestyle could explain their abundance in this sample.

The SE quadrant also contains a high number of 2OH-substituted FA (Fig. 6), which were rare in the overall sample set. The 2OH-substituted FAMES have been described as being characteristic of gram-negative bacteria generally, and specifically

for aerobic, gram-negative *Pseudomonads* and bacteria of the *Actinomycetales* group (specifically, *Mycobacteria*, a group characterized by a thickened cell wall similar in structure to that of gram-positive bacteria; Zelles, 1999). *Iso*- and *anteiso*-branched SFAs are also abundant in the SE quadrant: branched FAMES are known to occur in gram-negative, SO_4 -reducing bacteria (SRB), in gram-positive bacteria, and in members of the *Cytophaga* and *Flavobacteria* genera (Zelles, 1999). High abundances of *Cytophaga*–*Flavobacteria* and of SO_4 -reducing bacteria have been detected in offshore sediments in the North Sea (Büring et al., 2005); both groups are bacteria are known to cause diseases in fish (MicrobeWiki, 2007), and additionally *Cytophaga* have cellulose-degrading capability (Paustian and Roberts, 2007). The subtidal and offshore sediments plotting in the SE quadrant are likely to contain members of these groups, even though signature lipids for these organisms were not detected. FAMES diagnostic for these organisms may be of low abundance (as described for the 10-methyl FAMES for SRB), and therefore below detection in the complex GC patterns derived from these environmental samples.

Both the NE and SE quadrants contain samples with high abundances of fungal indicator FAMES (relative to total eukaryotic FAMES). Fungi are heterotrophs, and their abundance would not be restricted by light requirements. E301b (the lower portion of subtidal Ellamar sediment) seems a good possibility to have fungal FAMES based on these criteria (and it does), but sample T306 (peat-rich sediment) does not. This finding may not be unusual, however: in a study of 16 different microenvironments/depths in two Swedish boreal peatlands (total of 72 samples), Sundh et al. (1997) found that the fungal indicator FAME 18:2 ω 6 was less than 5 mol% of the total PLFA signature in the majority of samples, and exceeded 10 mol% only in samples collected within 5 cm of the ground surface. In accordance with ecological intuition, the relative abundances of diatom and fungal indicator FAMES are reversed in the upper and lower subtidal zone sediments at Ellamar (Fig. 4b). However, it was initially surprising that B301, a sample of ferric oxyhydroxide precipitate collected from a shallow pool, contained the most fungal FAMES of all samples examined in this study. However, as can be seen in Fig. 2e, the ferric oxyhydroxide is a fluffy coating on (mostly) dead plant material. The ferric oxyhydroxide sample was harvested by a suction method

that did not allow the large pieces of decaying plant material to pass, but could have picked up fungi adhering to the plant material, leading to the enhanced signal from this group. Significantly, the other samples from Beatson were collected from seep environments, not shallow pools of standing water, and did not show a preponderance of fungal indicator FAMES.

Inter- and supratidal zone Ellamar sediment in the western quadrants are distinguished from samples in the eastern quadrants primarily by three factors: (1) the presence of three or four SFA at >5% absolute abundance; (2) the presence and/or high abundance of cyclopropyl FAME 17:0 and branched MUFA i17:1 in samples of the NW quadrant and (3) and the presence of 3OH-SFA and branched, 3OH-SFA in the SW quadrant. Considered alone, the first criterion is not diagnostic, because SFA are found in nearly every type of organism. However, when coupled with other information, data for their abundance can be useful.

Supratidal, sulfide-rich samples from Ellamar are substantially enriched in cy17:0 (8–10% of total FAMES peak area). Along with i17:1ω7, this FAME has been used to document the presence of anaerobes, particularly SRB (Langworthy et al., 1998), but because additional signature lipids for *Desulfobacter* (10Me18:0), *Desulfovibrio* (i17:1ω7c, i15:1ω7c, and i19:1ω7c) and *Desulfobulbus* (17:1ω6 and 15:1; Sundh et al., 1997) nor those for several strains of SRB isolated from Arctic marine sediments (Knoblauch et al., 1999) were detected, members of this community are not thought to be abundant in these samples. Precedent exists for substantial populations of SO₄ reducers in SO₄-rich, low-pH sediment and water from one study in which the occurrence of significant numbers of δ-Proteobacteria (a group largely composed of SO₄ reducers) in low-pH sediment from a mining-impacted area was documented (Hao et al., 2007).

Thiobacilli and *Acidithiobacilli* contain high relative abundances of unbranched 3OH-FA (Kerger et al., 1987; Knief et al., 2003), and samples in the western part of the FAMES-PCA contain high numbers of 3OH-FAMES (especially relative to the number of identified peaks, which is low). Some *Thiobacilli* have methylated FAMES that are good signatures (Salomonova et al., 2003), but even in the pure cultures, their abundance is low (<5%) and might therefore be below detection in environmental samples. Additional evidence for the presence of *Thiobacilli* in these sediments is given by

the fact that at least some species contain 10:0 SFA (present in this dataset) and other known acidophiles such as *Acidithiobacilli* and *Leptospirillum* do not (Knief et al., 2003). The same study also demonstrated that *T. denitrificans* has approximately 3 times more cy17:0 than *Acidithiobacilli* and *Leptospirillum*, and no cy19:0, 11,12 (although this FAME is also absent in *Leptospirillum* species, *Acidithiobacilli* strains have been measured with 25–31% cy19:0, 11,12; Knief et al., 2003). *T. denitrificans* is unique in its ability to live under anaerobic conditions by coupling the oxidation of inorganic S to the reduction of several types of oxidized N (Kelly and Wood, 2000); other members of this genus are thought to be strict aerobes. Taken together, the three lines of evidence suggest that *T. denitrificans* may be a member of the microbial community in the low-pH sediments and pore waters in the supratidal zone, and other *Thiobacilli* and/or *Acidithiobacilli* are likely to be present in intertidal, sulfide-rich sediment. Alternatively, if the branched, 3OH-FA are taken as a strong indicator of gram-positive bacteria, one gram-positive, acidophilic bacterium (*Acidimicrobium*, a member of the phylum/class Actinobacteria) isolated from a mining impacted site that might serve as a model; unfortunately no compositional lipid data for this organism had been found at the time of writing.

4.4. Limitations of this study and directions of future work

The use of total FAMES for SLB analysis has been criticized on the grounds that non-PLFAs and non-viable PLFA (or free fatty acid) are also extracted during the procedures (Zelles, 1999), but these non-PLFA are easily removed from the FAMES assemblage prior to SLB or other analyses. Although the fraction of non-viable PLFA liberated with viable PLFA during the MIDI extraction procedure cannot be quantified easily, previous studies suggest that although differences in relative abundances of PLFAs are observed as a function of extraction method (MIDI or PLFA), the number of fatty acids unique to a given extraction type was low and similar degrees and patterns of ordination are obtained in multivariate analysis of lipid data from the two extraction procedures (Haack et al., 1994; Zelles, 1999; Schutter and Dick, 2000). These facts suggest that although there may be more bias in the total FAMES extraction, and although it samples the non-viable microbial com-

munity as well as the viable, an analysis based on total FAMES, if tailored appropriately, targets roughly the same portions of the microbial community as does an analysis based on PLFAs. It may be that the examination of tidal zone sediments (as opposed to terrestrial ones) was a fortunate choice, as the signal from exogenous, plant-derived FAMES was relatively low in the study.

There are several limitations on the resolving power of PLFA patterns derived from natural sediment or water samples relative to those derived from pure cultures, and even more limitations on the interpretation of total FAME patterns relative to PLFA patterns. The primary limitation of both analyses is that there are far fewer lipids than species (Zelles, 1999; Curtis and Sloan, 2004). Although there is evidence that 3–4 times more PLFAs could be recovered using additional processing steps not common to the standard PLFA and total FAMES extraction techniques (Zelles, 1999), there is clearly a limit to the information contained in cellular lipids with respect to identifying members of high-diversity natural microbial communities to the genus and species level.

In addition to the aforementioned intrinsic limitations, there are additional ones based on the specifics of this study. FAMES extraction and analyses replicates were not performed; these are needed to assign error to peak area quantifications. Also, the standard gas chromatography and peak analysis performed by the commercial lab does not give a reliable indicator of the conformation of *cis-trans* isomers, and probably provides lower-resolution peak patterns than do techniques that link GC with mass detection (Pinkart et al., 2002). The latter technique is probably also more sensitive to lower quantities of FAMES than that used in this study. The lack of data from appropriate background sites was also a limiting factor in this study; although upland drainages were sampled to deduce “background” water chemistry at each site, these freshwater samples are not similar to the samples under study, except at Beatson. Only two sediment samples were collected to serve as background samples; a better approach would have been to sample a shoreline transect through the intertidal zone at a site quite distant from known ARD-impacted sites.

No characterization of microbial community composition is truly complete, because of the huge number of bacteria estimated to be present in the environment, limitations on time and resources, and the limitations of the characterization techniques. However, the community characterization

reported here will be improved by a parallel analysis of the 16S rDNA, which has less ambiguity in the identification of microorganisms at the genus level; this work is currently underway. The identity and quantity of Archaea in sulfide-rich sediments from the subarctic PWS (intertidal/supertidal Ellamar sediments and T308, the ore pile remnant) clearly could be a focus of future work, because if they are present they are likely to at least be psychrotolerant and therefore may exhibit adaptations quite different from those of the mildly- to moderately-thermophilic Archaea that have been characterized in ARD drainage to date (Bond et al., 2000).

5. Conclusions

The geochemistry and microbiology of tidal zone sediments impacted by historical mining of VMS deposits located in subarctic Prince William Sound, AK, were investigated using standard geochemical techniques and total fatty acid methyl ester (FAMES) derived from the viable and non-viable microbial community. When compared within and among sites, biomass/diversity estimates based on groups of total FAMES relate samples by the local (meter to centimeter-scale) geochemistry within a site, rather than by site. They also suggest that the locations of highest microbial diversity and productivity are: (1) suboxic, organic matter-rich sediment from sub- and intertidal zones and (2) zones of Fe and/or Mn reduction. Very low diversity and biomass were measured in acid-generating, metal-rich sediments with abundant weathering sulfides. Although a positive bias in biomass estimate due to particle size is anticipated for these samples, it is not solely responsible for the observed trends, because other samples of similar particle size did not have similarly high values.

Independent assessments of the controls on the variance of water chemistry, sediment chemistry, and total fatty acid methyl ester (FAMES) datasets show a strong concordance among the three ordinations with regard to the relatedness among samples. Although this concordance gives no proof of causality, it can provide insight into the environmental parameters that change along with microbial community composition, which can be used to infer the key processes responsible for the relationships observed. Key distinctions among the water and sediment-PCAs were the role of DOC in the water-PCA (the analogous parameter, TOC was not included in the sediment-PCA) and the importance

of trace metals in secondary precipitates on sample relationships as mapped by sediment-PCA (these relationships were not captured in the water data). The FAMES-PCA indicated that site-specific and locality-specific factors were both important in defining the microbial community present in the solid phase, but not as much as sample type (largely a function of chemistry and location along salinity/moisture gradients).

Analysis of the kingdom and group-level data of FAMES grouped by structural type, and of the scores of individual FAMES from the PCA indicates that: (1) the contribution of terrestrial plant matter

(recent or ancient) to the total FAMES pattern is not overwhelming, allowing meaningful conclusions about the viable + non-viable microbial community to be made; (2) the relative abundance of various FAMES groups agrees generally with the distributions expected from a simple understanding of the ecological niches occupied by each groups. Most of the oxic ferric oxyhydroxide mats are enriched in MUFA characteristic of Gram-negative bacteria, and fungal FAMES are abundant in some samples. Acid-generating, jarosite- and sulfide-rich sediments in the intertidal zone at Ellamar have low-diversity bacterial communities; at least for the supratidal

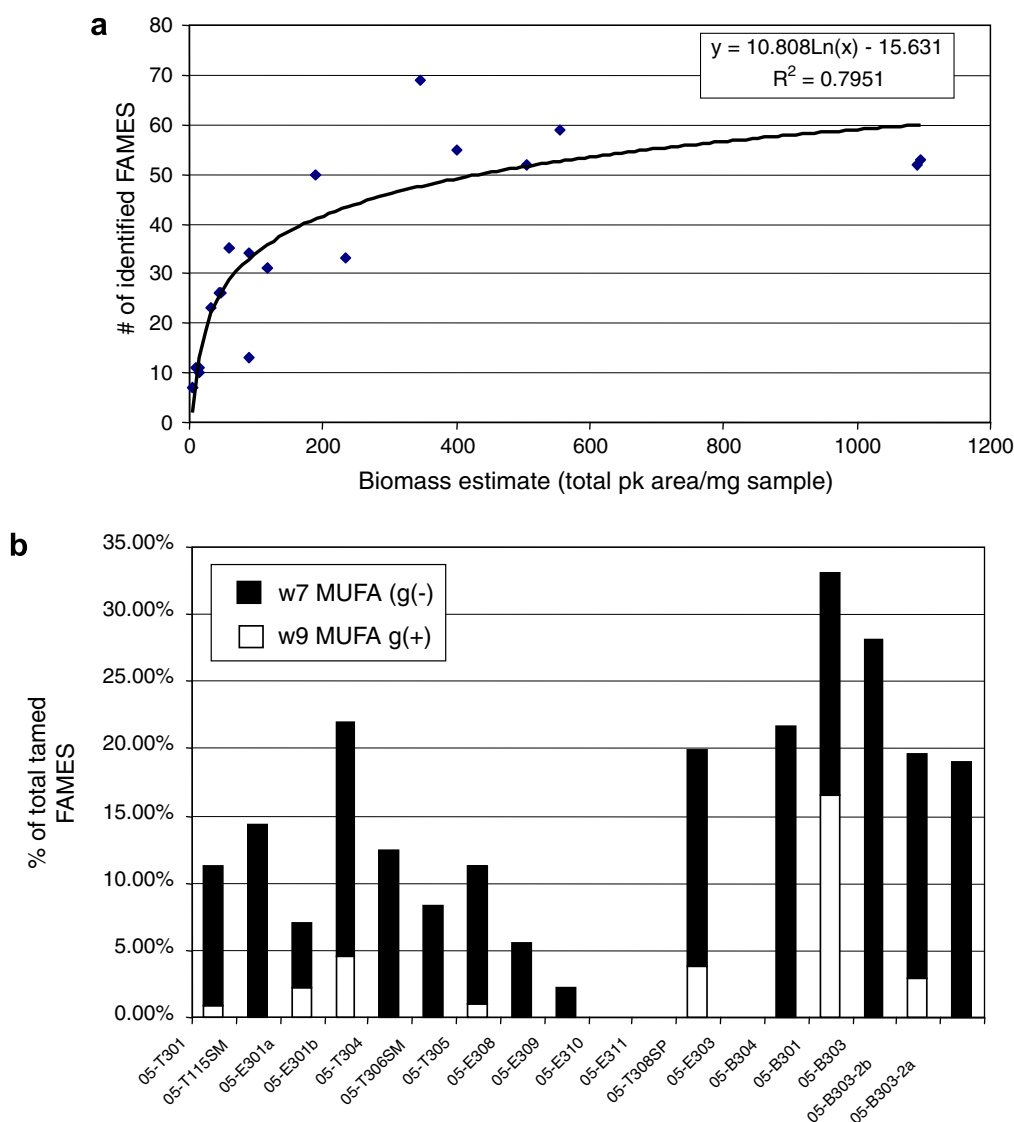


Fig. A.1. (a) plot of the relationship between the estimated biomass and the number of identified peaks in PWS samples, (b) plot of ω Gram (+) and Gram (-) bacterial abundance calculated on the basis of omega 7 and 9 FAMES. See text for discussion.

Ellamar sediments, a substantial portion of that community may be composed of *T. denitrificans*, a facultative anaerobe that oxidizes S with N compounds. Results are not as clear for the ARD-impacted Ellamar intertidal zone sediments. However, the presence of *Acidimicrobium* is suggested by the high proportion of branched FA in these samples. In addition, the low-diversity FAMES profile might be attributed to Gram-negative bacteria such as *Thiobacilli* and *Acidithiobacilli*. Although several species of Archaea are known to oxidize sulfide minerals, their presence in sulfide-rich tidal sediments from Ellamar has not been verified.

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Appendix

As discussed in Section 3.2, the approach to a constant value of the number of identified FAMES in samples with high estimated biomass (Fig. A.1(a)) suggests that the analytical limitations of the gas chromatographic methods employed may have underestimated the true FAMES diversity in these samples. The relative abundances of *iso* and *anteiso*-branched SFA are commonly used to quantify G(–)/anaerobes and G(+) aerobes, respectively, but as several of the sulfide-rich Ellamar sediments (in intertidal and supertidal zones) had no *iso/anteiso*-branched SFA to quantify, other metrics were explored. An alternative estimate relies on the $\omega 7$ and $\omega 9$ MUFAS to indicate G(–) aerobes + strict anaerobes and G(+) bacteria, respectively (Table 1) and references therein. G(–) aerobes + strict anaerobes are more than four times as abundant as G(+) bacteria in 14 of the 19 samples, and G(+) bacteria are present in samples E301b, T306, and B303-2a, at approximately the same levels (5%) as they were in the *isolanteiso* metric (Fig. A.1a). Now, however, sample B301 was found to have the highest proportion of G(+) bacteria, about the same as G(–) aerobes + strict anaerobes (Fig. A.1b), and fewer samples have G(+) FAMES by this metric (7 of 19). Unfortunately, $\omega 7$ and $\omega 9$ MUFAS were not

extracted from most of the Ellamar sediment samples at quantifiable levels either.

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