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Original Article

Comparison of boreal acid sulfate soil microbial communities in oxidative and reductive environments

Stephan Christel [a, *, 1], Changxun Yu [b, 1], Xiaofen Wu [a], Sarah Josefsson [c], Tom Lillhonga [d], Eva Högfors-Rönnholm [d], Gustav Sohlenius [c], Mats E. Åström [b], Mark Dopson [a]

[a] Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, SE-39182 Kalmar, Sweden
[b] Department of Biology and Environmental Science, Linnaeus University, SE-39182 Kalmar, Sweden
[c] Geological Survey of Sweden, Box 670, 751 28 Uppsala, Sweden
[d] Research and Development, Novia University of Applied Sciences, FI-65200, Vaasa, Finland

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ABSTRACT

Due to land uplift after the last ice age, previously stable Baltic Sea sulfidic sediments are becoming dry land. When these sediments are drained, the sulfide minerals are exposed to air and can release large amounts of metals and acid into the environment. This can cause severe ecological damage such as fish kills in rivers feeding the northern Baltic Sea. In this study, five sites were investigated for the occurrence of acid sulfate soils and their geochemistry and microbiology was identified. The pH and soil chemistry identified three of the areas as having classical acid sulfate soil characteristics and culture independent identification of 16S rRNA genes identified populations related to acidophilic bacteria capable of catalyzing sulfidic mineral dissolution, including species likely adapted to low temperature. These results were compared to an acid sulfate soil area that had been flooded for ten years and showed that the previously oxidized sulfidic materials had an increased pH compared to the unremediated oxidized layers. In addition, the microbiology of the flooded soil had changed such that alkalinity producing ferric and sulfate reducing reactions had likely occurred. This suggested that flooding of acid sulfate soils mitigates their environmental impact.

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1. Introduction

Metal sulfide-rich sediments occur widely in many coastal areas surrounding the Gulf of Bothnia region of the Baltic Sea [1]. Since the last ice age, land uplift has resulted in these sediments rising above the sea level and due to the typically high economic value of coastal areas, being drained for e.g. infrastructure or agricultural uses. This exposes the previously stable and pH-neutral 'potential acid sulfate soil materials' (PASS materials) [2,3] to air, allowing for their oxidation and turning them into acid sulfate soils (ASS) [4]. Oxidation of PASS material results in the release of acidic waters containing high concentrations of metals such as Cd, Ni, Mn, and Al into receiving water bodies [5–7]. This causes severe environmental as well as economic damage and potentially impacts human health. The presence, environmental impact, and remediation of Finnish ASS has been extensively studied [3,6,8–10]. However, only a few studies have been carried out on the presence and effect of ASS on the Swedish side of the Baltic Sea [11–14].

The oxidation of metal sulfides in PASS material (leading to ASS) is similar to the well studied generation of acid mine drainage from sulfide minerals and the commercial process of biomining [15,16]. In these processes, the metal sulfide bond is chemically oxidized by ferric iron to produce soluble metal(s) and depending on the type of mineral, either thiosulfate or elemental sulfur [17]. The mineral dissolution is catalyzed by acidophilic microorganisms (optimal pH for acidophile growth < 5 and extreme acidophiles < 3) that oxidize the ferrous iron to regenerate the chemical oxidant and convert the reduced sulfur to sulfuric acid. The acidophilic microorganisms responsible for catalyzing oxidation of PASS material have been studied in a boreal ASS profile at the Risöfladan experimental area, Vaasa, Finland where a mixture of populations related to either acid and metal contaminated sites or low temperature environments are present [4]. These acidophiles included *Acidithiobacillus ferrivorans* [18] that is a low temperature, ferrous iron and sulfur oxidizing acidophile [19–22]. However, there is a general lack of

* Corresponding author.
E-mail address: stephan.christel@lnu.se (S. Christel).
[1] These authors contributed equally to this work.

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studies addressing the microbiology of PASS material and ASS and in particular, the acidophile community in relation to sulfide oxidation and acid generation in boreal settings is poorly understood.

The oxidation of PASS material to ASS typically results in layers within the soil with acidic, ferric iron bound oxidized materials close to the surface, with an oxidation horizon below, and then unoxidized PASS material in the deepest anoxic layers. Methods to halt oxidation of PASS material and consequently, the release of acidic, metal laden solutions to the environment include the addition of e.g. lime to raise the pH and inhibit the activity of acidophilic microbes [10] or organic compounds to promote sulfate and ferric iron reduction to raise the pH and bind the metals [8] as well as increasing the ground-water table to create an anaerobic environment that halts the oxidation [23]. In an Australian study on flooding coastal ASS, the distribution of sulfate and ferric iron reducing microbes overlapped and were controlled by factors including acidity, redox potential, and mineralization [24]. However, the effect of flooding boreal ASS on the acidophilic microbes present has not been reported.

In this study, five sites in northern Sweden were investigated for the presence of acid sulfate soils and community DNA extracted to identify the microbes present by culture free amplification of the 16S rRNA gene. In addition, the soils below a pond that was created to flood an ASS area was tested to identify how this affected the microbial community. This is the first microbiological study of Swedish ASS and also the first to address changes in a boreal ASS microbial community as a result of flooding.

2. Materials and methods

2.1. Collection of samples and soil classification

Six soils in northern Sweden were selected for the study. The sampling was carried out on 29th and 30th September 2016 using an extendable Edelmann corer. The soil profiles reached from the surface down into the reduced sulfidic (i.e. anoxic) sediment. The latter was identified by its blackish color and/or near neutral pH that was measured in-field every 10 cm with a Hamilton 340i pH/Conductivity Pocket Meter with a flatrode electrode (Supplementary File 1). The definition of PASS material is a field pH $\geq$ 6.0 and a pH $\leq$ 4.0 after a 9-week-incubation in the laboratory, and the soils were classified as an ASS if its pH was either <4.0 or <4.5 if the underlying deposit was classified as PASS material. Ånäset (N 64° 16′ 8.51″, E 21° 2′ 1.43″) and Flarkbäck (N 64° 17′ 5.43″, E 20° 55′ 42.04″) are sites representative of classical boreal ASS formed as a result of farmland drainage. These soils contained blackish sulfidic material at the bottom (PASS material) and had pH values < 4.5 at depths of 50–160 and 60–160 cm, respectively (Supplementary File 1). The Vebomark pond (N 64° 24′ 17.13″, E 21° 1′ 6.57″) and Vebomark site (N 64° 24′ 15.10″, E 21° 1′ 10.10″) are also located in an area with ASS. The pond site represents a soil that has been covered with a 1.2-m-thick soil layer to create an artificial island in the pond leading to the ASS becoming submerged and its profile was blackish at the bottom with a pH < 4.5 at 160 cm. The Vebomark site is located in a field nearby the pond and its deepest parts were not blackish but had an incubation pH of 3.2 and from 70 to 120 cm a pH mainly <4.5 (Supplementary File 1). The fifth site, Bygdeå (N 64° 3′ 27.83″, E 20° 52′ 16.58″), was chosen since the wetland at this site is thought to be underlain with ASS and is planned to be restored by increasing the ground water table. However, although the deepest parts of the soil had an incubation pH well below 4.0, it did not have a pH < 4.5 except in the plough layer and was therefore not classified as an ASS (Supplementary File 1). Finally, Kålboda (N 64° 22′ 57.64″, E 20° 55′ 26.01″) was selected as a reference as it is a farmland site underlain with non-sulfidic fine-grained sediments. This soil had a pH value > 4.5 throughout its depth profile (Supplementary Fig. 1) and was therefore not an ASS.

Single replicate samples for detailed chemical and microbiological analysis were taken from the PASS material as well as from several depths in the ASSs and in the Kålboda and Bygdeå non-ASS profiles (Supplementary Fig. 1). Samples were collected from Ånäset between 50 and 180 cm below the surface (four samples designated as An-50 to An-180); Bygdeå (five samples; By-30 to By-220); Flarkbäck (five samples; Fl-50 to Fl-220); Kålaboda (four samples; Ka-50 to Ka-220); Vebomark (six samples; Ve-50 to Ve-220); and Vebomark Pond (five samples; VePo-130 to VePo-230; the uppermost 1.2 m artificial material was not sampled). The microbiological samples were collected aseptically and stored on ice until returned to the laboratory (within 36 h) where they were frozen until analysis.

2.2. Chemical analysis

In the laboratory, the samples were dried, pulverized, and analyzed for Co, Cr, Ni, Zn, and Mn concentrations by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer) after extraction of 2 g soil material with 10 mL 7M HNO_3 for 40 min at 115 °C. Additionally, total sulfur concentration was measured with inductively coupled plasma optical emission spectometry (ICP-OES). Total carbon (TC) and total organic carbon (TOC) contents were measured using a LECO TruMac at the Department of Soil and Environment, Swedish University of Agricultural Sciences. Total inorganic carbon (TIC) content was calculated as the difference between TC and TOC contents.

2.3. DNA extraction, amplification, sequencing, and analysis

To avoid extraction and amplification of extracellular DNA bound to the soil particles, intact cells were separated from the soil particles according to Alavi et al. [25] and adapted by Högfors-Rönnholm et al. [8,26]. DNA was extracted using the PowerSoil DNA Isolation Kit and the concentrations quantified with a Qubit fluorometer (Invitrogen).

Total community DNA was amplified in a two-step PCR whereby the first amplification was carried out using primers 341F and 805R targeting the V3–V5 region of the 16S rRNA gene [27] and then individual tags attached for Illumina sequencing according to Hugerth et al. [28]. Library preparation and sequencing was carried out at the Science for Life Laboratory, Stockholm, Sweden on the Illumina MiSeq platform to provide 2 × 300 bp pair-end sequences [29]. Bioinformatic analysis was performed using the UPARSE pipeline [30], OTUs annotated against the SINA/SILVA database (SILVA 119; [31]), and data interpreted in R 3.4.2 using the phyloseq package (v1.20) [32]. The presented 16S rRNA data are relative abundances and hence compositional in nature. Therefore, the multivariate structure of the data is analyzed using the compositional version of PCA (CoDaPCA) described in Högfors-Rönnholm et al. [33]. Maximum likelihood phylogenetic trees were constructed in MEGA7 [34]. The nucleic acid data is available in the NCBI database with the BioProject accession number: PRJNA420211.

3. Results and discussion

3.1. Geochemical characteristics

The Ånäset and Flarkbäck profiles were typical ASS developed on sulfidic clay/silt sediments. Both of the profiles contained well-

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developed acidic layers (An-80 and An-120 plus Fl-110 and Fl-140) with low pH (>4) and relatively low total sulfur concentrations, likely caused by sulfide oxidation followed by sulfate leaching [4,35]. Additionally, these two profiles showed typical trace-metal losses (via leaching) in the acidic layers as compared to the PASS material (An-180, Fl-220), in particular for Co, Ni, Zn, and Mn (Table 1). The clay/silt profile from Vebomark pond that had been covered with a 1.2-m-thick soil layer and been entirely under water (below the groundwater table) for ten years was, in terms of sulfur and trace-metal (Co, Ni, Zn, and Mn) patterns, very similar to Ånäset and Flarkbäck (Table 1). However, Vebomark pond had higher pH values (>4.3). This indicated alkalinity-producing reactions, such as reduction of sulfate and ferric iron phases, might have occurred during the ten-year-long waterlogging.

The clay/silt profile at Kålaboda, which was not an ASS, had throughout the depth profile low sulfur concentrations (0.01–0.05%) and relatively high pH (5.2–6.2). The three uppermost samples for detailed analyses (Ka-50, Ka-80, and Ka-110) were however weakly acidic (pH 5.2–5.6) and had, consistent with the ASS, lower trace-metal (Co, Ni, Zn, and Mn) concentrations than the deepest sample (Ka-120) with a pH of 6.2 and slightly elevated sulfur concentrations (Table 1). Therefore, it is possible that some metal-sulfide oxidation and associated sulfur and trace-metal mobilization and leaching had also occurred in this profile. The relatively high pH of this profile was not due to enhanced buffering from carbonate phases, as TIC was very low in this profile (Table 1). The remaining two profiles (Vebomark and Bygdeå) were more difficult to interpret from a geochemical point of view, as they had clay/silt material overlying coarser (sandy) material (Table 1). This was most likely caused by inherent geochemical heterogeneity, as indicated by the distribution pattern of chromium which is an excellent tracer for assessment of geochemical homogeneity of ASS [36]. In the sandy samples (i.e. By-70 and By-100 plus Ve-140, Ve-160, and Ve-190), chromium was <22 ppm and in the clay/silt samples 26–39 ppm (Table 1). As a comparison, chromium was very stable in the other four clay/silt profiles with values between 32 and 38 ppm. The Vebomark and Bygdeå profiles had sulfur concentrations that varied but were overall higher than in Kålaboda.

3.2. Microbiological characterization

Sequencing of the soil microbiome resulted in 4250 to 408,596 merged reads per sample (mean: 96,134; Supplementary Table 1), of which on average 1.86% were filtered as they did not meet quality criteria. Clustering based on 97% sequence identity revealed a total of 6630 operational taxonomic units (OTUs).

Alpha diversity analysis with Shannon's H index on the OTUs did not give any clear patterns between the samples, sample depths, or pH (Supplementary Fig. 2). This suggested that although the populations changed with the different conditions, the extreme acidity in the ASS did not inhibit the growth of a diverse population of microorganisms. At the family level, there was a general increase in the relative proportion of 16S rRNA gene sequences most similar to uncultured clones with increasing depths in the soil profiles (Fig. 1 & Supplementary Fig. 3). Although the bacterial primer set used in this study is also reported to have amplified archaeal sequences [37,38], in contrast to

Table 1
Physical and chemical analyses (single samples were analyzed) of the soil profiles from the six sampling sites.

Sample name	Sample depth (cm)	Soil type	pH (field)	S (%)	TIC (%)	Organic C (%)	Metal concentrations (ppm)				
							Co	Cr	Mn	Ni	Zn
Ånäset (ASS)											
An-50	50	Clay	4.2	0.11			3.9	38.9	158	10.3	34.8
An-80	80	Clay	3.7	0.16	0.1	2.1	4.3	38.5	176	11.5	39.8
An-120	120	Clay	3.6	0.22			3.9	34.8	166	10.2	36.4
An-180	180	Clay	6.6	0.69	0.1	2.3	7.9	33.7	349	19.2	59.1
Flarkbäck (ASS)											
Fl-50	50	Silty clay	4.6	0.11			3.1	31.5	124	8.9	29.7
Fl-80	80	Silty clay	4.4	0.14	0.1	3.4	3.0	31.7	125	8.8	31.0
Fl-110	110	Clay	3.6	0.37			3.2	31.5	142	8.9	29.8
Fl-140	140	Clay	3.9	0.78			6.2	34.1	274	14.8	51.8
Fl-220	220	Clay	6.9	0.73	0.1	1.7	7.6	32.3	452	17.3	57.5
Vebomark Pond (Flooded ASS)											
VePo-130	130	Clay	5.5	0.05			3.4	34.2	138	9.2	36.9
VePo-160	160	Clay	4.4	0.14	0.1	2.0	3.9	35.1	149	9.9	39.9
VePo-190	190	Clay	5.1	0.18			4.2	37.3	169	12.0	48.0
VePo-240	240	Clay	6.0	1.58			8.9	37.5	469	20.9	73.3
VePo-260	260	Clay	6.1	1.56	0.1	2.2	8.7	36.3	435	19.9	70.7
Vebomark (ASS)											
Ve-50	50	Silty clay	4.8	0.01			3.4	32.3	149	9.0	33.7
Ve-80	80	Silty clay	4.5	0.03	0.1	0.8	4.2	39.3	186	10.6	41.0
Ve-110	110	Clayey silt	4.3	0.12			2.7	30.7	122	7.4	28.4
Ve-140	140	Sandy silt	4.9	0.13			5.0	18.2	86	13.4	26.3
Ve-160	160	Sandy silt	5.0	0.12			4.3	21.7	91	13.8	33.1
Ve-190	190	Sandy silt	5.8	0.07	0.0	0.1	2.8	14.1	70	8.9	24.8
Bygdeå (Non-ASS)											
By-30	30	Silty clay	4.7	0.16			3.5	33.7	115	9.9	32.7
By-45	45	Silty clay	4.9	0.07			3.0	26.0	104	8.0	27.8
By-70	70	Silty sand	5.1	0.02	0.0	0.2	1.3	9.9	53	3.5	11.7
By-100	100	Silty sand	6.6	0.41			4.3	12.6	84	7.0	21.0
By-120	120	Silty clay	7.5	0.30	0.0	0.2	11.3	38.8	390	23.3	80.7
Kålaboda (Non-ASS)											
Ka-50	50	Clay	5.4	0.02			4.4	35.3	202	10.3	39.3
Ka-80	80	Clay	5.1	0.02	0.1	0.5	5.2	37.0	231	12.7	48.8
Ka-110	110	Clay	5.6	0.01			6.4	38.0	258	15.2	56.8
Ka-120	120	Clay	6.2	0.05	0.0	0.3	13.9	36.1	388	25.9	92.8

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Fig. 1. Stack bar graph of the family level (except where the hits are to uncultured 16S rRNA genes) of the relative abundance of the 16S rRNA gene sequencing (single community DNA extractions were performed). Only families with >1% relative abundance are included.

an Australian coastal ASS study [24], no archaea were identified in the dominant 16S rRNA gene sequences. This may be due to the typically increased incidence of archaea at the higher temperatures encountered in Australia compared to the boreal soils studied here. There were also large differences between the microbial community on the western Baltic Sea coast in this study compared to the eastern coastline [4]. For instance, the acidophilic ferrous iron and/or inorganic sulfur compound oxidizing *Acidithiobacillus* genus was lacking in this study but was identified in two reports on ASS on the eastern (Finnish) coastline [4,10].

Few of the most abundant microbial families were consistently present in both the low pH Ånäset (An-80 and An-120) as well as the Flarkbäck (Fl-110 and Fl-140) communities (Fig. 2). This was confirmed by compositional principle coordinate analyses of the microbial populations (Fig. 3), also in reference to either pH or soil depth (data not shown). While 43.8% and 18.7% of the observed diversity could be explained by the first and second principal component, the analysis confirmed the weak influence of most measured chemical parameters on the microbial community, with exception of the concentration of sulfur in the soil. This parameter positively correlated with the occurrence of predicted heterotrophic acidophilic organisms of the Acidimicrobiacaea and Acidimicrobiales. The closest correlation however was observed to unassigned members of the Proteobacteria (Fig. 3), which could indicate their placement in sulfur-oxidizing acidophilic clades. Nevertheless, the generally low predictive strength of the measured chemical parameters strongly suggests that low pH and high metal mobility were not the dominant factors that selected for the microbial communities. This is in contrast to findings in ASS on the eastern (Finnish) coastline [33]. Other factors that could play more important roles include availability of organic carbon and nutrients, the age of the ASS and consequently, the degree of sulfidic material oxidation.

As the Ånäset and Flarkbäck sites exhibited typical ASS soil profiles, these soils will be focused on in the microbiological analysis (Fig. 2 & Supplementary Fig. 4). The most dominant 16S rRNA gene sequences in the pH < 4.0 Ånäset samples were most similar to the uncultured clone ODP1230B8.23 [39]. These OTUs included e.g. OTU_000013, _005741, and _000016 (Fig. 2 & Supplementary Fig. 4) that also aligned to uncultured Halanaerobiales spp. that were identified from the extremely acidic Rio Tinto river [40] and acid mine drainage in Svalbard [41]. This suggested they were adapted to the acidic pH and metal rich conditions as well as to the low temperatures occurring in northern Sweden. A further family present in the An-80 and -120 samples was the Acidimicrobiaceae that included OTU_002215, _001883, and _000054 (totaling 13.9, 5.6, and 25.1% of the relative populations, respectively). These OTUs were most similar to an Acidimicrobiales sp. identified from an acid mine drainage at temperatures down to 9 °C [42]. A third family from the Ånäset site at 50 and 80 cm soil

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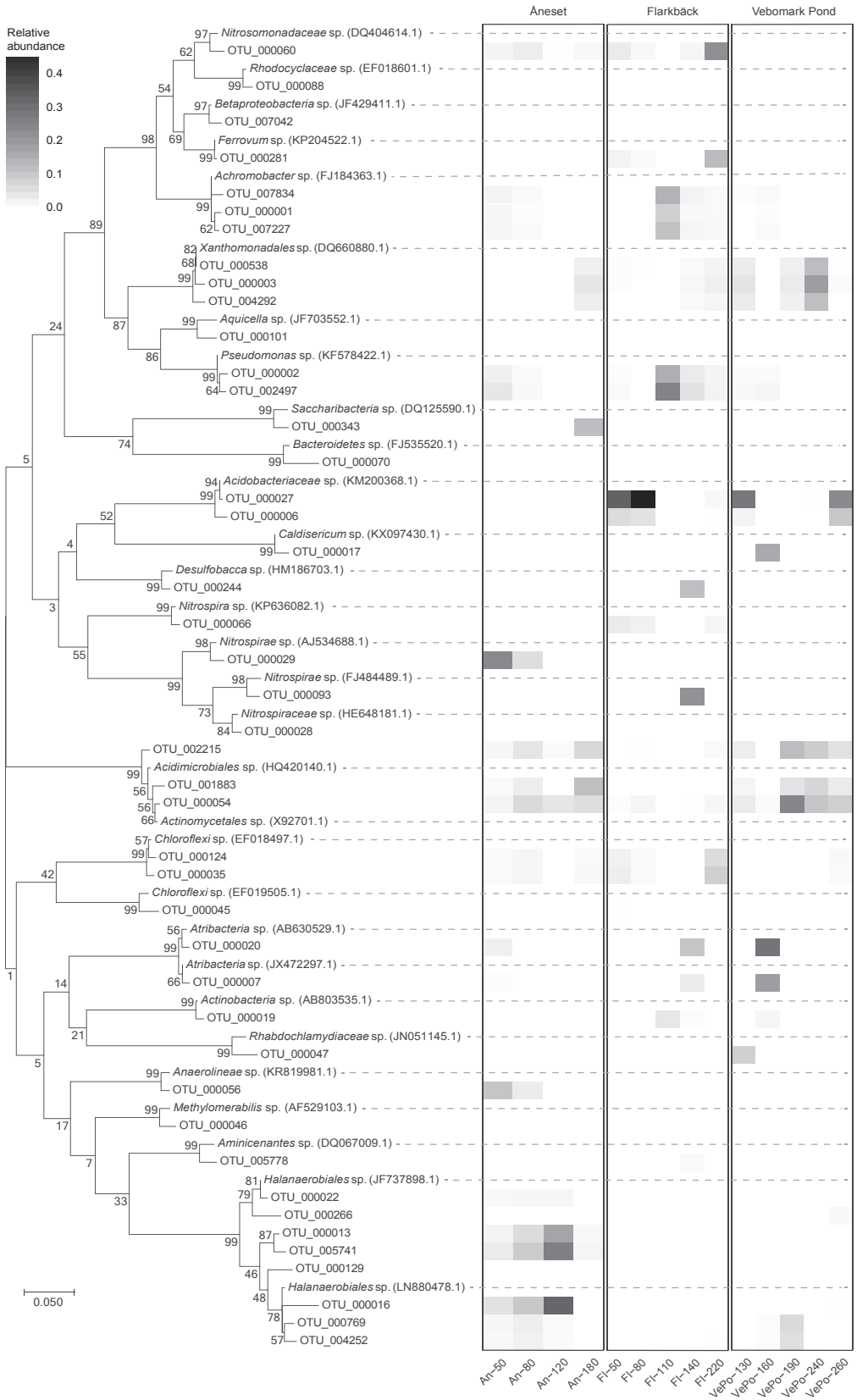

Fig. 2. Phylogenetic tree and heatmap showing the top OTUs (i.e. ≥ 10% relative abundance in at least one of the samples) derived from the 16S rRNA gene analysis from the Åneset (ASS), Flarkmark (ASS), and Vebomark Pond (Flooded ASS) samples. Reference sequences downloaded from NCBI Genebank have a dashed line in the heatmap. The scale bar represents nucleotide substitutions per site. The evolutionary history was inferred by using the Maximum Likelihood method and the highest log likelihood tree (−7832.1568) is shown that utilized a total of 427 positions in the final dataset. Bootstrap values are shown next to the branches.

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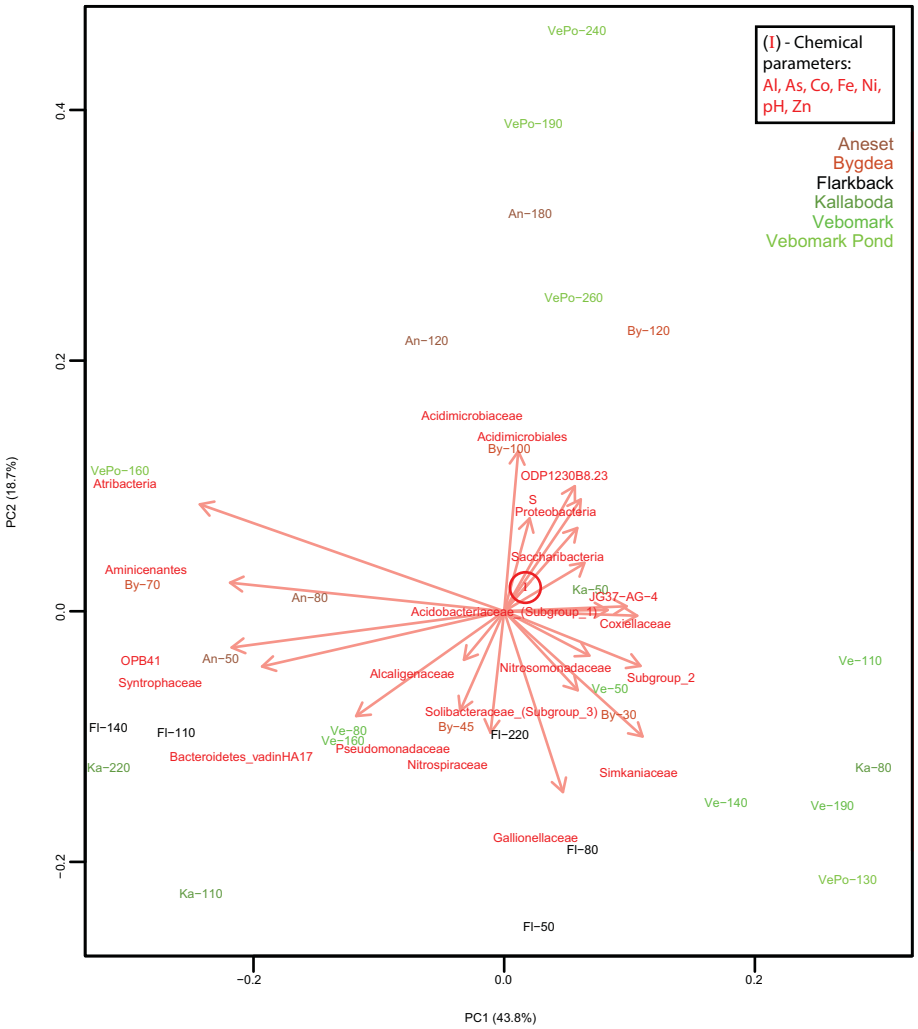

Fig. 3. Compositional principle component analyses of the 16S rRNA gene amplicon data and chemical analyses for the Ånäset, Flarkbäck, and Vebomark ASS; the Vebomark Pond flooded ASS; and Bygdeå and Kålaboda non-ASS. Sample abbreviations are as given in Table. For clarity, the location of a tight cluster of chemical parameters is indicated by (I).

depths was the Nitrospiraceae (e.g. OTU_000029 constituting 24.1 and 5.7%, of the respective relative communities). Members of this family are ubiquitous in oxic and anoxic soil environments and fulfill a wide range of metabolic functions [43]. The Nitrospiraceae include the acidophilic genus *Leptospirillum* [44] that is more commonly found at pH values of <2.0. The possible presence of Leptospirilli raises in question the existence of micro-niches of extreme acidity within the soil. Samples An-80 and An-120 also contain 16S rRNA gene sequences (e.g. OTU_001186, _000232, and _000051) that align with the ferrous iron oxidizing Gallionellaceae [45] that were also identified in Finnish ASS [4]. Finally, the Ånäset pH < 4.0 soils contained sequences that aligned with the Acetobacteraceae [46] that include several acidophilic phyla such as the *Acidiphilium* and the moderately acidophilic, low temperature genus *Acidisoma*.

The Flarkbäck soils at 110 and 140 cm (pH < 3.9) contained sequences from the Pseudomonadaceae [47] including OTU_000002 and _002497 that totaled 40.8 and 8.5% of the relative population in Fl-110 and -140 communities, respectively. These OTUs aligned with a *Pseudomonas* sp. isolated from a soil at 10 °C suggesting they were adapted to the low temperature. A second abundant family in Fl-110 and -140 was the Alcaligenaceae (e.g. OTU_007834, _00001, and _007227) that are found in a variety of environments [48]. ASS with a pH < 4.0 from Flarkbäck also had 16S rRNA genes most similar to Ferrovaceae that are low temperature ferrous iron and sulfur compound oxidizing acidophiles [49] as well as the ferrous iron oxidizing Gallionellaceae [45]. The Fl-140 soil also had a large relative abundance of OTUs most similar to bacteria within the Nitrospiraceae (e.g. OTU_000093; 22.1% of the relative abundance); the Syntrophaceae including OTU_000244 most similar to the sulfate reducing genus *Desulfobacca* with 12.0% of the relative population [50]; and the candidate phylum Atribacteria (e.g. OTU_000020; 10.7% of the relative population) identified from an Antarctic lake [51] suggesting it was adapted to the low temperature. The deepest Flarkbäck sample (Fl-220) had very different geochemical characteristics such as a higher pH and Co, Ni, Zn, and Mn concentrations typical for PASS material. However, the relative proportions of OTUs aligning with ferrous iron and sulfur compound oxidizing Nitrosomonadaceae (OTU_000060; 9.5%) and Ferrovaceae (OTU_000281; 5.4%) raises the possibility that this soil depth was in the process of being oxidized into an ASS.

In contrast to the ASS profiles for the Ånäset and Flarkbäck sites, the other soils had typical geochemical characteristics for boreal zones. These samples had 16S rRNA genes assigned to the obligately

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intracellular Simkaniaceae found in eukaryotes [52] that may reflect a higher proportion of eukaryotes in the pH neutral soil. A second family present in several of the non-ASS soils was the Alcaligenaceae that are found in a range of environments. Finally, the non-ASS soil profiles had a comparably low proportion of uncultured clones without a phylogenetic assignment that highlights the need to carry out further work to identify community members associated with ASS.

3.3. Comparison with the microbial community in the flooded ASS

The trend of increasing 16S rRNA sequences most similar to uncultured clones was particularly strong in the Vebomark Pond samples (Fig. 1 & Supplementary Fig. 3), potentially due to the deeper samples taken at this site. The VePo-160 sample had a high relative proportion of 16S rRNA sequences that aligned with the Caldisericaceae that in the literature is represented by a single species that grows chemoheterotrophically via anaerobic reduction of sulfur compounds [53] that is consistent with the geochemical data indicating alkalinity-producing reduction reactions had occurred [54]. The dominant families in the deeper Vebomark pond soils aligned within the Acidimicrobiaceae such as OTU_002215, _001883, and _000054 that together made up 42.0% of the relative population in VePo-190 and were also present at lower proportions in VePo-240 and -290. These OTUs were also identified in the pH < 4.0 Ånäset soils and were most similar to uncultured clones identified from acid mine drainage. However, in the waterlogged and likely reduced conditions in the Vebomark Pond samples, the Acidimicrobiales related OTUs potentially grew via anaerobic ferric iron reduction [55] and could thereby have elevated the pH of the former ASS. Within the uncultured portion of the community, the VePo-240 soil contained OTUs (e.g. OTU_000538, _000003, and _004292) that aligned with a Xanthomonadales sp. identified from acid mine drainage. 16S rRNA gene sequences similar to Xanthomonadaceae identified from acid mine drainage were also enriched from ASS on the Finnish side of the Baltic Sea [4]. Finally, the Vebomark pond soils contained 16S rRNA gene sequences most similar to an Acidobacteriaceae sp. (such as OTU_000027 and _000006) that was in turn most similar to species typically found in pH neutral environments. The high relative proportion of acidophiles in the Vebomark pond soils supports the geochemical analyses showing that this soil was once oxidized. However, it should be noted that the pH in these soils were higher than the typical ASS at Ånäset and Flarkbäck and the increased pH may have inhibited the acidophiles from catalyzing further acid and metal release. Additional studies will need to be carried out to identify if the metal sulfides continue to oxidize and the acidophilic microbes are active at this remediated site.

4. Conclusion

Despite environmental concerns related to the release of metals and acid, the occurrence of acid sulfate soils has scientifically been largely neglected in boreal environments. The results of this study showed that Swedish acid sulfate soils are being oxidized, that this oxidation is likely catalyzed by acidophilic microorganisms, and that creating an anoxic environment by flooding an acid sulfate soil has begun to reverse the process of acid generation. However, in contrast to studies on the eastern Baltic coast, principal coordinate analysis does not indicate low pH and high metal concentrations to be the dominant factors shaping the microbial community. These data can be used to identify areas of acid sulfate soils and to help design strategies to mitigate their environmental impact in the northern Baltic area.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resmic.2019.06.002.

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