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Dredging and deposition of metal sulfide rich river sediments results in rapid conversion to acid sulfate soil materials

Anders Johnson [a,*], Eva Högfors-Rönnholm [b], Sten Engblom [b], Peter Österholm [c], Mats Åström [d], Mark Dopson [a]

[a] *Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, SE-39231 Kalmar, Sweden*
[b] *Research and Development, Novia University of Applied Sciences, FI-65200 Vaasa, Finland*
[c] *Department of Geology and Mineralogy, Åbo Akademi University, FI-20500 Turku, Finland*
[d] *Biology and Environmental Sciences, Linnaeus University, SE-39231 Kalmar, Sweden*

HIGHLIGHTS

- Baltic Sea coastal sediments are rich in metal sulfides.
- Dredging of sediments exposes metal sulfides to oxygen.
- Oxidation of metal sulfide rich estuary sediments releases metals and acid.
- Metal sulfide oxidation coincides with increase of extremely acidophilic bacteria.
- Liming poorly neutralized acid generated from oxidized dredge sediments.

GRAPHICAL ABSTRACT

Dredging and Deposition of Metal Sulfide Rich River Sediments Results in Rapid Conversion to Acid Sulfate Soil Materials

Investigate geochemistry & microbiology of oxidation to acid sulfate soils

- Baltic Sea coastal sediments are rich in metal sulfides
- Dredging of sediments exposes metal sulfides to oxygen
- Addition of lime buffers the dredge spoils

- Oxidation of metal sulfide rich sediments releases metals and acid
- Rapid oxidation catalyzed by extreme acidophiles
- If uncontrolled, deposition of sediments can cause severe environmental damage

Authors: Anders Johnson, Eva Högfors-Rönnholm, Sten Engblom, Peter Österholm, Mats Åström, & Mark Dopson

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ABSTRACT

Sediments along the Baltic Sea coast can contain considerable amounts of metal sulfides that if dredged and the spoils deposited such that they are exposed to air, can release high concentrations of acid and toxic metals into recipient water bodies. Two river estuaries in western Finland were dredged from 2013 to 2018 and the dredge spoils were deposited on land previously covered with agricultural limestone to buffer the pH and mitigate acid and metal release. In this study, the geochemistry and 16S rRNA gene amplicon based bacterial communities were investigated over time to explore whether the application of lime prevented a conversion of the dredge spoils into acid producing and metal releasing soil. The pH of the dredge spoils decreased with time indicating metal sulfide oxidation and resulted in elevated sulfate concentrations along with a concomitant release of metals. However, calculations indicated only approximately 5% of the added lime had been dissolved. The bacterial communities decreased in diversity with the lowering of the pH as taxa most similar to extremely acidophilic sulfur, and in some cases iron, oxidizing *Acidithiobacillus* species became the dominant characterized genus in the deposited dredge spoils as the oxidation front moved deeper. In addition, other taxa characterized as involved in oxidation of iron or sulfur were identified including *Gallionella*, *Sulfuricurvum*, and *Sulfurimonas*. These data suggest there was a rapid conversion of the dredge spoils to severely acidic soil similar to actual acid sulfate soil and that the lime placed on the land prior to deposition of the spoils, and later ploughed into the dry dredge spoils, was insufficient to halt this process. Hence, future dredging and deposition of dredge spoils containing metal sulfides should not only take into account the amount of lime used for buffering but also its grain size and mixing into the soil.

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1. Introduction

Soils are a 'critical' surface layer used by humans for services including food production, extraction of drinking water, and building of infrastructure. Coastal regions (along with some inland areas) in e.g. Australia, North and South America, Europe, Asia, and Africa encompass more than 17 million hectares of potential acid sulfate soil (PASS) materials containing metal sulfide rich deposits (Michael, 2013; Rickard and Luther, 2007). Increasing pressure on coastal areas for urban, infrastructure, and agricultural development coupled with natural isostatic Scandinavian land uplift results in drainage of the PASS materials that exposes the metal sulfides such as pyrite (FeS_2) to oxygen. Abiotic reactions then occur that convert the PASS materials to actual acid sulfate soils (AS soils) with pH values typically between 2.5 and 4.0 (Boman et al., 2010; Yli-Halla et al., 2006). Actual AS soils are described as the "nastiest soils in the world" (Dent and Pons, 1995) as they release metal laden (e.g. Ni, Cd, Al, and Zn) acidic solutions that can harm the environment such as by large scale fish kills, construction due to concrete and steel corrosion, and human health (Cook et al., 2000; Fältmarsch et al., 2008). Consequently, proper management of PASS and the mitigation of metal and acid release from actual AS soil is crucial.

Oxidation of the metal sulfide bond in PASS materials by ferric iron, and to a lesser extent oxygen, results in the generation of ferrous iron and reduced inorganic sulfur compounds (RISCs) in a similar manner as the generation of acid mine drainage and the biotechnological process of 'biomining' (Schippers and Sand, 1999; Vera et al., 2013). The abiotic oxidation is catalyzed by regeneration of the primary oxidant, ferric iron, and acidophilic microorganisms generate acidity by oxidation of RISCs to sulfuric acid (Bonnefoy and Holmes, 2012; Dopson and Johnson, 2012). These microorganisms have an optimum pH below 5 (extreme acidophiles pH <3) and survive in the acidic (Slonczewski et al., 2009) and metal laden (Dopson et al., 2014) conditions by a combination of biotic systems and abiotic complexation of the free metal by sulfate ions. Extreme acidophiles such as *Acidocella* and *Acidithiobacillus* spp. are identified when PASS material is rapidly oxygenated in laboratory reactors (Wu et al., 2013), *Acidithiobacillus* spp. were identified from actual AS soils (Arkesteyn, 1980), and *Acidithiobacillus* genus cell numbers correlated to metal and acid discharge from Finnish AS soils (Niemela and Tuovinen, 1972). In contrast, the microbial communities in older and more established actual AS soils tend to be dominated by iron and RISC oxidizing moderate acidophiles (Christel et al., 2019; Wu et al., 2013). Although ferrous iron and RISC oxidizing microbial species are present, little detailed microbial characterization has been performed during the transition of PASS dredge spoils into actual AS soils.

Boreal actual AS soil has been intensely investigated including at the Risöfladan experimental field, Vaasa, Finland (Engblom et al., 2014; Österholm et al., 2014; Wu et al., 2015; Wu et al., 2013). Here the old sediments have developed into a typical actual AS soil during 60 years and mitigation techniques for reducing the environmental impact of acidic and metal-rich drainage waters have been studied (e.g. (Dalhem et al., 2019; Högfors-Rönnholm et al., 2018a; Högfors-Rönnholm et al., 2020)). The Laihianjoki (Toby å) and Sulvanjoki (Solf å) Rivers drain large geographical areas into an estuary close to the Risöfladan experimental field on the Finnish west coast. The catchment areas of those rivers contain large areas of actual AS soils and leached metals often precipitate close to the river mouths (Nordmyr et al., 2008) where reducing conditions are favorable for sulfide formation. Dredging of these river mouths and drying of the spoils on land results in their oxidation with the concomitant leaching of large amounts of metal-rich acidic waters into the surroundings (Nordmyr et al., 2008). To mitigate this release of toxic metals and acid, the material is limed as it is deposited and the drainage water is led through a lime barrier before being released into the bay. Despite the attempts to mitigate metal and acid release, the eventual rate and total release of toxic drainage waters from dredge spoils is unknown.

In this study, a new river dredging campaign was investigated with the aim to elucidate the spatial and temporal geochemical processes and bacterial community diversity and composition occurring in the lime-treated sulfidic dredged materials and the potentially large environmental impacts of these dredge spoil systems (Ohimain et al., 2004). The study contributes to an improved understanding of the environmental consequences of depositing PASS materials, the effect of lime applications, the early development of actual AS soils, and ultimately provides valuable information on the safe use of river dredge spoils for agriculture.

2. Materials and methods

2.1. Study site

The study site was located south of Laihianjoki River estuary, approximately 10 km south of Vaasa in Western Finland where an area of approximately 20 ha had been prepared for the dredge spoils (Fig. 1; longitude and latitude found in Supplemental File S3). The prepared area was part of an embankment that had previously been drained (between 1940 and 1970) and used as a commercial birch forest. Prior to the deposition of the dredge spoils, the forest was logged and stumps and other tree remnants were removed from the area. Dredging of the Laihianjoki and Sulvanjoki River estuaries was initiated in the winter of 2013–2014 and was executed by excavating the sediment from the ice-covered estuaries. Before depositing the dredge spoils, the land area was covered with 240 tons of agricultural limestone ($CaCO_3$, particle diameter < 3.150 mm) spread over the 20 ha area. Dredged sediment was surface drained and allowed to dry until the following summer when approximately 10 kg agricultural limestone (liming rates were consistent with current Finnish recommendations) per m^3 of dredged sediment was ploughed into the dry dredge spoil. The amount of lime needed for neutralizing the acidity from the oxidized sulfidic sediment had been calculated based on laboratory analyses of the sediment. The dredging was completed in the winter of 2018, when a total of 115,000 m^3 of sediment had been removed from the estuaries (Environmental Permit Authorities of Western Finland, permit decision 97/2008/3; information from the Centre for Economic Development, Transport and the Environment in Southern Ostrobothnia).

In this study, only a limited part of the total area was used for sample collection (Fig. 1C) and the sediments in this restricted area had been dredged during the winter of 2015–2016 from Laihianjoki River estuary. These sediments were therefore recently deposited prior to sampling and were also representative of the reference samples described below.

2.2. Sample collection

Sampling from the dredge spoils was performed on five occasions: November 2016, May, August, and October 2017 plus May 2018. At every sampling time, three replicate sites (Fig. 1C) were used where an approximately 30 × 50 cm wide and 70 cm deep hole was excavated with a shovel. The dredge spoil layer was approximately 50 cm and underneath was the old surface layer, a forest floor with lots of roots and plant parts. Samples were aseptically taken by pushing sterile 50 mL trimmed syringes into the soil at 10, 20, 30, and 60 cm below the surface (giving $n = 3$ per sampling depth). Due to ground frost, the 10 cm samples could not be taken during the November 2016 sampling. The syringes were immediately covered with parafilm, stored at 4 °C in the dark, and transported to the laboratory. From each sampling depth, parallel syringes were taken for bacterial and geochemical analyses.

As reference, intact sediment was sampled from the Laihianjoki River estuary in April 2018 (three sampling sites each with two replicates giving $n = 6$; Fig. 1C). As the river was still covered with ice, three holes were generated with an ice drill and 2 m long polyethylene sampling tubes were pressed down into the sediment. The tube openings were covered with an airtight cap, lifted onto the ice, and sealed at the bottom before transporting them to the laboratory. Each replicate sampling tube was cut at four different sediment depths, and since the dredge spoils were mixed sediments from different depths, the four aseptically taken sediment samples per replicate were mixed before filling two 50 mL tubes for bacterial analyses and
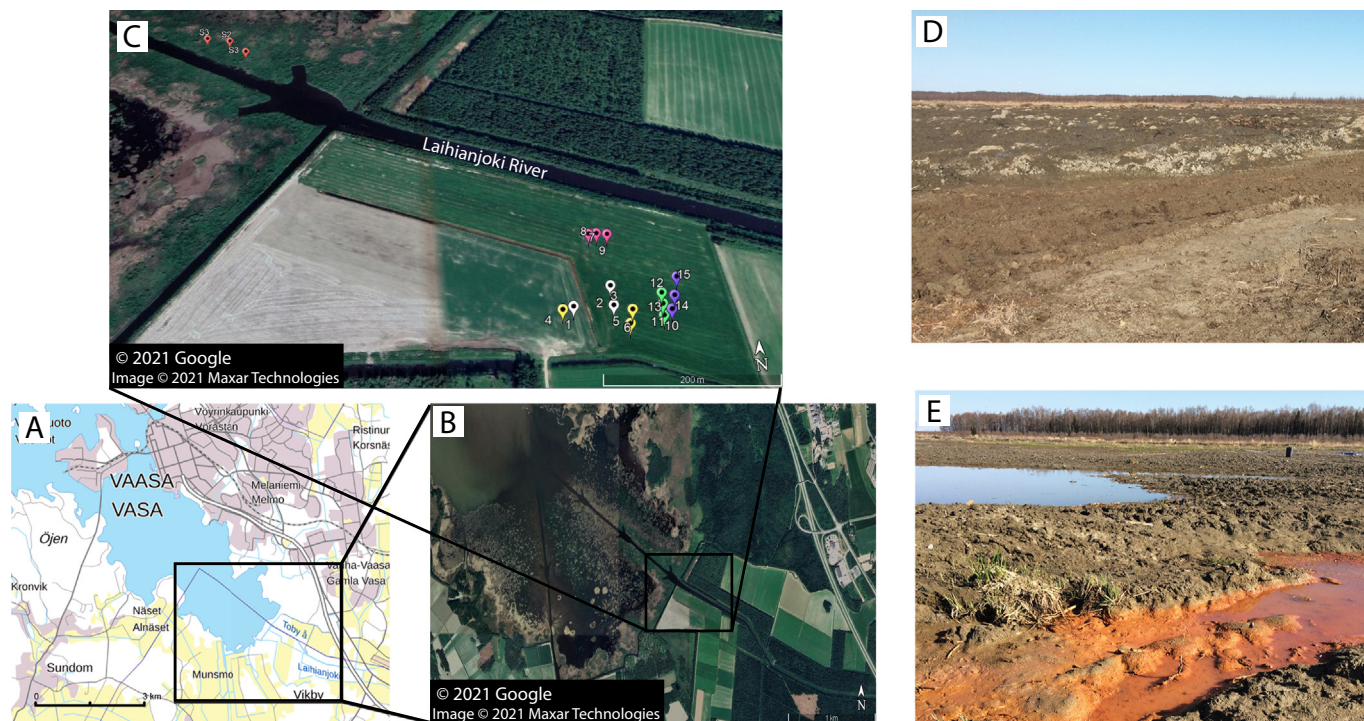
Fig. 1. Map of the dredge spoil study site located south of Laihianjoki River estuary (Sulvanjoki river not shown) near Vaasa, Western Finland (A) with the lower orthophoto showing a zoomed in section of the map (B) and the upper orthophoto shows the dredge spoil sampling sites (C). Sites 1–3 (white dots) were sampled in November 2016, sites 4–6 (yellow dots) in May 2017, sites 7–9 (pink dots) in August 2017, sites 10–12 (green dots) in October 2017, sites 13–15 (purple dots) in May 2018, and reference sediment samples (S1-S3, red dots) in April 2018. The two photographs (both credits: E. Högfors-Rönnholm) show the sampling site in May 2017 (D) and May 2018 (E). The figure contains data from the National Land Survey of Finland Topographic Database (06/2021) and NLS orthophotos (03/2019).

two 50 mL tubes of 15 g for geochemical analyses with mixed sediment and handled as described below.

2.3. Geochemical analyses

An equal amount (15 g) of MilliQ-ultrapure water was added to the dredge spoil and reference sediment sample tubes. The heterogeneous dredge spoil-water mixture was slowly stirred with a spatula for 1 min in order to establish a pH value by the interaction of the exposed dredge spoil surfaces and the added water. Complete homogenization was carefully avoided, and only very loose soil aggregates disintegrated in this mixing step. For pH measurements, a FlaTrode electrode (Hamilton) with the Everef reference, the Polisolve electrolyte, and a ring diaphragm junction was used. After pH measurements, all samples were stored frozen at -20 °C until further geochemical analyses were performed under oxygen free conditions. Total reducible sulfur (TRS) was determined in the dredge spoil samples using a simplified distillation method for sulfur speciation (Dalhem et al., 2021). Briefly, a 10 mL deoxygenated mixture of 6 M HCl and 2 mL of 1 M ascorbic acid followed by 12 mL of an acidic Cr(II) solution were injected into a 50 mL reaction vessel with circa 300 mg sample and heated to 60 °C under magnetic stirring for 4 h. Solid sulfide (pyrite and metastable iron sulfides such as mackinawite and greigite) and elemental sulfur (assumed to occur in low amounts) transformed into hydrogen sulfide gas (H_2S) were carried by a steady flow of N_2-gas to a 50 mL polypropylene tube filled with an alkaline Zn trapping solution and precipitated as Zn-sulfide. The TRS was then determined by iodometric titration of the Zn-sulfide precipitate. The water extractable concentration of ferrous iron and chemical elements were analyzed on dredge spoil-water extracts, as previously described (Högfors-Rönnholm et al., 2018a). Briefly, after agitating the dredge spoil with deoxygenated MilliQ-ultrapure water (dredge spoil-water wet weight ratio of 1:2.5) for 1 h on a Vortex mixer, the suspension was centrifuged (2000g, 10 min), the supernatant filtered (0.2 μm), and

the final extract divided into aliquots. Ferrous iron was determined using a 1,10-phenanthroline method with the absorbance measured with a Hach 2010 spectrophotometer. The water extractable concentrations of S, Ca, Fe, and Mn were determined with inductively coupled plasma-optical emission spectroscopy (ICP-OES) and Mg, Sr Al, Co, Ni, Zn, Cu, As, and Cr with inductively coupled plasma-mass spectrometry (ICP-MS) at Activation Laboratories LTD, Canada. The obtained results were normalized against dredge spoil dry weight. Finally, excess dredge spoil in syringes and homogenized sediment samples were used for estimation of dry weight (SFS-EN 12880)) and loss on ignition (LOI) of dry mass (SFS EN 12879).

2.4. 16S rRNA gene amplicon sequencing analysis of bacterial communities

All dredge spoil samples and reference sediment samples (hereafter called soil) were stored at 4 °C until DNA extraction was performed within 48 h after sampling. DNA from intact bacterial cells was extracted from the soil using an indirect DNA extraction method, developed for acidic soil with high clay content, that excludes extraction of extracellular DNA (Högfors-Rönnholm et al., 2018b). Briefly, bacterial cells were released from the soil by suspending and stirring the soil in a sodium phosphate buffer (500 mM Na_2HPO_4, 500 mM NaH_2PO_4, 2% NaCl, pH 7.2) three times. Between each suspension step, the stirred suspension was centrifuged (500g, 15 min) to remove soil particles from the bacterial cell-containing supernatant. The bacterial cells were finally harvested from the pooled supernatants by centrifugation (10,000g, 15 min) and DNA was extracted from the bacterial cell pellets using the DNeasy PowerSoil Kit (QIAGEN) according to the manufacturer's instructions. The final DNA concentrations were measured with a Qubit fluorimeter (Invitrogen).

A two-step PCR procedure was used in order to amplify the total community DNA in the samples as previously described (Högfors-Rönnholm et al., 2020). Briefly, in the first amplification step, primers 341F and 805R were used to target the V3-V4 region of the 16S rRNA gene

(Herlemann et al., 2011). In the second amplification step, individual tags for Illumina sequencing were attached to the amplicons (Hugerth et al., 2014). The final amplicons were sequenced (2 × 300 bp pair end reads) at the Science for Life Laboratory, Sweden on the Illumina MiSeq platform (Högfors-Rönnholm et al., 2020).

2.5. Bioinformatic and statistical analyses

Sequencing analysis computations were provided by the Swedish National Infrastructure for Computing (SNIC) at UPPMAX by employing the Ampliseq pipeline (v1.2; (Straub et al., 2020)) that processes Illumina reads with QIIME2 (Bolyen et al., 2019) and infers amplicon sequencing variants (ASVs) with DADA2 (Callahan et al., 2016) as previously described (Bellenberg et al., 2021). After initial filtering and trimming (280 and 220 bp sequence length for forward and reverse reads, respectively; 21 bp trimmed for primers), residual primer sequences were removed with cutadapt (v2.8, (Martin, 2011). On average, 24.3% of raw reads were filtered out. The generated ASVs were annotated against the SILVA database (v138.1, (Quast et al., 2013)) and 22,298 unique ASVs were generated. The resultant data were analyzed in R (v 4.0.2; (Team, 2020)) and RStudio (Team, 2019) using the Tidyverse (v1.31, (Wickham et al., 2019)), vegan (v2.5-7, (Oksanen et al., 2020)), and asbio (v1.6-7, (Aho, 2020)) packages following previously described methods (Bellenberg et al., 2021). The full reproducible R code is available in Supplemental File S1. As described in Högfors-Rönnholm et al. (2018a), due to the compositional nature of the relative abundance of the 16S rRNA gene sequences, single variables were not free to vary separately from the rest of the total composition, regardless of whether or not all bacterial species are analyzed. Therefore, the raw data were transformed prior to analysis of variance (Aitchison, 1986).

2.6. Data availability

16S rRNA gene amplicon nucleic acid sequences are available with the National Center for Biotechnology Information (NCBI) under the BioProject accession number: PRJNA763750.

3. Results and discussion

3.1. Geochemistry

The elemental concentrations presented below (Table 1 and Fig. 2) represent the water-extractable fraction, except for TRS and pH that were measured on solid-phase materials. The water extractable fraction is dynamic as oxidation and weathering release elements into this fraction while subsequent leaching and solid-phase scavenging removes elements from it. High or increased concentrations are thus an indication of domination of processes that release over those that remove an element from water-soluble, i.e. easily leachable, form. Each value (concentration) for the dredge spoil is a mean of triplicate measurements.

3.1.1. Sulfur and pH

pH was near neutral (6.8–7.1) in the six reference sediment samples, the TRS concentrations in these were 0.50 to 0.65%, and the water extractable S concentrations (hereafter called S) in soil-water extracts were below the detection limit indicating that no significant oxidation of sulfide to sulfate had occurred in this water-saturated reduced sediment. In the dredge spoil (10–30 cm sampling depths), there was a consistent distribution of pH, TRS, and S values (Fig. 2 & Supplemental File S2). In the first two sampling events the mean pH values ranged from 4.7–6.7 (all $n = 3$), the TRS concentrations were 0.32–0.65%, and the S concentrations were 0.05–0.19%. In contrast, the following three sampling events showed pH values from 3.0–3.7, decreased TRS concentrations (0.06–0.17%, Student's t-test, $p \leq 0.01$), and strongly elevated S concentrations of 0.17–0.55% ($p \leq 0.01$). However, there was considerable variability among the replicates likely due to heterogeneous metal sulfide oxidation rates due to for

Table 1

Additional chemical parameters (i.e., not shown in Fig. 2) presented as means ($n = 3$) from the dredge spoil sampling at different depths and times. Full data are presented in Supplemental File S2.

	Depth	Mg	Co	Zn	Cu	As	Fe	LOI[a]
	cm	mg/kg			%			
Nov 2016	10	Ns[b]	ns	ns	ns	ns	ns	ns
	20	132	0.2	1.9	0.2	0.1	155	12
	30	71	0.01	4.1	0.2	0.1	75	14
	60	89	0.2	2.0	0.2	0.1	236	6.9
May 2017	10	299	1.4	10	0.3	0.1	9.2	8.0
	20	284	1.3	7.3	0.4	0.04	9.5	11
	30	105	0.4	1.7	0.2	0.1	72	5.8
	60	50	0.1	2.1	0.3	0.1	413	16
Aug 2017	10	785	5.7	23	2.2	0.4	144	8.4
	20	722	3.2	13	1.0	0.2	67	10
	30	598	2.8	11	0.5	0.1	92	12
	60	29	0.1	1.9	0.3	0.04	456	9.1
Oct 2017	10	506	1.7	8.6	0.8	0.2	54	7
	20	667	3.2	21	1.0	0.3	45	8.1
	30	576	3.5	15	0.5	0.2	6	9.7
	60	208	0.6	4.4	0.3	0.1	165	8.2
May 2018	10	259	1.2	11	0.7	0.1	7.6	8.3
	20	390	1.5	53	1.3	0.2	98	5.9
	30	630	2.7	11	1.5	0.2	75	7.6
	60	397	0.8	4.4	0.3	0.04	6.5	7.6
Reference	10–60	47	0.01	6.4	0.3	0.1	41	9.8

[a] LOI, loss on ignition of dry mass.
[b] ns = not sampled due to frozen ground.

example, cracks forming in the dredge spoils allowing ingress of air. For example, at 20 cm depth in November 2016 the TRS concentrations varied between 0.34 and 1.1% and pH varied between 4.1 and 6.5 whereas in May 2017 the values were between 0.05 and 0.82% and 3.8 to 6.5, respectively. These data revealed several features and mechanisms: (i) the TRS concentrations were higher in part of the dredge spoil than in the reference sediment, (ii) oxidation of sulfides had occurred to some extent by the second sampling event (approximately 1.5 months after deposition) causing a decline in pH and elevation in S concentrations (certainly as sulfate) in part of the dredge spoil, (iii) from summer 2017 (three months after the second sampling event), sulfide oxidation had become extensive causing the pH to strongly drop and S concentrations to strongly increase, and (iv) the increase seen in S concentrations over time in the soil substrate at 60 cm depth was explained by accumulation of sulfate leached from the overlying dredge spoil. The data support that the applied lime failed to prevent rapid development of actual AS soils. This has also been observed elsewhere for similar dredged materials (Borma et al., 2003).

3.1.2. Calcium, magnesium, and strontium

The results for Ca, Mg, and Sr are presented in Fig. 2, Table 1 and Supplemental File S2. Concentrations of Ca (range: 32–41 mg/kg), Mg (44–49 mg/kg), and Sr (0.34–0.40 mg/kg) were low in the reference sediment. On the first sampling event, the 10–30 cm dredge spoils showed a two-to-three fold increase relative to the reference samples in the concentrations of Ca (range of triplicate means: 62–113 mg/kg, $p = 0.20$), Mg (71–131 mg/kg, $p \leq 0.01$), and Sr (0.7–1.3 mg/kg, $p = 0.21$). These concentrations had further increased in May 2017 (second sampling event) and were strongly elevated during the final three sampling times with maximum concentrations of 1624 ($p = 0.01$), 785 ($p \leq 0.01$), and 9.1 mg/kg ($p \leq 0.01$), respectively. These large increases were explained by proton-mediated reactions including cation exchange, aluminosilicate alteration, and for Ca dissolution of added lime.

To assess how much of the added lime (CaCO₃: 10 kg/m³ = 100 mol/m³) had been dissolved, calculations were made based on the results for Ca, Mg, and S. It was assumed that the bulk density of the dredged material was 500 kg/m³, which gave approximately 0.2 mol lime or Ca per kg. Both the Ca and Mg concentrations increased over time (Fig. 2 and Table 1), which is a result of the release of Ca^{2+} and Mg^{2+} in cation-
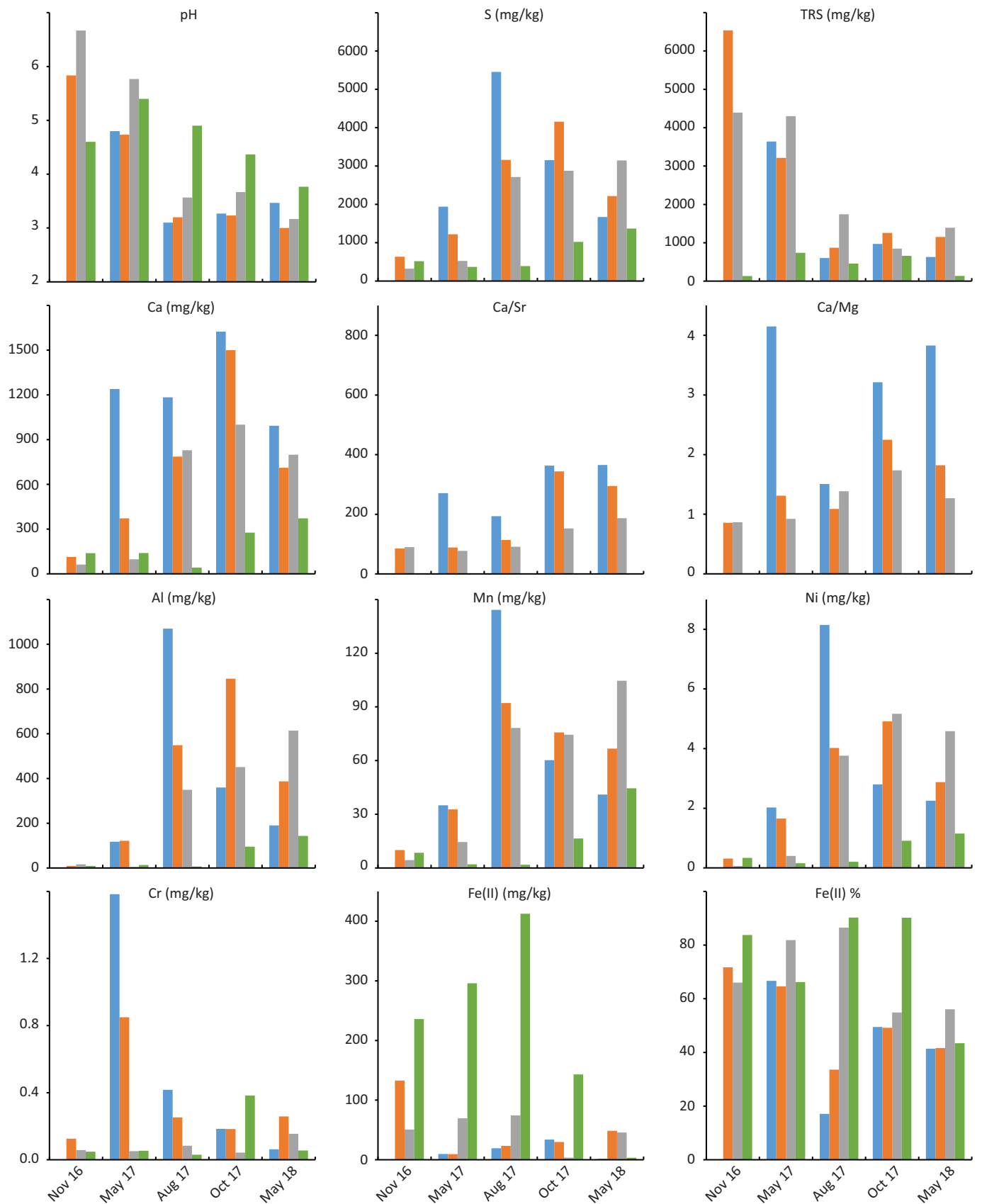
Fig. 2. Selected chemical parameters for the 10 (blue), 20 (orange), 30 (grey), and 60 (green) cm depths in the dredge spoils at the five sampling times. Each bar is a mean of triplicate measurements.

exchange reactions and aluminosilicate weathering in the acidic material (Åström and Åström, 1997; Hartikainen and Yli-Halla, 1986). It was therefore assumed that the stronger increase in Ca (see Ca/Mg in Fig. 2) was due to the dissolution of added lime. Consequently, lime-released Ca was determined by performing a subtraction: Ca - Mg. The mean of this difference for the samples from 10 to 30 cm depth was for the final (5th) sampling event was 0.0033 mol, whereas the summed means for all five sampling events was 0.018 mol. The former difference will have underestimated the lime-derived Ca as some of the Ca certainly had been leached and thus not accounted for, whereas the latter difference assumed that in-between each sampling event all the Ca in the pore waters had been replaced that is unlikely and thus caused an overestimation of the amount of lime dissolved. For the final sampling event, the Ca losses were two orders of magnitude lower and for all sampling events combined an order of magnitude lower than the added lime (0.2 mol/kg). A similar assessment was based on the S results assuming that oxidation of 1 mol of sulfide-S produced two mols of H^+ that was neutralized by 1 mol of $CaCO_3$. Hence, the molar ratio of S (sulfate) to Ca should have been close to 1.0 if all sulfide oxidized was neutralized by the added lime. The final sampling event had an average S concentration of 0.073 mol/kg, which was approximately 20 times higher than the corresponding calculated lime-released Ca (0.0033 mol/kg) and all five sampling events summed up had 0.35 mol S/kg which was also approximately 20 times higher than the corresponding lime-released Ca (0.018 mol/kg). This showed that approximately 5% of the sulfide-oxidation produced acidity was neutralized by the lime. Although involving uncertainties and assumptions, taken together these data were in line with the measured very low pH in the dredge spoils and indicated that the lime dissolution was inefficient and hindered. This may have been caused by a number of factors such as too large grain size of the lime (Högfors-Rönnholm et al., 2020), inefficient mixing of the lime and soil, and possibly passivation of lime-grains by precipitated Fe(III) phases (Hammarstrom et al., 2003). The limited dissolution of added lime was also in line with typically slow and poor dissolution of lime added to the surface of acid sulfate soils in the region (Österholm and Åström, 2002; Österholm and Åström, 2004).

3.1.3. Trace elements (Al, Mn, Co, Ni, Zn, Cu, As, and Cr)

In the dredge spoil, the concentration-variation patterns for Al, Mn, Co, Ni, Zn, Cu, and As were overall similar to those of S (and pH) with low concentrations during the first two-sampling events (as well as in the reference sediments) followed by elevated concentrations during the final three sampling times (Fig. 2, Table 1, & Supplemental File S2). Mean values (mean ± SD) for the reference samples ($n = 6$) increased in the order: Co (0.01 ± 0.0005 mg/kg) < Ni (0.04 ± 0.006) < As (0.05 ± 0.01) < Cu (0.26 ± 0.05) < Mn (2.3 ± 0.20) < Al (6.4 ± 0.73) = Zn (6.4 ± 6.5) compared to increased mean values for the three final sampling times in the order: As (0.20 ± 0.14) < Cu (1.0 ± 0.83) < Co (2.8 ± 1.9) < Ni (4.3 ± 2.7) < Zn (18 ± 25) < Mn (76 ± 41) < Al (535 ± 452). The latter order of elements indicated the relative extent to which these will likely leach from the dredge spoil upon oxidation and hydrological activation (during rainy periods and snow melting). This was the same order as these elements are leached from actual AS soils in the region as measured in streams draining these soils: arsenic is typically not enriched, Cu was enriched to some extent whereas there is extensive enrichment of Co, Ni, Zn, Mn, and Al (Åström and Åström, 1997; Roos and Åström, 2008). It was thus evident that the liming not only failed to prevent a severe drop in pH, but also permitted mobilization of potentially toxic metals that are typically leached from actual AS soils. The pattern for Cr was different to the other seven trace elements with the highest concentrations in the reference samples (2.4 ± 0.42) plus the dredge spoil concentrations were lower during the final three sampling times than the second (Fig. 2). This showed that Cr was relatively soluble and thus, can potentially be leached in early stages but was immobilized as oxidation and acidification progressed. This was in line with the generally low (background) concentrations of Cr in streams draining AS soils (Roos and Åström, 2006) and was possibly related to scavenging of Cr by hydroxides and oxyhydroxysulfates formed when the iron-sulfide rich sediments oxidize.

3.1.4. Iron

The behavior of Fe was different to all the other studied elements, as Fe (II) occurred in much higher concentrations in the soil substrate (60 cm depth) than in the dredge spoil above (Fig. 2). Transport of Fe(II) from the dredge spoil was an unlikely major mechanism as an enrichment in the soil substrate would in that case also have been seen for S and trace elements. The soil substrate, being an actual AS soil, likely contained Fe (oxy) hydroxides and possibly Fe oxyhydroxysulfates (Collins et al., 2010; Karimian et al., 2018). The addition of fresh dredge spoil rich in organic matter (mean 9% LOI) on top of the soil substrate restricted oxygen supply and thus created reducing conditions. Therefore, the soil substrate itself was a potential source of the mobilized Fe(II) with a general decline in the dredge spoil Fe(II) to Fe(III) ratio over time consistent with on-going oxidation.

3.2. Microbiology

3.2.1. General 16S rRNA gene results

Sequencing of the 16S rRNA gene amplicons (total 63 samples) produced a mean of 314,233 (min: 60,241 and max: 582,575) sequences that after quality control and filtering gave 43,377 to 455,846 sequences (Supplemental File S3). Rarefaction curves of the number of unique ASVs in the individual samples were predominantly asymptotic suggesting that the majority of the bacterial diversity in the dredge spoils had been identified (Supplemental File S4).

3.2.2. Bacterial community diversity indices

The reference sample bacterial Bray-Curtis dissimilarity (Fig. 3) showed a separation on axis one compared to the majority of the 10 to 30 cm deep dredge spoil samples. The three dredge spoil samples that grouped with the reference samples were two from the first sampling point (no 10 cm deep samples were collected on November 2016 as the sampling field was frozen at that depth) and one at the 30 cm depth from the second sampling point and therefore, were likely collected before the bacterial community had altered due to the conversion of dredge spoil to actual AS soils. In addition, the 60 cm samples from all sampling times grouped separately on axis two compared to the other samples. This was potentially as these samples approximated the depth of the former forest floor that now resides under the dredge spoil deposits and that the communities were not exposed to the large geochemical fluctuations that occurred in the shallower depths within the dredge spoils.

The reference samples had the highest Shannon H, species richness, and Pielou's evenness indices along with the highest, circumneutral pH values (Fig. 3). In contrast, the 10–30 cm depth deposited dredge spoil diversity indices decreased over time that followed the decrease in pH below 4 by August 2017, which satisfied the requirements for classification as an actual AS soil. These indices suggested that the decreasing pH along with the mobility of S, trace metals, and Fe (as described in Section 3.1) selected for a limited consortia of dominating microorganisms adapted to the extreme conditions. These data were similar to laboratory experiments investigating (bio)remediation of actual AS soils from the nearby Risöfladan experimental field by the addition of e.g. lime that was mixed with the dredge spoils in this study (Högfors-Rönnholm et al., 2020). The later 10–30 cm depth dredge spoil Shannon's H index values were also similar to Swedish actual AS soils that were between approximately 2 and 3.5 (Christel et al., 2019). In contrast, the initial bacterial diversity and richness were lower at 60 cm depth as well as a lower initial pH when compared to the other depths that increased until May 2017 before decreasing again. This was potentially as these samples were taken from the mature actual AS soils upon which the dredge spoils were deposited that initially increased in pH with the lime mixed into the dredge spoils and then decreased as the overlying spoils were converted into an actual AS soil. In summary, the data supported a decreased bacterial diversity as the pH decreases during conversion to actual AS soils but increases again with time in a mature AS soil. A similar control of bacterial diversity by pH was observed in a coastal AS soil environment in Australia, where lowest diversities were found in the
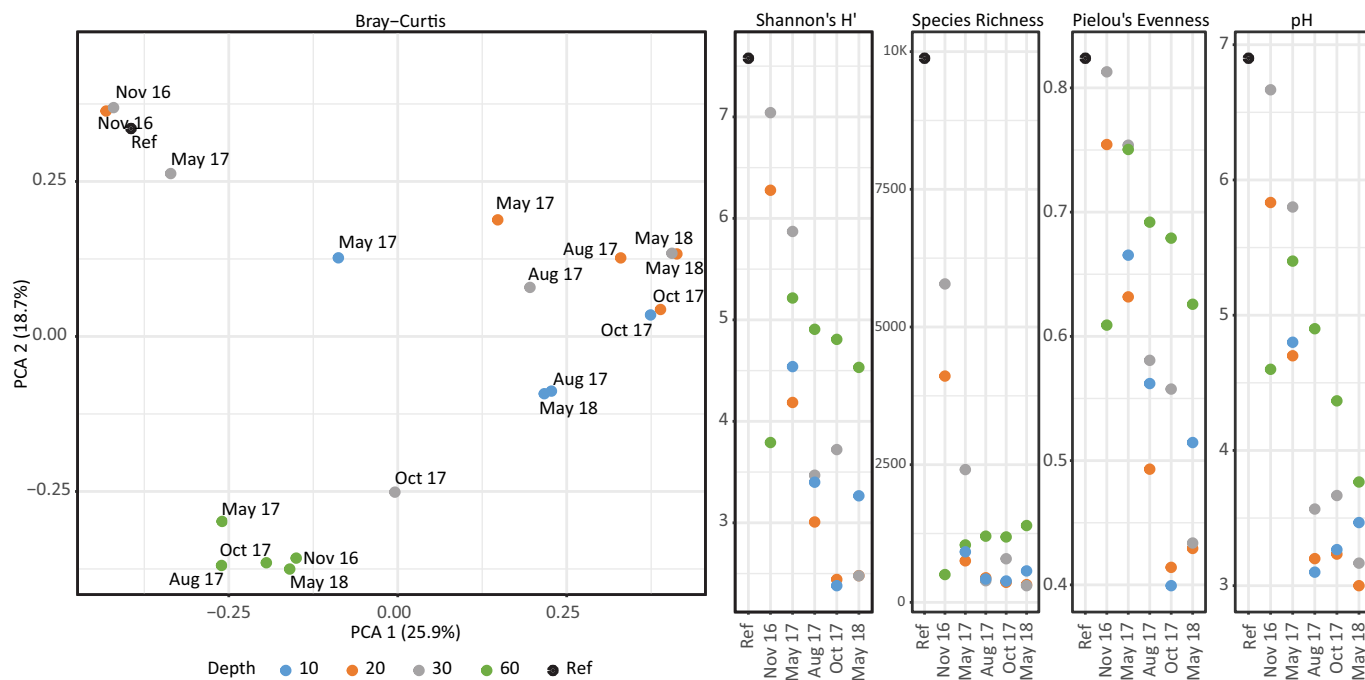
Fig. 3. Diversity indices of the 16S rRNA gene amplicon sequence data showing Bray-Curtis dissimilarity, Shannon's H, and evenness, Pilou's evenness together with pH for the reference samples (ref) and 10, 20, 30, and 60 cm depths in the dredge spoils at the four sampling times.

upper parts of the wetland AS soil most exposed to air with pH values ranging between 3 and 5 and higher diversities were found in reduced PASS sediments with pH values > 6 (Ling et al., 2015).

3.2.3. Effects on bacterial community composition

The reference samples were collected from an old anoxic sulfidic sediment that was high in metastable iron sulfides and therefore, similar to the dredged sediment (Fig. 4 and Supplemental File S5). The 16S rRNA gene amplicons were dominated by ASVs that aligned with Proteobacteria, Chloroflexi, and Bacteroidetes with 32.9, 26.4, and 10.8% relative abundances (i.e. % of total reads/amplicons), respectively. The genera with highest relative abundances in the reference samples were identified as the anaerobic *Syntrophus* (10.7%) identified in sediments (Mountfort et al., 1984), *GOUTA6* (1.6%) previously found in soils (Truu et al., 2020), and *Gallionella* (1.7%) that are characterized as iron-oxidizing chemolithotrophs found in low oxygen environments (Fabisch et al., 2013; Hallbeck and Pedersen, 2015). These data support a pH neutral soil or sediment environment containing iron.

The dredge spoils altered in 16S rRNA gene amplicon based bacterial community with both depth from 10 to 30 cm in the spoils and time from November 2016 until May 2018 (Fig. 4 and Supplemental File S5). The phyla with highest relative abundances at 10 to 30 cm depth included ASVs most similar to Proteobacteria ($\leq 77.8\%$), Actinobacteria ($\leq 58.6\%$), Epsilonbacteraeota ($\leq 25.0\%$), Chloroflexi (17.9%), Patescibacteria (12.1%), and Bacteroidetes (10.4%). In contrast, the taxa were generally more diverse at 60 cm where small fluctuations in relative abundances were observed over time of e.g. Actinobacteria ($\leq 32.9\%$; genus *Actinomycetales*), Proteobacteria ($\leq 27.0\%$), Bacteroidetes ($\leq 25.7\%$), Patescibacteria ($\leq 15.6\%$), Chloroflexi (17.9%), WPS-2 ($\leq 16.2\%$), and Epsilonbacteraeota ($\leq 11.0\%$). The phyla at 60 cm were consistent with this depth being the previous forest floor and will not be discussed further.

The rapid oxidation of the displaced dredge spoils facilitated the severe drop in pH values that was reflected in an altering bacterial community (Fig. 4 and Supplemental File S5) and increased relative abundance of taxa commonly found in acidic environments such as acid sulfate soils, biomining, and acid mine drainage (Bonnefoy and Holmes, 2012; Christel et al., 2019; Dopson and Johnson, 2012; Wu et al., 2013). The pH drop at 10 cm in the dredge spoils over the course of sampling from pH 4.8 to 3.4

(Fig. 2) was reflected in an initial community of genera such as *Gallionella* (Fabisch et al., 2013), the psychrotolerant, facultative anaerobic ferric iron reducer *Rhodanobacter* (Finneran et al., 2003), and the halotolerant sulfur oxidizing *Halothiobacillus* (Kelly and Wood, 2000) some of which have previously been identified in actual AS soils and laboratory remediation experiments (Christel et al., 2019; Högfors-Rönnholm et al., 2020). In August 2017, the relative abundance of ASVs that aligned with the sulfur and in some cases iron oxidizing acidophilic *Acidithiobacillus* genus (Moya-Beltrán et al., 2021) had risen to a peak of 33.1% while in October 2017 and ASVs that aligned with the acid-tolerant genus *Metallibacterium* that has previously been enriched from actual AS soils environments (Högfors-Rönnholm et al., 2018a; Wu et al., 2013) reached a relative abundance of 41.7%. In addition, ASVs aligning with the acidophilic and iron oxidizing genus *Ferrithrix* (Johnson et al., 2009) also identified in actual AS soils (Högfors-Rönnholm et al., 2018b) reached a peak relative abundance of 13.9% in May 2018.

On the first sampling date in November 2016 at 20 cm depth in the dredge spoils, the facultative anaerobic, chemolithoautotrophic, sulfur-oxidizing *Sulfuricurvum* (Kodama and Watanabe, 2004) was the dominant genus that was consistent with this depth having the highest concentration of TRS of all sampling depths and dates. A similar temporal trend in altering bacterial community was observed at 20 cm as seen at 10 cm except that the *Acidithiobacillus* genus reached higher relative abundances of 23.6% in May 2017, peaking at 65.3% in August 2017, and remaining at $\geq 37.2\%$ for the final two sampling times. In addition, other genera with lower relative abundances included *Sulfuricurvum* ($\leq 7.3\%$), *Rhodanobacter* ($\leq 7.2\%$), and *Gallionella* ($\leq 5.6\%$). The initial (November 2017) amplicon based bacterial community at 30 cm depth contained the sulfur oxidizing *Sulfurimonas* genus (8.2% relative abundance; (Inagaki et al., 2003)) also identified from actual AS soils and laboratory experiments (Christel et al., 2019; Högfors-Rönnholm et al., 2020) along with the *Syntrophus* genus (7.7%). Subsequently, this depth also followed the same pattern of increasing relative abundances of extreme acidophiles from the *Acidithiobacillus* ($\leq 59.9\%$) along with other taxa. However, it took until August 2018 for these acidophiles to become dominant due to the oxidation front taking time to reach this depth and the pH values to decrease. In summary, the 16S rRNA gene based community supports the geochemical data with the increased abundance of acid tolerant and acidophilic genera. Acidophilic taxa had previously been
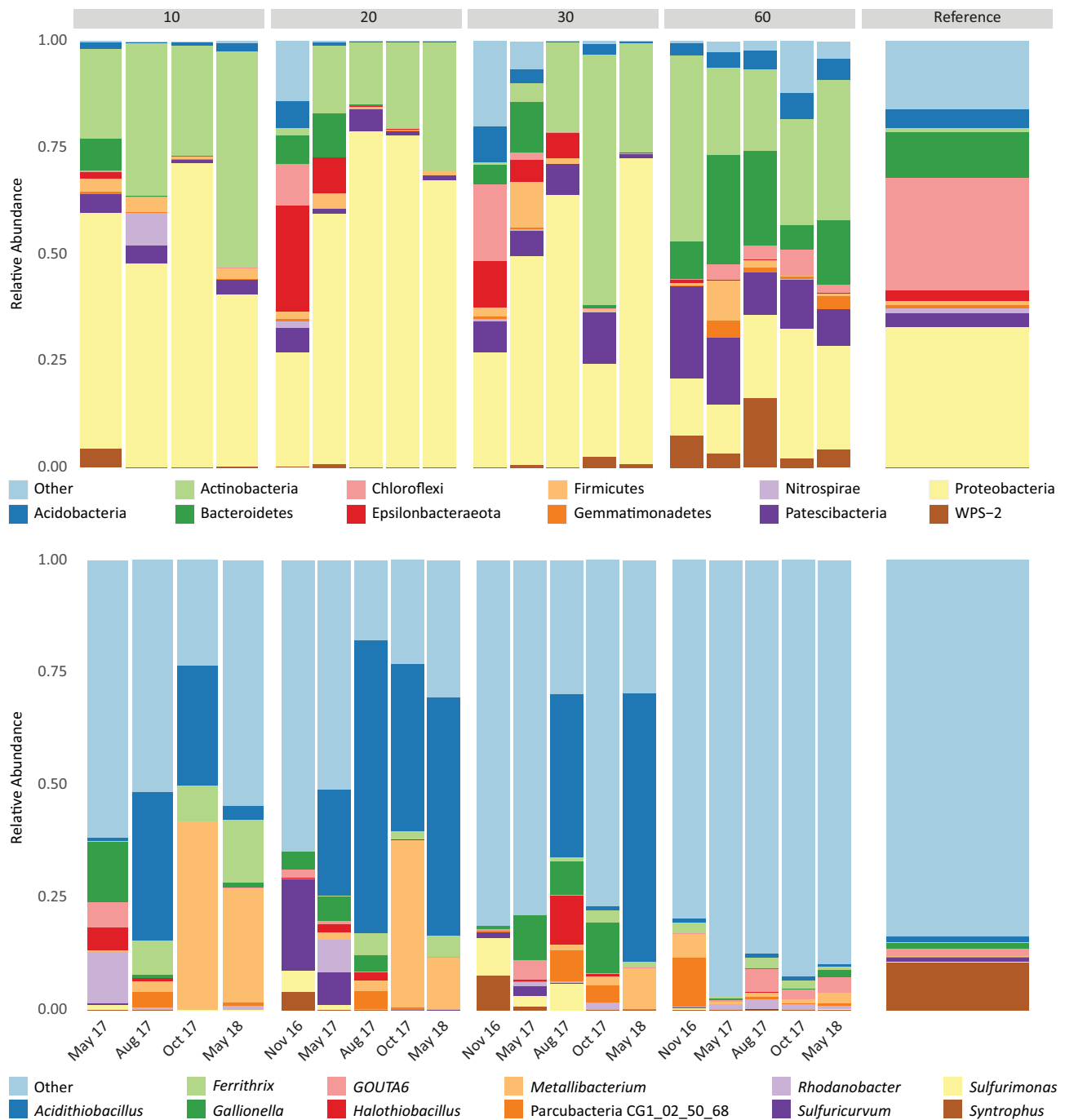
Fig. 4. Stacked bar graph of the bacterial community composition at the level of phyla (top) and genera (bottom) for the 10, 20, 30, and 60 cm depths in the deposited dredge spoils plus reference sample. The figure shows the relative abundances of the top eleven phyla or genera with the remaining proportion to 100% (1.0) grouped as 'Other'.

identified when PASS from the Risöfladan experimental field was rapidly oxidized in a laboratory experiment when air was driven through bioreactors causing rapid oxidation, quickly dropping the pH (Wu et al., 2013).

4. Conclusions

Both the geochemical and microbiological data supported that the liming strategy applied to the field up to the time of sampling was unsuccessful. The pH dropped to very low values, despite that calculations indicated that only in the order of 5% of the added lime had been dissolved. As a result of the sulfide oxidation and strong decrease in pH, the water-extractable concentrations of a number of potentially toxic metals substantially increased and will thus, gradually leach into the relatively small (and thus sensitive)

river (catchment area 506 km^2) and estuary systems in the area. In addition, the bacterial communities showed the development of extremely acidophilic genera typically found in acidic and metal containing environments. Since the utilized method of lime addition to the dredge spoils was largely unsuccessful in mitigating acid and metals release, other factors must also be considered such as the size of the lime grains and the soil-lime mixing in future depositions of dredge spoils containing metal sulfides. The efficacy of these additional considerations should be explored in future investigations.

Credit authorship contribution statement

All authors participated in the development of the manuscript. The following contains an accurate description of their contributions to the work:

Eva Högfors-Rönnholm and **Sten Engblom** conceived the study.

Eva Högfors-Rönnholm and **Sten Engblom** collected samples.

Eva Högfors-Rönnholm, **Sten Engblom**, and **Peter Österholm** carried out analysis.

Anders Johnson, **Peter Österholm**, **Mats Åström**, **Mark Dopson** analyzed data.

Anders Johnson drafted the manuscript with contributions from all authors.

All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151864.

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