RESEARCH ARTICLE

Biodegraded peat and ultrafine calcium carbonate result in retained metals and higher microbial diversities in boreal acid sulfate soil

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ABSTRACT

To efficiently mitigate bacterial mediated acid and metal discharge from acid sulfate soils, iron- and sulfur-oxidizing microorganisms that catalyze the iron sulfide dissolution should be inactivated. An organic carbon source could further be introduced into the soil to promote the growth of iron- and sulfur-reducing bacteria. In this study, acid sulfate soil was amended with a mobile form of ultrafine calcium carbonate alone or in combination with fractions of peat, sodium acetate, or sodium lactate. The introduction of ultrafine calcium carbonate resulted in a raised pH that appeared to inactivate the acidophiles, but did not reactivate iron- or sulfur-reducing bacteria. The addition of organic matter resulted in higher microbial diversities and retention of metals, although acid-tolerant and acidophilic microbes still dominated. A low abundance of an iron-reducing bacteria was identified in the all treatments with both peat fractions and pure organic carbon compounds. These results indicated that biodegraded peat could be used as an energy source for at least iron-reducing bacteria in the acid sulfate soil at the same time as it retains metals in the soil. These findings are of value for further developing mitigation methods for the sustainable use of acid sulfate soils.

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1 Introduction

Coastal boreal acid sulfate soils (ASS) around the Baltic Sea originate from past Litorina Sea (8000–4000 years B.P.) and later sea stage sediments formed under eutrophicated and anoxic conditions (Sohlenius et al., 1996). These sea

sediments, rich in fine grains of metal sulfides such as pyrite (FeS_2), have naturally been exposed due to post glacial sea level change and land uplift and then been covered by peat and vegetation. When these sediments are exposed to atmospheric oxygen, metal sulfide oxidation is initiated and catalyzed by microorganisms that generates sulfuric acid and leachable toxic metal(loid)s. The biogeochemical reactions in the formed ASS, that are often low in carbonate alkalinity, thus lead to acidification and an increase in metal concentrations in recipient waters with harmful effects on the environment (Dent, 1986). In the Baltic Sea coastal area, the abundant

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ASS affected by human activity have resulted in watercourses showing very poor chemical and ecological status without signs of improvement (Wallin et al., 2015). ASS management practices and mitigation treatments are therefore urgently needed.

The role of microorganisms in catalyzing pyrite oxidation in ASS is well established (Wu et al., 2013). Sulfur-oxidizing bacteria are a collective name for bacteria capable of utilizing several reduced sulfur compounds as electron donors with sulfate as the ultimate end product (Reddy and DeLaune, 2008). The generated sulfate can undergo microbial mediated reduction under anaerobic, oxygen depleted conditions to form sulfide that can subsequently react with iron to re-form pyrite or monosulfides. Sulfur-reducing bacteria are the collective name for bacteria that can utilize diverse oxidation states of sulfur (e.g., sulfate, elemental sulfur, and sulfite) as electron acceptors with sulfide as the final end product (Reddy and DeLaune, 2008). To inactivate iron- and sulfur-oxidizing microorganisms and to reactivate iron- and sulfur-reducers in ASS (thereby mitigating acid and metal discharge), these microbes need to be identified and their energy sources and growth optima characterized.

A few investigations have characterized microbial populations in ASS through 16S rRNA gene sequencing (Wu et al., 2013, 2015; Stroud et al., 2014). These studies showed unclassified operational taxonomic units (OTUs) at the species level as the dominant microbial populations, but known acidophilic (optimal pH for growth < 5) iron- and sulfur-oxidizing bacteria were also identified. In addition, species most similar to iron- and sulfur-oxidizers found in low-temperature acid and metal contaminated environments were identified in boreal ASS (Wu et al., 2013, 2015).

In a recent study of boreal ASS by Högfors-Rönnholm et al. (2018a), a low relative abundance of OTUs aligning with the iron-reducer *Rhodospirillum rubrum* sp. was identified. However, this population as well as other known iron- and sulfur-reducing bacteria could not be identified in the ASS mixed with peat as an additional energy source. This indicates that although an organic carbon source is needed in the ASS to reactivate sulfate reduction and pyrite formation (Kölbl et al., 2017), the added peat was unavailable as an energy source for ASS iron- and sulfur-reducers during the experimental time frame.

The aim of this study was to further investigate the effect of addition of organic material on a boreal ASS microbial community in order to find the optimal mitigation treatment for the simultaneous inactivation of acidophiles and reactivation of iron- and sulfur-reducing bacteria. Treatment with ultrafine calcium carbonate, separately and in combination with organic material, was investigated in a small scale laboratory experiment under both oxygen rich and oxygen depleted conditions. Ultrafine-grained calcium carbonate was used to add carbonate alkalinity to the soil. This both increases the pH of the soil that is expected to inactivate acidophiles as well as adding buffering capacity to prolong the treatment effect. Using calcium carbonate with an extremely small particle diameter considerably increases the reactive

surface, which decreases the amount of calcium carbonate needed. Calcium carbonate is moreover a safe material to handle giving circumneutral pH values ideal for crop growth. Peat was used as organic material since it is an abundant material in the boreal climatic zone often covering potential ASS sediments in coastal Baltic Sea areas. Major intermediate breakdown products of peat include acetate and lactate that can be utilized as energy sources by iron- and sulfur-reducing bacteria (Reddy and DeLaune, 2008) also in acidic environments (reviewed in Ayangbenro et al., 2018). Treatments with sodium acetate and sodium lactate were therefore additionally tested separately in order to investigate if these pure low molecular weight organic compounds have more effect on the ASS microbial population than the more complex organic material peat.

2 Materials and methods

2.1 Soil sampling

Soil was sampled at the Risöfladan experimental field, Vaasa, Finland (63°02' N, 21°42' E) in April 2017. The experimental field is situated on an area that was drained in the 1950's in order to convert it into agricultural land. The old marine sulfidic rich sediments have since then oxidized and developed into a typical boreal ASS low in carbonate alkalinity and with a pH value < 4 and that leaches significant amount of metals (Nordmyr et al., 2008). Samples were taken as soil cores with an excavator by pushing black polyethylene tubes (150 cm long; 14.2 cm inner diameter) vertically into the ground. The soil core tubes were sealed airtight and stored at 4°C.

2.2 Soil treatments

The treatments applied in the study were: 1) Control incubation with no additions (Con), 2) 1% (wt/wt) CaCO₃ EnrichBio (EB), 3) 1% (wt/wt) peat fraction H1 (sieved to < 0.5 mm) mixed equally with CaCO₃ C2 (P1/CaCO₃), 4) 1% (wt/wt) peat fraction H5 (sieved to < 0.5 mm) mixed equally with CaCO₃ C2 (P5/CaCO₃), 5) 0.1% (wt/wt) sodium acetate (NaAc), and 6) 0.1% (wt/wt) sodium lactate (NaLa).

EnrichBio was supplied by Nordkalk Corporation, Finland and is a mixture of 37% ultrafine-grained CaCO₃ with a median particle diameter of 0.3 µm and 4% per dry weight CaCO₃ Alcoguard® H 5941 (Akzo Nobel), which is a biodegradable dispersant agent preventing flocculation and promoting transport of CaCO₃ through soil pores. The technical grade CaCO₃ (trade name C2) was also supplied by Nordkalk and is ultrafine-grained with a median particle diameter of 2.5 µm and previously tested for mitigation of ASS (Wu et al., 2015; Högfors-Rönnholm et al., 2018a). The peat fractions were chosen based on their biodegradation level according to the von Post scale (from slightly biodegraded H1 to fully biodegraded H10) and were supplied by Vapo Fibers, Finland that also determined the biodegradation level. The sodium acetate (Merck) used was a dry powder and the

sodium lactate (Sigma-Aldrich) was a sodium DL-lactate solution syrup, 60% (w/w).

2.3 Experimental design

Three different soil core tubes were used in each experiment as biological triplicates and the soil was aseptically collected from each core at the ASS horizon that was in the 70–85 cm soil zone below the surface. The dry weight (SFS-EN 12880) of all three soil samples was determined to 54%. Soil treatments were performed according to Högfors-Rönholm et al. (2018a) with minor modifications. Briefly, each homogenized triplicate soil sample was divided into different beakers and mixed with the treatment substances under both anaerobic (oxygen depleted) and aerobic (oxygen rich) conditions. The anaerobic treatments were performed inside an anaerobic tent (Aldrich® AtmosBag, Sigma-Aldrich) filled with N₂-gas and the absence of oxygen was confirmed with resazurin. For microbiological analyses, untreated and treated soil was placed into separate 50 mL sterile tubes with 15 g soil per tube, summing up to 12 tubes per each homogenized triplicate soil sample, six tubes for anaerobic and six tubes for aerobic conditions. Tubes for geochemical analyses were prepared in the same way, adding up to the same amount of tubes as for the microbiological analyses. The tubes were incubated for ten weeks at 10°C in the dark. This temperature was chosen as it is the average field temperature at the ASS horizon at Risöfladan experimental field (Wu et al., 2013). Additionally, soil was sampled from each homogenized triplicate soil for microbial zero time point (ZTP) and geochemical starting point analyses.

2.4 Analytical methods

After incubation, geochemical samples were stored at –20°C until analysis. pH, conductivity (EC), and ferrous iron were analyzed from soil leachates as described by Högfors-Rönholm et al. (2018a). Sulfur speciation was performed on soil material according to Högfors-Rönholm et al., (2018a). Multi-element analysis was further conducted on the soil leachates using ICP-MS at the Activation Laboratories LTD, Canada.

Microbiological samples were stored at 4°C until DNA was extracted within four days according to the indirect DNA extraction method described by Högfors-Rönholm et al. (2018b). DNA was extracted from the separated bacterial cells using the DNeasy PowerSoil Kit (QIAGEN). The V3–V4 region of the 16S rRNA gene was amplified using the 341F and 805R primers (Herlemann et al., 2011), followed by an amplification step that attached individual tags for Illumina sequencing (Hugerth et al., 2014). The amplicons were sequenced on the Illumina MiSeq platform at the Science for Life Laboratory, Sweden. Downstream bioinformatics analysis was performed using the UPARSE pipeline (Edgar, 2013) by clustering the sequences into OTUs that were annotated against the SINA/SILVA database (SILVA 119; Quast et al., 2013). Data interpretation and plotting were performed in R (v. 3.4.2; R

Core Team 2015) using the phyloseq package (v. 1.20; McMurdie and Holmes 2013). Phylogenetic trees were constructed by FastTree (v. 2.1.8; Price et al. 2010) and drawn using iTOL (v. 4.2.1). The raw nucleic acid sequences were archived in the NCBI database with the BioProject accession number: PRJNA433814.

Beta diversity was calculated as a principal coordinate analysis (PCoA) derived from weighted UniFrac distances on the sequencing data. One-way ANOVA analysis followed by Tukey's post hoc test identified significant differences in alpha diversity ($p < 0.05$) between the control and treatments. R (v. 3.1.3) with the additional package 'robCompositions' was used to prepare compositional principal component analysis (cPCA) biplots of the 16S rRNA gene and geochemistry data as the amplicon sequencing data consist of proportional rather than absolute values, as described in Högfors-Rönholm et al., 2018a.

3 Results and discussion

16S rRNA gene amplicon sequencing resulted in 1.14 million merged reads, with single samples yielding on average 23 837 sequences (min 4290; max 64 149). A portion (36.7%) of obtained reads were discarded due to not meeting set quality criteria (Table S1) giving a total of 585 OTUs that were retrieved from all samples. Similar to our previous studies on boreal ASS (Högfors-Rönholm et al., 2018a; Christel et al., 2019). Bacteria dominated the microbial populations, and the populations with >1% relative abundance were assigned to eight phyla. Less than 0.1% of the OTUs were assigned to the Archaea domain, and as Archaea typically occur in more temperate zones, the microbial community found in the studied ASS reflects the colder environment in the boreal zone. The indirect DNA extraction method (Högfors-Rönholm et al., 2018b) used in this study removes relic DNA in the soil and gives a valid description of the microbial community in the various samples.

3.1 Community structure of boreal ASS was dominated by sulfur- and iron-oxidizers

The alpha diversity was low in the field, and ZTP samples (Fig. 1A) significantly increased during incubation when the pH raised and/or organic carbon was added (one-way ANOVA plus Tukey post hoc test, $p < 0.05$ for the aerobic and anaerobic P1/CaCO₃, P5/CaCO₃, and NaAc samples as well as for the anaerobic NaLa replicates). This suggests that a broader microbial community was supported in the organic carbon treated samples than in the acidic untreated soil, where only inorganic electron donors from the metal sulfides were available as energy sources (Li et al., 2017). The high variation in the aerobic NaLa replicates likely reflects extremely heterogeneous nature of the soils with one replicate having a much lower alpha diversity than the other samples. In addition, the ZTP and Con samples were clustered together in the beta diversity biplot (Fig. 1B), suggesting the controls were a valid representation of the field samples.

Fig. 1 (A) Shannon alpha diversity index and (B) beta diversity calculated as a principal coordinate analysis derived from weighted UniFrac distances on 16S rRNA gene amplicon sequencing data from acid sulfate soil zero time point (ZTP, ♦) samples, untreated control samples (Con, ●), and samples treated with EnrichBio (EB, ▲), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃, ■), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃, ▽), sodium acetate (NaAc, ★) and sodium lactate (NaLa, ✕). Closed symbols are aerobic samples and open symbols are anaerobic samples. All controls and treatments were carried out as biological triplicates.

Although assigning metabolic function based on 16S rRNA gene similarity between species must be carried out with care (e.g., the 16S rRNA gene sequences most similar to an alkaliphile identified from an ASS described below), inferences are drawn on potential metabolic capacities in the various treatments. The microbial communities in the ZTP and Con samples (with a pH of 3.7–4.2; Tables S2 and S3) were dominated by OTUs belonging to the Gammaproteobacteria KF-JG30-C25 (56%–79% relative abundance; Figs. 2 and S1) that were most similar to the alkaliphilic sulfur-oxidizer *Thioalkalivibrio nitratireducens* (Fig. 3; Sorokin et al., 2003) and the Acidimicrobia (4%–12% relative abundance; Figs. 2 and S2) most similar to the acidophilic iron-oxidizer *Acidimicrobium ferrooxidans* (Fig. 3; Clark and Norris, 1996). As *Thioalkalivibrio* sp. has previously only been isolated from alkaline environments (Ahn et al., 2017), it is unlikely that the KF-JG30-C2 OTUs represent these species. It is more likely that these belong to another unknown sulfur-oxidizing *Thioalkalivibrio*-like species of the Ectothiorhodospiraceae family able to tolerate acidic conditions. The high relative abundance of the KF-JG30-C25 OTUs negatively correlated with total sulfur and total reduced sulfur (TRS) in the cPCAs and were clustered together with the typical ASS metals such as Al (Fig. 4), which indicated a high activity of these supposed sulfur-oxidizers in the ZTP and untreated control soils. The microbial community also contained smaller relative abundances (<4%) of OTUs most similar to acidophilic or acid-tolerant bacterial families including Acidobacteraceae and Rhodanobacteraceae (Fig. 5).

3.2 Raising the pH reduces the relative proportion of acidophiles

Although the EB treatment increased the soil pH from 3.7–4.2

in the ZTP and Con samples to 6.0–6.6 (Tables S2 and S3), no statistically significant differences could be found in the alpha diversities. However, the microbial community in the EB treatment has changed compared to that in the ZTP and Con samples, seen as a distinct separation in the PCoA along the first principle coordinates (Fig. 1B). The microbial community was dominated by OTUs belonging to the Rhodanobacteraceae family (75%–89% relative abundance; Fig. 5) and were most similar to species from the acid tolerant genus *Rhodanobacter* (Figs. 2, 3 and S4). This contrasted with a previous study where a *Rhodanobacter* population decreased with an increase in pH when ASS was treated with 2.5 µm CaCO₃ (C2; Högfors-Rönnholm et al., 2018a). As the *Rhodanobacter* spp. in this and the previous study were not identified on a species level, the difference may have been due to identification of different species. *Rhodanobacter* spp. have previously been known to be abundant only in low-pH conditions (Green et al., 2012). However, a new species, *Rhodanobacter humi*, has recently been identified that has a pH optimum at >6.5 (Dahal and Kim, 2017). The other abundant populations in the EB treatments were OTUs most similar to the ecologically diverse, neutrophilic Burkholderiaceae family (3%–14% relative abundance; Figs. 2, 3 and 5). The lack of OTUs aligning with KF-JG30-C25 (Fig. 2) suggested they were inactivated by the higher pH and this was illustrated by the clustering of soil pH, total sulfur, TRS, and the EB samples in the cPCAs (Fig. 4). However, the increased pH was not suggested to have affected metal(loid) leaching since the EB samples also grouped together with As and Zn (Fig. 4).

3.3 Effect of organic matter addition

With the exception of adding sodium lactate ('NaLa') in

Fig. 2 Heatmap showing the relative abundance of the top 26 OTUs in the aerobic (A) and anaerobic (B) triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa). All amplicon sequencing was carried out separately on the triplicate samples giving three values per treatment. The greyscales give percentage relative abundance of the OTUs.

Fig. 3 Maximum likelihood (unrooted) phylogenetic tree (100 × bootstrap) and heat map of the OTUs (>5% relative abundance) from the 16S rRNA gene sequencing of triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO_3 (P1/ CaCO_3), peat fraction H5 mixed equally with CaCO_3 (P5/ CaCO_3), sodium acetate (NaAc) and sodium lactate (NaLa). The greyscale gives percentage relative abundance of the OTUs and the scale bar gives the number of substitutions per site. An expanded version of the tree and heatmap of the *Rhodanobacter* OTUs is given in Fig. S4.

Fig. 4 Biplots from the compositional principal component analyses of the chemical and microbiological parameters from the aerobic (A) and anaerobic (B) triplicate acid sulfate soil zero time point (ZTP; red) samples, untreated control samples (Con; orange), and samples treated with EnrichBio (EB; blue), peat fraction H1 mixed equally with CaCO_3 (P1/ CaCO_3 ; purple), peat fraction H5 mixed equally with CaCO_3 (P5/ CaCO_3 ; pink), sodium acetate (NaAc; dark green) and sodium lactate (NaLa; light green).

Fig. 5 Microbial community structure (>1% relative abundance) on family level in acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO_3 (P1/ CaCO_3), peat fraction H5 mixed equally with CaCO_3 (P5/ CaCO_3), sodium acetate (NaAc) and sodium lactate (NaLa). All amplicon sequencing was carried out separately on the triplicate samples, giving three stacked bars per treatment.

aerobic conditions that had a very high variation in alpha diversity, addition of organic matter in the form of peat fraction H1 mixed with CaCO_3 ('P1/ CaCO_3 '), peat fraction H5 with CaCO_3 ('P5/ CaCO_3 '), and sodium acetate ('NaAc') further increased the alpha diversity (Fig. 1A; one-way ANOVA plus Tukey post hoc test, $p < 0.05$ for the aerobic and anaerobic P1/ CaCO_3 , P5/ CaCO_3 , and NaAc plus the anaerobic NaLa replicates). The higher diversity was unlikely to be due to introduced bacterial taxa from the peat as a similar effect on diversity was seen in the NaAc and NaLa treatments. However, the beta diversity PCoA suggested the samples split into two groups with the addition of peat on one hand and lactate or acetate on the other (Fig. 1B). The similarity of the P1/ CaCO_3 and P5/ CaCO_3 treatment beta diversities was supported by their clustering in the cPCAs (Fig. 4) and these treatments resulted in an increased pH that ranged from 4.8 to 5.7 (Tables S2 and S3). However, OTUs belonging to the Rhodanobacteraceae family (28%–70% relative abundance; Figs. 2, 5 and S4) still dominated. Other abundant species in the P1/ CaCO_3 and P5/ CaCO_3 treatments were OTUs belonging to the order KF-JG30-C25 (6%–33% relative abundance; Figs. 2 and S1) and class Acidimicrobiia (5%–13% relative abundance; Fig. 2 and S2). Finally, small relative abundances of OTUs aligning with the Burkholderiaceae family (1%–4% relative abundance) and *Mucilaginibacter paludis* (1%–5% relative abundance) isolated from an acidic peat bog (Figs. 2 and 3; Pankratov et al., 2007), were identified in the P1/ CaCO_3 and P5/ CaCO_3 treatments. Some

OTUs belonging to the Alphaproteobacteria family Micropepsaceae (0.3%–2% relative abundance) were most similar to the iron-reducer *Magnetospirillum magneticum* (Figs. 2 to 5; Suzuki et al., 2006), indicating that both peat fractions could be used as energy source for iron reducing bacteria. However, as these iron-reducers are very slow growing (Schüler and Schleifer, 2005), their growth could be limited by other microorganisms able to utilize the available organic material. The addition of peat seemed to prevent metal leaching as illustrated by the P1/ CaCO_3 and P5/ CaCO_3 treatments negatively correlating with Al, Cd, Co, Mn, and Ni (Fig. 4). The retention of metals after organic material addition is also reported for subtropical ASS (Dang et al., 2016) and is due to binding of cations to negatively charged carbon functional groups in the organic material. A pH increase of the soil increases the metal binding to organic materials additionally, as a higher pH both reduces metal solubility and increases the negative surface charge of organic materials (see ref. in Dang et al., 2016). The decrease in Al and other metals could also be due to chelation with organic acids produced by the OTUs aligning with the Burkholderiaceae, a process previously reported in ASS (Panhwar et al., 2014) and supported by the negative correlation between Burkholderiaceae and Al, Co, Mn, and Ni in the cPCAs (Fig. 4).

The higher pH in the NaAc and NaLa treatments (pH ranged from 3.9 to 5.3; Tables S2 and S3) compared to the untreated control samples was most likely due to sodium acetate and sodium lactate dissociating in solution and

consuming hydrogen ions (Reddy and DeLaune 2008). As for the P1/CaCO₃ and P5/CaCO₃ treatments, the NaAc and NaLa treatments clustered together in the cPCAs (Fig. 4). In the NaAc and NaLa treatments, OTUs belonging to the Rhodanobacteraceae family (2%–45% relative abundance) were abundant, but not as dominating as in the EB, P1/CaCO₃ and P5/CaCO₃ treatments (Figs. 2, 5 and S4). *Rhodanobacter* spp. are known to assimilate both sodium acetate and lactic acid to some degree (Dahal and Kim, 2017). Other abundant populations in the NaAc treatment were OTUs aligning with the order KF-JG30-C25 (9%–22% relative abundance; Figs. 2 and S1) and to a WPS-2 sp. (Figs. 2 and 3) that has previously been identified in high abundances from slightly acidic Antarctic soil (Ji et al., 2016). Other abundant OTUs in the NaLa treatment aligned with the Burkholderiaceae family (2%–70% relative abundance; Figs. 2 and 5), order KF-JG30-C25 (4%–13% relative abundance; Figs. 2 and S1), and phylum WPS-2 (1%–9% relative abundance; Figs. 2 and S3). Of these, only species belonging to the Burkholderiaceae family are previously known to assimilate acetate and lactate (e.g., Lee et al., 2018; Sato et al., 2006). Low abundance (0.3%–6%) populations in the NaAc and NaLa treatments included OTUs belonging to the family Acetobacteraceae as well as unclassified Alphaproteobacteria (Figs. 2–5 and S2). Although the Acetobacteraceae consists of several acidophilic species, the OTUs in this study were most similar to the neutrophilic *Roseomonas mucosa* (Fig. 3; Komagata et al., 2014) while the unclassified OTUs correlated negatively with S and TRS (Fig. 4) and were most similar to the sulfur-oxidizer *Eliaera tepidiphila* (Fig. 3; Albuquerque et al., 2008). OTUs from the Micropepsaceae family, most similar to the iron-reducer *Magnetospirillum magneticum* (Figs. 2 to 5), were also identified in the NaAc and NaLa treatments (0.3%–3% relative abundance). *Magnetospirillum* spp. use lactate as an energy source (Schüler and Schleifer 2005) that likely supported their growth in the biodegraded peat. The small iron-reducing population identified in the NaAc and NaLa treatments negatively correlated with ferrous iron (Fig. 4). However, the fact that these treatments were also dominated by sulfur- and iron-oxidizers that correlated negatively with S and TRS in the cPCAs (Fig. 4), indicated that oxidizing conditions were still prevalent in these treatments. However, the addition of these organic compounds seemed to prevent metal release (Fig. 4) and sodium acetate has previously been shown to act as a chelating agent by removing heavy metals from green mussel (*Perna viridis*). The chelating effect was shown to work at pH values between 4 and 9, but to be highest at neutral pH values (Azelee et al., 2014). Lactate has furthermore been shown to form simple one-to-one metal chelates in aqueous solutions at acidic conditions to pH values over 6.5 (Hamada et al., 2005).

4 Conclusions

In conclusion, the addition of calcium carbonate (EB) raised

the ASS pH and appeared to inactivate the acidophiles, but not the desired outcome to activate iron- or sulfur-reducing bacteria (Fig. 2). In contrast, although the addition of organic materials had similar effects, they were at a lower level than desirable. Overall, addition of organic materials to the ASS resulted in higher microbial diversities and retention of metals, although acid-tolerant and acidophilic microbes still dominated (Fig. 2). No significant differences in metal release were measured between the two peat fractions and the low molecular weight organic compounds, which indicated different biodegradation levels of peat are equally good organic materials to use to retain metals. The low relative abundance of iron-reducers identified in the treatments with added organic materials (Fig. 2) indicated that both fractions of peat could be used to reactivate iron-reducers in ASS. However, with the methods used in the study, no known sulfur-reducers were identified. Since many of the OTUs could not be assigned on a species level, ongoing metagenomic and metatranscriptomic studies regarding boreal ASS are now focusing on characterizing the microbial species living in this environment in more detail.

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Conflict of interest

Authors have no conflict of interest to declare.

Electronic supplementary material

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