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Chemical and microbiological evaluation of novel chemical treatment methods for acid sulfate soils

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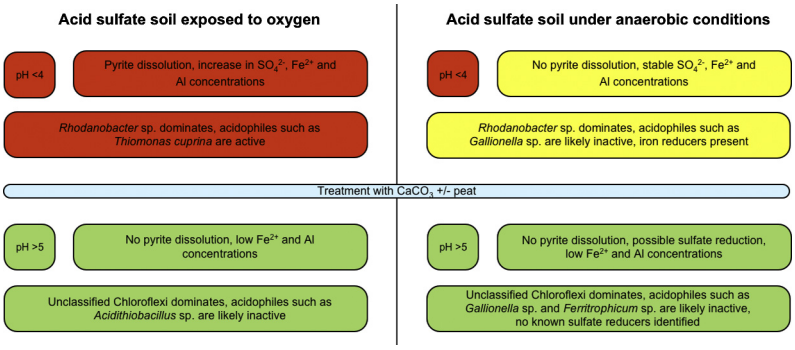
HIGHLIGHTS

- Naturally occurring sulfidic deposits are common and cause acid and metal release.
- Addition of $CaCO_3$ (plus or minus peat) decreased metal and acid release.
- Addition of peat alone was a poor candidate for mitigation.
- 16S rRNA gene sequencing identified moderate and extreme acidophiles.
- Identified acidophiles were inactivated by pH in the presence of $CaCO_3$.

GRAPHICAL ABSTRACT



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ABSTRACT

Naturally occurring sulfide rich deposits are common along the northern Baltic Sea coast that when exposed to air, release large amounts of acid and metals into receiving water bodies. This causes severe environmental implications for agriculture, forestry, and building of infrastructure. In this study, we investigated the efficiency of ultrafine-grained calcium carbonate and peat (both separately and in combination) to mitigate acid and metal release. The experiments were carried out aerobically that mimicked summer conditions when the groundwater level is low and acid sulfate soils are exposed to oxygen, and anaerobically that is similar to autumn to spring conditions. The ultrafine-grained calcium carbonate dissipated well in the soil and its effect alone and when mixed with peat raised the pH and reduced pyrite dissolution while peat alone was similar to the controls and did not halt metal and acid release. High throughput 16S rRNA gene sequencing identified populations most similar to characterized acidophiles in the control and peat treated incubations while the acidophilic like populations were altered in the calcium carbonate alone and calcium carbonate plus peat treated acid sulfate soils. Coupled with the geochemistry data, it was suggested that the acidophiles were inactivated by the high pH in the presence of calcium carbonate but catalyzed pyrite dissolution in the controls and peat incubations. In conclusion, the anaerobic conditions during winter would likely be sufficient to mitigate acid production and metal release from acid sulfate soils and in the summer, treatment with calcium carbonate was the best mitigation method.

Abbreviations: AS, acid sulfate; EC, conductivity; eDNA, extracellular DNA; ilr, isometric logratio; NTA, nitrilotriacetic acid; OTU, operational taxonomic units; PCA, principal component analysis; PMA, propidium monoazide; TRS, total reducible sulfur; ZTP, zero time point.

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1. Introduction

Naturally occurring sulfide rich deposits cover over 17 million ha of coastal areas. These soils occur in North America, Europe, Asia, Africa, Australia, and Latin America (Michael, 2013) and are common along the Finnish Baltic Sea coast. As long as they remain anoxic, i.e. below the water table, these deposits are stable with a neutral pH and bound metals (Boman et al., 2010; Nordmyr et al., 2006). However, when land uplift and artificial drainage for uses such as agriculture, forestry, and building of infrastructure expose these sulfidic materials to atmospheric oxygen, the iron sulfide minerals are rapidly oxidized. This process turns the sulfide rich soils into acid sulfate (AS) soils and mobilizes large quantities of acidity and leachable toxic metals (Boman et al., 2010; Nordmyr et al., 2006). The metals and acidity are leached from the soil and ultimately transported to the surrounding waters where they cause severe environmental problems such as ecotoxicological effects in aquatic organisms (Michael, 2013). AS soils can furthermore have a considerable negative economic impact by causing concrete and steel corrosion along with having poor geotechnical properties (Fanning et al., 2004). Due to these negative effects, efficient remediation methods for AS soils are severely needed.

It is known that acidophilic microorganisms living in AS soils catalyze iron sulfide mineral oxidation. However, very few studies regarding the microorganisms in AS soils have been published. In Finland, the acidophilic microorganisms have been identified to be similar to iron- and inorganic sulfur-oxidizing bacteria and archaea living in acidic environments such as acid mine drainage as well as microbes living in Arctic soils (Wu et al., 2013; Wu et al., 2015). Previous research has also found that treating AS soil from the oxidized horizon at 70–85 cm with solutions of ultrafine-grained calcium carbonate ($CaCO_3$) and calcium hydroxide ($Ca(OH)_2$) increases the pH of the soil and consequently, decreases the acid and metal discharge. However, the treatments did not change the composition of the microbial population in the soil (Wu et al., 2015) and it is still unknown if the resulting increase in pH inhibits microbial catalysis of the iron sulfide minerals.

The lack of observable changes in the 16S rRNA gene based microbial community after treatment with basic $CaCO_3$ and $Ca(OH)_2$ is potentially due to the clay rich soil preserving extracellular DNA (eDNA) as a result of the strong adsorptive binding of nucleic acids to clay particles (Nielsen et al., 2006). In addition, the clay suppresses DNA degradation due to the adsorption of DNases (Corinaldesi et al., 2008). Since the previous results by Wu et al. (2015) were based on direct DNA extractions, there is a possibility that the co-extraction of both intracellular DNA and eDNA resulted in an overestimation of the microbial diversity in the soil and gave a misleading picture of the intact and potentially viable microbial populations (Dlott et al., 2015).

An alternative strategy to mitigate iron sulfide oxidation is to deplete the AS environment of oxygen and thereby, inhibit the aerobic acidophiles. Previous studies have shown that addition of organic matter in the form of ground dry leaves into AS soil can promote sulfate reduction under aerobic conditions (Michael et al., 2015). The subsequent anoxic conditions are suggested to favor sulfate-reducing bacteria which re-alkalinize the AS soil and bind metals as insoluble sulfides (Michael et al., 2015). In addition to stimulating sulfate reduction, organic matter also functions as an antioxidant (Aeschbacher et al., 2012) and as it complexes dissolved ferrous iron (Yu et al., 2015), it immobilizes dissolved metals in the AS soil. An abundant organic material in northern wetlands is peat and one of its major intermediate breakdown products is acetate (Hines and Duddleston, 2001). Since acetate has already been found to inhibit iron oxidation in acidophilic microorganisms (Fang and Zhou, 2006), peat could be a good candidate for treatment of AS soils.

The aim of the study was to screen the chemical and microbiological effects of ultrafine-grained $CaCO_3$ and peat after incorporation with AS soil both separately and in a combination under both aerobic and anaerobic laboratory conditions. To obtain a more comprehensive image of the microbial population inhabiting the AS soil and the possible effect the treatments have, microbial community DNA was extracted and the 16S rRNA gene sequenced both directly from the soil and indirectly by first separating intact cells from the soil prior to DNA extraction. To our knowledge this is the first study to use compositional statistical data analysis for combined geochemical and 16S rRNA gene sequencing data.

2. Materials and methods

2.1. Soil description and sampling

The used AS soil was taken from an untreated field in the Risöfladan experimental area, Vaasa, Finland (63.045°N, 21.711°E). The parent sediment (>2 m below surface) contains 40% clay and 0.9% total reducible sulfur (TRS) that decreases in the acidified zone (~1 m below surface) to 33% and 0.2%, respectively (Nordmyr et al., 2006). Soils were collected by removing the plow layer (0–50 cm) and then pushing polyethylene tubes (14.2 cm inner-diameter) into the ground. The cores were dug up, sealed airtight, and stored at 4 °C. At the time, samples from the AS soil horizon were taken for estimation of dry weight (SFS-EN 12880) and loss on ignition of dry mass (SFS-EN 12879) that were determined to be 65.3% and 4.8%, respectively.

2.2. $CaCO_3$ and peat used for soil treatments

The technical grade $CaCO_3$ was ultrafine-grained with a median particle diameter of 2.5 μm (C2, Nordkalk Corporation, Finland). Previous analyses of the $CaCO_3$ (Wu et al., 2015) verified that it does not significantly contribute to metal and sulfur concentrations in the treated AS soils. The peat was supplied by Vapo Oy, Finland and was dried before ultrafine-milling using a Planetary Ball Mill PM 400 (Retsch GmbH, Germany) to a particle size ranging from 4 to 8 μm in diameter.

2.3. Experimental procedure

To investigate the effect of ultrafine-grained $CaCO_3$ and peat on AS soil, a modified method from Michael et al. (2015) was used. In order to avoid oxygen exposure, preparation steps were performed inside an anaerobic tent (Aldrich® AtmosBag, Sigma-Aldrich) as confirmed with resazurin. Treatments were conducted on three biological replicates, i.e. three different soil cores taken several meters apart. Immediately before the incubations, the cores were cut at the AS soil horizon (i.e. 75–90 cm), the top of the soil core was aseptically removed, and approximately 500 g of soil untouched by the polyethylene tube was placed in a sterile bowl for manual homogenization. A sample for microbial zero time point (ZTP) analysis was taken from the homogenized soil by completely filling a 50 mL sterile polypropylene tube with soil, sealing the tube cap tightly, and storing it at 4 °C until DNA was extracted the following day. The rest of the homogenized soil was divided into separate bowls for each treatment and manually mixed with 1% (wt/wt) $CaCO_3$, 1% (wt/wt) peat, or a 1% (wt/wt) mixture of equal parts $CaCO_3$/peat per dry weight soil. Untreated soil was used for control samples. To investigate the effect of the treatments under anaerobic conditions, 15 g from the treated and untreated soil was placed into separate 50 mL sterile tubes, completely filled with autoclaved MilliQ-ultrapure water, and sealed. Aerobic samples were prepared in the same way but outside the anaerobic tent and without the addition of water. The sample tubes were incubated for ten weeks at 10 °C (Wu et al., 2013) in the dark with the tube caps sealed tightly during the whole period. Anaerobic and aerobic samples for geochemical analyses were prepared and incubated in the same way except that three tubes per treatment and oxygen condition were prepared; one for starting point analyses (0 w), one for five weeks of incubation (5 w), and one for ten weeks (10 w).