Research article

Soil microbial community responses to acid exposure and neutralization treatment

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ABSTRACT

Changes in microbial community induced by acid shock were studied in the context of potential release of acids to the environment due to chemical accidents. The responses of microbial communities in three different soils to the exposure to sulfuric or hydrofluoric acid and to the subsequent neutralization treatment were investigated as functions of acid concentration and exposure time by using 16S-rRNA gene based pyrosequencing and DGGE (Denaturing Gradient Gel Electrophoresis). Measurements of soil pH and dissolved ion concentrations revealed that the added acids were neutralized to different degrees, depending on the mineral composition and soil texture. Hydrofluoric acid was more effectively neutralized by the soils, compared with sulfuric acid at the same normality. Gram-negative ß-Proteobacteria were shown to be the most acid-sensitive bacterial strains, while spore-forming Gram-positive Bacilli were the most acid-tolerant. The results of this study suggest that the Gram-positive to Gram-negative bacterial ratio may serve as an effective bio-indicator in assessing the impact of the acid shock on the microbial community. Neutralization treatments helped recover the ratio closer to their original values. The findings of this study show that microbial community changes as well as geochemical changes such as pH and dissolved ion concentrations need to be considered in estimating the impact of an acid spill, in selecting an optimal remediation strategy, and in deciding when to end remedial actions at the acid spill impacted site.

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1. Introduction

Acid spill accidents have occurred on a number of occasions in Korea recently, including an incident involving hydrofluoric acid in 2012 (An et al., 2015; Na et al., 2013). Spilled acids may migrate into subsurface environments either directly or indirectly in diluted forms, reacting with the soil components in the process and subsequently altering soil mineralogy, soil carbon cycling, plant productivity, microbial processes, microbial community composition, and so on. Whereas there have been many studies on spills of oil and organic solvents such as glycol ether (Braddock and McCarthy, 1996; Cooper, 2014; Farrington, 2014), few studies have reported on spills of inorganic acids and their effects on the subsurface environments.

Spilled acids will have direct impacts on the subsurface environments through increased acidity and anion concentrations. The acidity will exert a negative impact on the microbial community health before the acids are eventually neutralized in the subsurface environments by dissolution (e.g. carbonate or oxide minerals) and chemical weathering (e.g. phyllosilicate minerals) of soil minerals (Denham, 1998; Totsche et al., 2003) or reaction with soil organic matter (Michael et al., 2015). Therefore the degree of the

environmental impact caused by the increased acidity on the soil microbial community is expected to vary depending on acid neutralizing or buffering capacity of the soils. Dissociation of the acids will also release anions, such as fluoride, sulfate, nitrate, or chloride depending on the type of spilled acids, to the subsurface environments. The increased concentrations of the acid-derived anions may also affect the microbial community by changing the surrounding chemical environments (Waldron et al., 2009).

Most studies on the effect of acid on soil microbial communities have focused on prolonged acid stresses due to processes such as acid rain (Pennanen et al., 1998), acid mine drainage (Mills and Mallory, 1987), and soil acidification (Blagodatskaya and Anderson, 1999), as well as general characteristics of microbial communities with respect to soil pH (Bååth and Anderson, 2003; Fierer and Jackson, 2006). For example, the Hoosfield acid strip (Aciego Pietri and Brookes, 2008; Avery and Catt, 1995) has been studied as a model contaminated site that underwent a long-term acid impact. However, the microbial communities at the Hoosfield site and other similar sites are likely adapted to the acidified environments caused by prolonged acid exposure, including the establishment of acid-tolerant or acidophilic bacteria. These communities therefore may not reflect the acute impact of an acid spill event on the community in a previously pristine, non-acidified soil.

The aim of the present study is to investigate soil microbial communities' responses to accidental spills of sulfuric and hydrofluoric acid, and to subsequent neutralization treatments. This study also tries to find a bio-indicator which can easily be applied in estimating the degree of acid impacts and recovery from the impacts. Spill accidents are generally characterized by a sudden release of a large amount of acid from a point source such as an overturned tanker truck in a short time period. Under such circumstances, the knowledge obtained from the above mentioned studies may not directly apply. The acute and chronic impacts of spilled acids are expected to have multiple aspects, including chemical, mineralogical, biological, and ecological changes. Among these, this study focuses on the microbial community changes caused by spilled acids to assess and predict how common microbial communities respond to and recover from accidental acid spills. It will also be beneficial to develop a simple yet useful indicator that reflects the changes in microbial community in response to spilled acids similar to those that have been developed for bacterial and fungal communities with respect to general soil pH conditions (Bååth and Anderson, 2003; Rousk et al., 2010). Furthermore, the effect of a neutralization treatment on microbial community recovery is unclear and requires investigation.

2. Experimental

2.1. Soil preparation and chemicals

Three different soil samples were collected from two industrial complex sites (labelled IG and IM) and one forest site (FG) in Daejeon, Korea. The IG and IM soils were collected from a granitic and a metamorphic terrain, respectively. The forest soil sample was collected in a granitic terrain. The properties of the soils are listed in Table 1 and more detailed methods descriptions are given in Supplementary Information (SI Text S1). After collection, the soils were stored at 4 °C during transport and directly used in experiments without drying or sieving. Sulfuric acid (H_2SO_4, 98%, J.T. Baker) and hydrofluoric acid (HF, 48%—51%, J.T. Baker) were selected based on the amount of use and toxicity reported in our previous study (Shin et al., 2015). All other chemicals used in experiments were reagent grade.

2.2. Acid exposure and neutralization treatment

After removing plant roots and gravels, a 10 g of soil was placed in a 50 mL conical tube containing a 20 mL of a 10-fold-diluted nutrient broth (NB, pH 6.8) and then incubated in a shaking incubator at 30 °C and 180 rpm for 1 d. This incubation step was performed to grow a sufficient biomass to allow acid exposure without a complete loss of biomass. Even though the soil samples were pre-incubated, the soil samples exposed to 1 N of H_2SO_4 or HF were excluded from this analysis due to the destruction of DNA in the samples by acid exposure. The microbial community counts before and after the incubation and the detail description on the microbial community changes are provided in SI Fig. S1.

A 0.001, 0.01, 0.1, and 1 N H_2SO_4 or HF were added to the pre-incubated soil samples to simulate exposure to acid spill events. The acid-exposed soil samples were then incubated in the shaking incubator at 30 °C and 180 rpm for up to 10 d. Identical sample tubes were prepared for each soil sample, and a tube was taken for analysis as a function of time: before the acid addition (0 d), after 1 d and 10 d. The same experimental sets were prepared and 1 d after the acid exposure a 1 N NaOH solution was added until the slurry pH reached the soils' original pH before the acid exposure. The neutralized samples were further incubated and then taken for analysis 10 d after the neutralization treatment.

The solid and liquid phases separated by centrifugation at 5000×g for 30 min at 4 °C. The liquid phases were filtered using a 0.22 μm syringe filter. The filtered solutions were analyzed for major anions (F^-, Cl^-, Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) and cations (Si^{4+}, Al^{3+}, Ca^{2+}, total Fe, K^+, Mg^{2+}, total Mn, Na^+, Ti^{4+}) by ion chromatography (ICS-3000, Dionex, CA, USA) and inductively coupled plasma optical emission spectrometry (Optima 5300DV, Perkin Elmer, MA, USA; JY 38, Jobin Yvon, USA; ICAP 6500 DVO, Thermo Scientific, CA, USA), respectively. The solid phases were washed with a phosphate buffer saline three times to prevent further reaction by removing residual acid. A 5 g subsample of the washed solid was submitted for DNA extraction (see below), and the remainder was analyzed by x-ray diffraction (RTP 300 RC, Rigaku, Japan).

Table 1
Properties of the soils used in this study.

Soil type	Location	pH	Organic matter content (%)	Soil texture	CEC (cmol/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	Al (mg/kg)
Forest — granite (FG)	N 36°22′02.00 E 127°23′51.82	4.3	1.65	Sandy loam	9.46	14,257	326	1849	41	1627	17,471
Industrial — granite (IG)	N 36°22′10.14 E 127°24′00.55	7.3	2.42	Sand	2.80	49,860	2823	1447	246	1524	7886
Industrial — metamorphic rock (IM)	N 36°22′09.92 E 127°23′30.97	4.5	0.94	Silt loam	13.51	43,493	219	9155	93	7699	37,375