The effect of soil horizon and mineral type on the distribution of siderophores in soil

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Abstract

Iron is a key component of the chemical architecture of the biosphere. Due to the low bioavailability of iron in the environment, microorganisms have developed specific uptake strategies like production of siderophores. Siderophores are operationally defined as low-molecular-mass biogenic Fe(III)-binding compounds, that can increase the bioavailability of iron by promoting the dissolution of iron-bearing minerals. In the present study, we investigated the composition of dissolved and adsorbed siderophores of the hydroxamate family in the soil horizons of podzol and the effect of specific mineral types on siderophores. Three polished mineral specimens of 3 cm × 4 cm × 3 mm (apatite, biotite and oligioclase) were inserted in the soil horizons (O (organic), E (eluvial) and B (upper illuvial)). After two years, soil samples were collected from both the bulk soil of the whole profile and from the soil attached to the mineral surfaces. The concentration of ten different fungal tri-hydroxamates within ferrichromes, fusigen and coprogens families, and five bacterial hydroxamates within the ferrioxamine family were detected. All hydroxamate types were determined in both soil water (dissolved) and soil methanol (adsorbed) extracts along the whole soil profile by high-performance liquid chromatography coupled to electrospray ionization mass spectrometry (HPLC–ESI-MS); hence, the study is the most extensive of its kind. We found that coprogens and fusigen were present in much higher concentrations in bulk soil than were ferrioxamines and ferrichromes. On the other hand, the presence of the polished mineral completely altered the distribution of siderophores. In addition, each mineral had a unique interaction with the dissolved and adsorbed hydroxamates in the different soil horizons. Thus siderophore composition in the soil environment is controlled by the chemical, physical and biological characteristics of each soil horizon and also by the available mineral types.
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1. INTRODUCTION

In the soil environment, the microbial communities that colonize mineral surfaces differ from those of the surrounding soil particles (Certini et al., 2004). Microbial attachment to mineral surfaces leads to the formation of a microenvironment that protects the microorganisms against environmental stress (Beveridge et al., 1997; Liermann et al., 2000a;

Ojeda et al., 2006). In the microenvironments, mineral nutrients can be chelated directly from the soil minerals by certain microorganisms or shared among the surrounding microorganisms (Brown et al., 1994; Rogers et al., 1998; Roberts Rogers et al., 2001; Bennett et al., 2001; Roberts Rogers and Bennett, 2004). Most soil microorganisms can promote mineral weathering by production of siderophores which are defined as low-molecular-mass Fe(III)-binding compounds. Siderophores provide an efficient Fe-acquisition system due to their high affinity for Fe(III) complexation resulting in mineral dissolution (Kraemer, 2004). In soils that are enriched with iron oxide and clay silicate mineral phases, siderophores play a significant role in iron dissolution, making it available for microorganisms and

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plants (Hersman et al., 1995). There are several mechanisms for siderophore promoted iron dissolution (e.g., Holmén and Casey, 1996, 1998). The general mechanism is that the Fe-siderophore complex is formed at the mineral surface and is then transferred into the surrounding soil solution, thereby becoming available for uptake by the cell membrane of microorganisms or plants (Kalinowski et al., 2000a; Liermann et al., 2000b; Kraemer, 2004). Siderophores are either recycled or destroyed upon iron reduction, whereas the reduced iron Fe(II) that is not used by the cell can act as an electron donor in electron transport chains (Kalinowski et al., 2000b). The impact of siderophores on soil mineral weathering can be more effective compared to that of organic acids since siderophores form more stable complexes with Fe(III). Siderophores form 1:1 complexes with Fe(III), with binding constants ranging between $K = 10^{30}$ and $K = 10^{52}$ (Jalal and van der Helm, 1991; Matzanke, 1991), while the binding constants of oxalic and citric acids with Fe(III) are $K = 10^{7.6}$ and $10^{12.3}$, respectively (Perrin, 1979). The ligand protonation constant (pKa) values of siderophores were estimated between 7.3 and 13 dependent on the siderophore type (Raymond et al., 1984), whereas the pKa values of organic acids were ranged between 2.5 and 6.5 according to the acid type (Banaszak et al., 1999).

Microorganisms produce a wide range of siderophore types. Most of the bacterial siderophores are catecholates, and some of them are trihydroxamates and carboxylates, whereas most of fungal siderophores are hydroxamates (Schalk et al., 2011). The trihydroxamate ferrioxamine is produced by many soil bacteria, such as *Erwinia*, *Nocardia*, *Streptomyces*, *Arthrobacter*, *Chromobacterium* and *Pseudomonas* species (Meyer and Abdallah, 1980; Muller and Raymond, 1984; Berner et al., 1988; Gunter et al., 1993; Wei et al., 2007). In contrast, most hydroxamate siderophores produced by soil fungal species (i.e. *Suillus granulatus, Fusarium* spp and *Aspergillus* spp.) are of the ferrichrome family. Ferrichromes are divided into five groups depending on the side chain of the hydroxamate functional group: acetyl (ferrichrome, ferrichrome C, ferricrocin and ferrichrysin), malonyl (malonichrome), trans-b-methylglutaconyl (ferrichrome A), trans-anhydromevalonyl (ferrirubin) and cis-anhydromevalonyl (ferrirhodin) (Winkelmann and Huschka 1987; Renshaw et al., 2002).

Due to the importance of microbial siderophores in weathering and soil formation, the role of siderophores in the dissolution of iron minerals has been investigated intensively (Inoue et al., 1993; Watteau and Berthelin, 1994; Hersman et al., 1995; Hiradate and Inoue, 1998; Holmén and Casey, 1996, 1998; Kraemer et al., 1999; Liermann et al., 2000a; Kalinowski et al., 2000a; Stone, 1997; Reichard et al., 2005; Buss et al., 2007; Shirvani and Nourbakhsh, 2010). Hydroxamate siderophores produced by the ectomycorrhizal fungus *Suillus granulatus* are very efficient in the dissolution of goethite. High quantities (10^{-9} mol m^{-2} h^{-1}) of iron have been demonstrated to be mobilized in the presence of *Suillus* sp. because of the continuous production of siderophores (Watteau and Berthelin, 1994). Mineral dissolution is enhanced not only by siderophore-producing fungi but also by bacteria such as *Bacillus* sp., which have been documented to produce siderophores that promoted the dissolution of the surface of hornblende (Buss et al., 2007). The dissolution of Fe from the hornblende has been also observed in the presence of siderophore-producing actinomycetes such as *Streptomyces* and *Arthrobacter* (Kalinowski et al., 2000b). Therefore, the interactions between siderophores and iron minerals are directly related to the iron acquisition efficiency of the living cells in the soil environment (Shirvani and Nourbakhsh, 2010). For example, fungal siderophores (i.e. ferrichrome and ferricrocin) have been found to play a significant role in changing the surface structure of biotite and increasing its dissolution in podzolic forest soil (Sokolova et al., 2010).

Few studies have discussed the concentrations of siderophore in podzolic soil solution (Holmström et al., 2004; Essén et al., 2006; Ali et al., 2011). Many gaps still remain in understanding the relationship between siderophore content and mineral weathering in the field. Due to the wide variation of the chemical properties (e.g. pH and mineral nutrients) and microbial composition of each horizon in the podzol soil, the present study aimed to answer several questions: (1) How do the podzol soil horizon characteristics affect the concentration and distribution of hydroxamates? (2) Could the presence of different mineral types change the concentration and distribution of hydroxamates? (3) In which phase, dissolved or adsorbed, can siderophores be found in soil?

2. MATERIALS AND METHODS

2.1. Sampling site

Soils were sampled in September 2011 from central Sweden in the vicinity of the village Bispgården (63°07′N, 16°70′E). The site is located on a slope (angle 2°) at an altitude of 258 m above sea level and is forested with 80-yr-old Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The annual average precipitation is 700 mm, and is not acidic. The annual average temperature is +2 °C. The bedrock in the area is granite and gneiss. The soil is a typical haplic podzol (FAO, 1990). The soil horizons in the studied soil profile have the following depths: 12 cm for O (organic horizon), 10 cm for E (elluvial horizon), 9 cm for B (upper illuvial horizon) and finally C (parent material). Three polished minerals: biotite (purchased from Words, Canada), apatite and oligioclase (purchased from Krantz, Germany) with dimensions of 3 cm × 4 cm × 3 mm were used. The minerals were polished with 6, 3 and 1 μm diamond paste to get a fresh un-weathered surface. The three minerals were buried into the O-, E- and B-horizons at depths of 7, 14 and 28 cm, respectively from the top of the soil at June 2009 by Olofsson et al. (in preparation). The soil samples for this study were taken from the bulk soil of the whole profile and mineral surfaces and kept cold (+4 °C) until further analysis. The soil samples of each horizon were characterized chemically (Table 1).

2.2. Extraction of dissolved and adsorbed siderophores from soil

For extraction of dissolved siderophores, 1 g of air dried soil sample was added to 10 ml of Milli Q-water and shaken