



## Competitive sorption of microbial metabolites on an iron oxide mineral



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### ARTICLE INFO

#### Article history:

Received 16 April 2015

Received in revised form

22 July 2015

Accepted 25 July 2015

Available online 7 August 2015

#### Keywords:

Ferrihydrite

Sorption

Phosphate

Soil organic matter

Metabolomics

### ABSTRACT

A large fraction of soil organic matter (SOM) is composed of small molecules of microbial origin. However, the biotic and abiotic cycling of these nutrients is poorly understood and is a critical component of the global carbon cycle. Although there are many factors controlling the accessibility of SOM to microbes, sorption to mineral surfaces is among the most significant. Here, we investigated the competitive sorption of a complex pool of microbial metabolites on ferrihydrite, an iron oxide mineral, using a lysate prepared from a soil bacterium, *Pseudomonas stutzeri* RCH2. After a 24-h incubation with a range of mineral concentrations, more than half of the metabolites showed significant decreases in solution concentration. Phosphate-containing metabolites showed the greatest degree of sorption followed by dicarboxylates and metabolites containing both nitrogen and an aromatic moiety. Similar trends were observed when comparing sorption of metabolites with an equimolar metabolite mixture rather than a bacterial lysate. Interestingly, ectoine, lysine, two disaccharides and uracil were found not to sorb and may be more bioavailable in iron oxide-rich soils. Additionally, the highest-sorbing metabolites were examined for their ability to mobilize mineral-sorbed phosphate. All phosphate-containing metabolites tested and glutathione released phosphate from the mineral surface within 30 min of metabolite addition. These findings of preferential sorption behavior within a complex pool of microbial metabolites may provide insight into the cycling of SOM and specific nutrient availability. Finally, the release of highly-sorptive metabolites may be an underexplored mechanism utilized by microbial communities to gain access to limited environmental nutrients.

Published by Elsevier Ltd.

### 1. Introduction

Terrestrial ecosystems store more carbon than plant biomass and the atmosphere combined with soil organic matter (SOM) being the largest terrestrial pool of organic carbon (Batjes, 1996; Houghton, 2007). Many contradictory predictions exist regarding the effects climate change will have on microbial activity and composition and the role terrestrial ecosystems will play in the overall carbon cycle (Giardina and Ryan, 2000; Davidson and Janssens, 2006; Hopkins et al., 2012; Karhu et al., 2014). A critical step toward developing better terrestrial carbon cycling models is to understand not only the chemical composition of SOM, but factors controlling the stability, behavior and (microbial) accessibility of these compounds. This may ultimately allow us to predict

climate-induced alterations in microbial community composition and anticipate changes in carbon mineralization and respiration rates.

SOM is a complex mixture of compounds with a large fraction derived from microbial products (Schmidt et al., 2011; Ludwig et al., 2015). The soluble fraction (dissolved organic matter, DOM) accounts for less than 2% of SOM, but is the most accessible fraction for microbial degradation and therefore DOM turnover is likely an accurate representation of overall SOM cycling (Kalbitz, 2003; Kaiser and Kalbitz, 2012; Guigue et al., 2015). Subsoil DOM consists of microbial products (Kaiser and Kalbitz, 2012), but the specific chemical composition of DOM is just beginning to be more thoroughly characterized and includes carbohydrates, amino acids, organic acids, fatty acids, sterols and nucleosides (Kakumanu et al., 2013; Swenson et al., 2015; Warren, 2015). Microbes rely on DOM as an important carbon source and tend to prefer carbohydrates and amino acids over complex (possibly lignin-derived) aromatic molecules (Amon et al., 2001; Kalbitz, 2003). As a result of substrate

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preferences such as these, microbial function and community structure are highly influenced by the abundance and the composition of DOM (Hopkinson et al., 1998; Judd et al., 2006; Heckman et al., 2013a; Guigue et al., 2015). Hence, alteration of the DOM composition can have a drastic affect on microbial community structure.

In addition to microbial processing, many abiotic (e.g. sorption, precipitation, dissolution, diffusion) processes affect microbial accessibility and overall cycling of DOM (Zsolnay, 1996; Kalbitz et al., 2000; Kaiser and Kalbitz, 2012). Sorption to mineral surfaces is potentially the most impactful abiotic factor. Specific mineral content (clay, iron oxide minerals, etc) and surface area have a strong influence on the solid-solution partitioning and the accessibility of DOM components to microorganisms (Kalbitz et al., 2000). It would follow that the sorption behavior of specific metabolites may influence community structure by changing the bioavailability of DOM components favoring microbes that can best utilize the available components and access adsorbed metabolites. Indeed, recent studies show that soil composition, in terms of mineral and clay content, shapes microbial community structure (Heckman et al., 2013b; Vogel et al., 2014). For example, forest floor DOM sorption dynamics with goethite and gibbsite affect microbial community composition in a time-dependent manner (Heckman et al., 2013b).

There are limited studies aimed at examining the sorption behavior of well-characterized DOM components on minerals. However, there is general agreement that iron (hydr)oxide minerals such as goethite, ferrihydrite and hematite preferentially sorb molecules containing aromatic, nitrogen, phosphate and carboxylate groups (Zhou et al., 2001; Omoike and Chorover, 2006; Yeasmin et al., 2014). These strong, and often irreversible, mineral-nutrient interactions have a drastic influence on biological dynamics. While studies such as these have led to our understanding of sorption-based DOM stability of general classes of compounds, in order to link DOM cycling with soil microbial community dynamics, there is a need to investigate specific metabolite sorption behavior.

Another essential component of understanding nutrient availability in terrestrial systems is evaluating how mineral sorption of DOM molecules can affect the mobility of vital mineral-restricted nutrients such as phosphorus. Although total phosphorus in soils is generally high, available soluble forms are limited mostly due to phosphate sorption to mineral surfaces, especially iron and aluminum oxides (Arai and Sparks, 2007; Sharma et al., 2013; Wang et al., 2013). Microbes have developed many mechanisms for phosphate mobilization including production of phosphatases, lowering of soil pH and release of organic acids (Sharma et al., 2013). Possibly due to the limited availability of appropriate metabolomics technology, investigations into phosphate mobilization by high-sorbing DOM components, other than organic acids, are limited.

The objective of this study was to examine the sorption behavior of microbial metabolites as they exist in a competitive and complex, biologically-relevant mixture. Most studies to date tend to focus either on simple systems (Persson and Axe, 2005; Cagnasso et al., 2010) or complex mixtures of organics that are only roughly characterized (Kaiser and Guggenberger, 2007; Heckman et al., 2013a). We used lysates prepared from a soil bacterium to represent compounds commonly found in DOM. This mixture of microbial metabolites represents a unique system that is both highly complex and well-characterized (Swenson et al., 2015). Aged ferrihydrite, an iron (oxyhydr)oxide mineral, was used as our model sorbent due to its ubiquitous presence in natural systems and its nanoporous, highly reactive and large specific surface area (Hiemstra and Van Riemsdijk, 2009). Metabolite sorption as a function of mineral

concentration was monitored by liquid chromatography/mass spectrometry (LC/MS). Individual high-sorbing compounds were then selected to investigate the potential to desorb and mobilize phosphate from the mineral surface. These data may shed light onto exometabolite mechanisms by soil microbes to gain access to limiting nutrients such as phosphate.

## 2. Materials and methods

### 2.1. Materials

LC/MS-grade water and LC/MS-grade methanol (CAS 67-56-1) were from Honeywell Burdick & Jackson (Morristown, NJ). LC/MS-grade OmniSolv acetonitrile (CAS 75-05-8) was from EMD Millipore (Billerica, MA). MOPS (CAS 1132-61-2), HEPES (CAS 7365-45-9), 3,6-dihydroxy-4-methylpyridazine (CAS 5754-18-7), 4-(3,3-dimethyl-ureido)benzoic acid (CAS 91880-51-2), *d*<sub>5</sub>-benzoic acid (CAS 1079-02-3), 9-anthracene carboxylic acid (CAS 723-62-6), ammonium acetate (CAS 631-61-8), KH<sub>2</sub>PO<sub>4</sub> (CAS 7778-77-0) were from Sigma (St. Louis, MO). All compounds in the standards mixture were from Sigma (St. Louis, MO).

Ferrihydrite was synthesized according to the procedure of Schwertmann and Cornell (2000) and dry-aged in the dark for eight years. The resulting mineral was characterized by x-ray diffraction and shown to be low crystallinity, 2-line ferrihydrite. Bacterial lysates were prepared using the soil isolate, *Pseudomonas stutzeri* RCH2, obtained from Romy Chakraborty (LBNL). Bacteria were grown in M9 minimal media containing sodium acetate (0.2%) as the carbon source and lysates prepared according to the protocol in Swenson et al. (2015). In brief, cultures were grown in M9 minimal media until an optical density of 0.5 (at 600 nm) was reached. Cells were pelleted by centrifuging at 3220 × *g* for 10 min, washed with cold phosphate-buffered saline (pH 7.4) and re-pelleted. The supernatant was discarded and pellets resuspended in 1 mL cold methanol, sonicated for 2 × 20 s using a Q125 Qsonica sonicator then 5 min in a sonicating water bath (VWR symphony), centrifuged at 2348 × *g* for 5 min and the supernatant dried. Lysates were resuspended in sterile LC/MS-grade water resulting in a total organic carbon (TOC) content of 158 mg/L (Shimadzu TOC-V CSH analyzer, Kyoto, Japan), a pH of 6.8 and 55 (including six putative) metabolites identified by LC/MS analysis (Supplementary Table 1).

To explore the relative sorption behavior of bacterial metabolites when present in equimolar concentrations, the ferrihydrite sorption experiment was conducted with an equimolar mixture of standards. Each compound was prepared at 20 μM each (final concentration) in LC/MS-grade water, resulting in a pH of 6.8. The standards mixture contained 44 out of the 55 compounds reported for the bacterial lysates (the remaining 11 compounds were unavailable) plus five additional compounds (nicotinic acid, dodecanoic acid, hydroxy-proline, ornithine, raffinose) that were detected in the preliminary analysis of bacterial lysates (but not included in the final results of that experiment due to small and ambiguous peaks). Maltose and trehalose were included in the mixture to represent disaccharide 1 and 2, respectively. Of the metabolites added, five were not detected (coenzyme A, tyrosine, citramalic acid, glutathione and alanine) indicating that they may be present in higher concentrations in the lysate than in the standards mixture. The TOC of the standards mixture was 1.698 g/L.

### 2.2. Metabolite sorption assay

Resuspended lysates or standards mixture (1 mL) was added to 5 mL Eppendorf tubes containing mineral (0, 0.5, 1, 2, 4, 8, 16 or

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