Studies of Soil Mineral-Organic Matter Associations and Plant Nutrient Fixation Using Synchrotron X-ray Methods

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Abstract

To address some challenging biological and environmental problems, scientists at the Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (PNNL) have been pairing scientific capabilities at their home institution with the bright, tunable energy x-rays and high-resolution instruments at several national synchrotron user facilities. Synchrotron techniques can help answer a variety questions, and two examples featuring synchrotron-based structural and chemical imaging in bio/geochemical systems will be presented. In the first example, x-ray fluorescence spectromicroscopy and microtomography were used to visualize and better understand phosphorous uptake in poplar trees. Endophyte-promoted phosphorous uptake was seen inside poplar roots, where the fixated phosphorous appeared to be in the form of an organic phosphate. Analysis of the tomography data showing increased root mass for the plants inoculated with the endophytes supported the picture of increased nutrient uptake in those plants. These results along with in-house proteomics characterization point to the biological relevance of the symbiosis between endophytes and the host plant. In the second example, scanning transmission x-ray microscopy (STXM) combined with x-ray absorption near edge structure (XANES) helped us investigate soil mineral – soil organic matter (SOM) interactions in an alkaline soil from Washington state. Ca mineral–organic associations were found to be predominant which may play a critical role in the stabilization/degradation of SOM and mineral. Micro- and nanoscale characterization of the chemical state of both Ca from the mineral and C from the organic matter are crucial for understanding such stabilization mechanisms as well as soil nutrient dynamics.

Studies of Soil Mineral-Organic Matter Interactions and Plant Nutrient Fixation Using Synchrotron X-ray Methods

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Abstract

To address some challenging biological and environmental problems, we have paired scientific capabilities at the Environmental Molecular Sciences Laboratory (EMSL), PNNL with the bright, tunable energy x-rays and high-resolution instruments at two national synchrotron user facilities. Synchrotron techniques can help answer a variety questions, and two examples featuring synchrotron-based structural and chemical imaging in bio/geochemical systems are presented here. In the first example (middle panel), x-ray fluorescence imaging and spectroscopy as well as microtomography were used in combination with proteomics to visualize and better understand phosphorous uptake in poplar trees. In the second example (right side panel), scanning transmission x-ray microscopy (STXM) combined with x-ray absorption near edge structure (XANES) were used to investigate soil mineral – soil organic matter (SOM) interactions in an alkaline soil from Washington state.

Plant growth and harvesting

- Poplar samples inoculated with endophyte consortium (P-mix) and un-inoculated controls were prepared
- Nutrient mix prepared so that plants were nutrient limited
- Non-water soluble $Ca_3(PO_4)_2$ was chosen to confirm the capability of the endophytes to help the plant solubilize P from that compound and transform it into fixable nutrients
- Roots were harvested for physical measurements, x-ray imaging, and proteomics

Characterization

Proteomics

- Protein extraction and trypsin digestion prior to iTRAQ labeling and multiplexing of peptides, then iTRAQ Peptide Labeling
- High pH off-line C18 fractionation, followed by on-line low pH C18 separation and HCD MS/MS Qexactive detection
- Peptide sequence and relative abundance determination
- Significance determination and STRING Network Analysis

3D Imaging

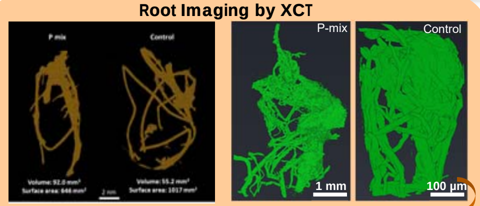
- X-ray Computed Tomography (XCT), both in-lab and synchrotron using the Advanced Light Source (ALS), beamline 8.3.2

2D Mapping & Spectroscopy

- Synchrotron X-ray Fluorescence Imaging (ALS 10.3.2 and APS 2-ID-E)
- XANES (ALS 10.3.2)
- STXM (mineral-SOM, ALS 5.3.2.2)

Results on P uptake in Poplar

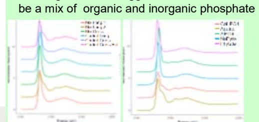
Root Imaging by XCT



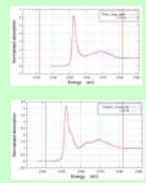
P-mix root V: 538.3 mm³, surface area: 1842.1 mm²; Control: V = 27.4 mm³, area 2940.6 mm²

XANES

P K-edge XANES suggests the chemical state of the new P fixated inside the root may be a mix of organic and inorganic phosphate



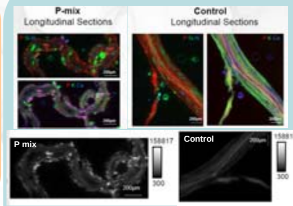
Linear Combination Fitting: P-mix sample has significant amount of organic phosphate, hot spots are inorganic.



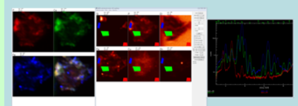
P-mix:
NaPyroP: 39.3 %
AlPO4: 11.8 %
Ca-phytate: 47.1 %

Control:
Amor. CaPO4: 18.6 %
Ca3(PO4)2: 66.0 %
Ca-(H2PO4)2: 14.3 %

Micro-XRF

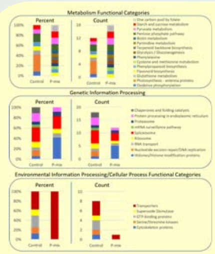


µ-XRF maps show evidence of P uptake in plant that has endophytic bacteria



P concentration consistently greater (by 20-30 %) for all P-mix samples than for controls

Proteomics



KEGG functional category enrichment distributions

STRING network analysis of proteins found to be significantly changed

- Abundance of POPTR_0003s14620, a protein involved in P sensing responses, significantly increased in P-mix samples
- More significantly changed proteins and more KEGG functional categories represented in P-mix roots than in the Control; greater access to P stimulates more metabolic pathways
- Enrichment of oxidative phosphorylation, photosynthesis-antenna protein and proteins involved in oxidation stress (e.g. glutathione metabolism and superoxide dismutase) in the Control
- ATPase POPTR_0009s12330, that plays a role in cell replication supporting increased growth of root cells with higher phosphate availability, increased in abundance in P-mix roots

Summary and Outlook

Endophyte-promoted phosphorous uptake was seen inside poplar roots, where the chemical form of the fixated phosphorous appears mixed (organic/inorganic). Analysis of the tomography data showing increased root mass for the plants inoculated with the endophytes supported the picture of increased nutrient uptake in those plants. These results along with in-house proteomics characterization point to the biological relevance of the symbiosis between endophytes and the host plant. Future work: investigate endophytic N fixation by STXM.

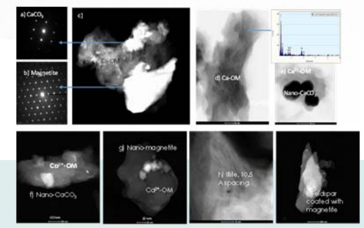
Ca mineral–organic associations were found to be predominant which may play a critical role in the stabilization/degradation of SOM and mineral. Micro- and nanoscale characterization of the chemical state of both Ca from the mineral and C from the organic matter are crucial for understanding such stabilization mechanisms as well as soil nutrient dynamics. Future work: identification of specific organics, modeling.

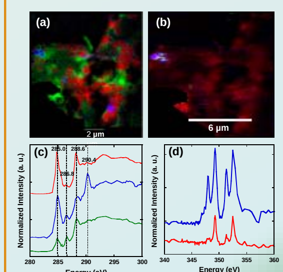
Soil Mineral-Organic Matter Interactions in Alkaline Soil from WA

Motivation:

- Significant amounts of SOM is stabilized by minerals (Fe minerals in e.g. acidic soils), pH dependence
- In alkaline soils, Ca appears to play a significant role
- Ca could exist in multiple forms; different minerals, Ca-OM complexes
- Role of Ca in OM stabilization is not fully understood
- Preliminary results and literature suggest that **understanding OM-Mineral interactions in this soil is critical**

- Ca- and Si- associated with OM
- OM is frequently associated with $CaCO_3$ and magnetite
- Sparsely coated on quartz and feldspar, and illite
- What is the nature of "coating" on Ca-minerals?

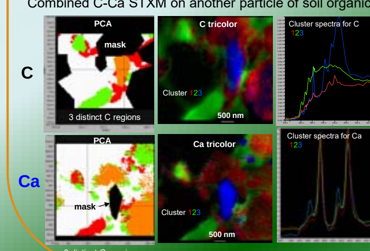


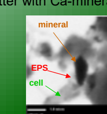


STXM on mineral-OM association from WA soil: (a) and (b) Tricolor STXM images depicting C and Ca distribution and C and Ca states, resp., on the same particle; (c) C K-edge NEXAFS spectra showing different functional groups on particle shown in (a). Red, blue, and green colors correspond to colored regions in tricolor (a).
285.0 eV: aromatic C;
286.8 eV: aliphatic C;
288.6 eV: carboxylic C;
290.4 eV: polysaccharide C*
(d) Ca K-edge NEXAFS spectra of corresponding regions of particle in. Red and blue spectra correspond to the colored regions in (b). Blue represents Ca from $CaCO_3$, red represents Ca primarily from Ca-OM bridges.

*Chen, *et al*., Environ. Sci. Technol. 2014, 48, 6678−6686, Henneberry *et al*., Organic Geochemistry 48 (2012) 81–89

Combined C-Ca STXM on another particle of soil organic matter with Ca-minerals:





- PCA and tricolor of C and Ca data show strong correlation between the C from OM and Ca, indicating Ca-OM bridging
- "Cell" spectrum matches the protein standard (BSA) spectrum well

EMSL, a U.S. Department of Energy national scientific user facility located at Pacific Northwest National Laboratory, provides integrated experimental and computational resources for discovery and technological innovation in the environmental molecular sciences to support the needs of DOE and the nation.

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