PROJECT REPORT

How can microbes help us fight climate change? The role of fungi in soil carbon stabilization

SUMMARY

Soils act as a major sink for atmospheric carbon (C) and, correctly managed, can help counterbalance the excessive CO_2 emissions. Organic C in soils can be physically stabilized and 'hidden' from its decomposers within soil aggregates and it is thought that soil fungi play a decisive role in "gluing together" and redistributing soil mineral particles and existing organic matter to form them. In this project we show that synchrotron X-ray radiation based techniques aid in disentangling nano- to macro- scale processes responsible for formation of organo-mineral interfaces and subsequent formation of soil aggregates. Specifically, STXM-NEXAFS images allow direct observations of fungal exudate depositions on minerals. Interacting with the mineral surface, these depositions can, for instance, reduce iron in goethite, which changes its properties, including solubility.

In the second part of the project we show that nitrogen rich organic matter in soil (here represented by litter of maze) generally facilitate formation of large macroaggregates in soils, with little influence from microbes. We also show that mineral agglomerations associated with this type of organic matter constitute larger fragments within soil microaggregates – a result that suggest bacterial influence on these formations. This points to the complexity of the many processes that govern soil aggregation.

Although further research is required to deepen the understanding of these processes, the results of this project provide insights to nanoscale processes of fungal exudation and their role in the formation of organo-mineral interfaces as well as subsequent soil aggregation.

PROJECT OUTCOMES

Synchrotron beamtimes:

Diamond Light Source, STXM beamline (I08), Oxfordshire, UK (2019-11)

Synchrotron facility SOLEIL, infrared microspectroscopy beamline (SMIS), Paris, France (2019-07 and 2019-11)

Synchrotron facility MAX IV, µXRF beamline (nanoMAX), Lund, Sweden (2020-04)

Conference contributions:

Milda Pucetaite, Per Persson, and Edith C. Hammer: Nanoscale STXM imaging of soil fungal exudates and organo-mineral interfaces. European Geoscience Union General Assembly EGU2020-4733.

Edith C. Hammer, Per Persson, and Milda Pucetaite: STXM analysis of fungal soil aggregation. European Geoscience Union General Assembly EGU2020-17614.

Manuscripts in preparation:

Milda Pucetaite, Adam Hitchcock, M. Obst, Per Persson, and Edith C. Hammer, Nano-scale STXM imaging of fungal-mineral interactions in soil, in preparation.

Milda Pucetaite, Per Persson, and Edith C. Hammer, Dynamics of soil aggregation as affected by organic matter inputs, in preparation.

Communication of the project goals and pilot results:

As a co-convener and chair of session 'Analytical methods as tools for new experimental approaches in soil science' within EGU2020: Sharing Geoscience Online, 4-8 May 2020

In an invited lecture in a weekly seminar series in the Vilnius National Center of Physical and Technological Sciences to present 'Bright light, big opportunities – use of synchrotron radiation based (micro)spectroscopy in environmental science' (2020-02-14)

In a workshop that I organized within Soil Science working group at Lund Institute for Advanced Neutron and X-ray Science (LINXS): Applications of X-ray and Neutron Imaging in Soil Sciences, 17-18 June 2019, Lund, Sweden (51 participant)

Sharing experience and expertise:

In a hands-on workshop that I have organized within Soil Science working group at Lund Institute for Advanced Neutron and X-ray Science (LINXS): Applications of X-ray Fluorescence (XRF) Microscopy in Soil Sciences, October 2020 (precise date to be announced), nanoMAX beamline, MAX IV, Lund, Swden

PROJECT COURSE AND RESULTS

BACKGROUND

Soils act as a major sink for atmospheric carbon (C) and, correctly managed, can help counterbalance the excessive CO_2 emissions. Organic C in soils can be physically stabilized and 'hidden' from its decomposers within soil aggregates and it is thought that soil fungi play a decisive role in "gluing together" and redistributing soil mineral particles and existing organic matter to form them (Schmidt et al., 2011). A significant contribution to the early aggregation process is adsorption of fungal exudates to the reactive surfaces of mineral particles. Fungi also move existing organic material, and include own EPS and dead cells into the forming aggregates. To uncover the mechanisms of C stabilization processes and to be able to increase the C sink potential of our soils, we need a deepened understanding of which fungi play key roles in the process, what mineral and organic matter properties promote it, and what type of fungal exudates are involved. Therefore, the dynamic processes of fungi-related soil aggregate formation, and the nature of fungal "gluing" agents are studied within this project. Different spectroscopic techniques, including synchrotron radiation based techniques at MAX IV laboratory and in collaboration with other large scale synchrotron facilities around the world, are used for this purpose.

METHODS

(1) At nano- to micro- scale – analysis of fungal 'gluing' agents

We have grown one species of each arbuscular mycorrhizal, ectomycorrhizal and saprotrophic fungi, alone or in the presence of two basic soil minerals – quartz (SiO_2) and goethite (α-FeO(OH)) – on top of X-ray transparent silicon nitride membrane windows. We have analyzed fungal hyphae and their exudates by high lateral resolution (50 nm) synchrotron based scanning transmission X-ray microscopy (STXM) in combination with near edge X-ray fine structure (NEXAFS) spectroscopy at absorption edges of C(K), K(L), N(K) and Fe(L). We focused on analyzing hyphal apexes – functionally the most active part of the hypha – and hyphal-mineral interfaces. We performed our experiments in the SM beamline at *Canadian Light Source*, Saskatoon, Canada and I08 beamline at *Diamond synchrotron facility*, Oxfordshire, UK. To confirm the results obtained, we performed additional measurements of the samples by optical photothermal infrared (O-PTIR) microspectroscopy at SMIS beamline at *SOLEIL synchrotron facility*, France.

(2) *At micro- to macro- scale – analysis of spatial arrangement of the building blocks of soil aggregates*

We have performed rare-earth element (REE) labelling experiments in sterile microcosms as described by (Gryze et al., 2006)and (Morris et al., 2019). Initially de-aggregated and sterile soil particles were labelled with different REEs (Nd2O3 and Sm2O3, 300 mg/kg). Milled maze leaves (low C:N) and straw (high C:N) were added to the batches of Nd and Sm labelled soil respectively, as well as water and microbes from natural soil. The batches were incubated for 4 weeks; then the formed aggregates containing different REE tracers were mixed and incubated for subsequent 6 weeks. The final aggregated soil was fractionated to large macroaggregates (>1mm), small macroaggregates (250µm–1mm), microaggregates (53–250µm) and small microaggregates (20-53 µm). The quantification of REE labels within each size fraction of aggregates is done by inductively coupled plasma mass spectroscopy (ICP-MS). Visualization of the labels has been performed by micro X-ray fluorescence (µXRF) imaging at nanoMAX beamline at *MAX IV synchrotron facility*, Sweden. The sub-micrometer scale µXRF mapping is the most suitable for the microaggregate analysis, while large macroaggregates were excluded from the µXRF experiments.

For the µXRF experiments, epoxy resin embedded individual aggregates were sectioned to approx. 1 µm using microtomy and placed on silicon nitride membrane windows. The sections were mapped for Nd and Sm labels with 200nm resolution.

RESULTS

STXM-NEXAFS analysis of fungal exudates. We have performed principal component analysis (PCA) and cluster analysis of the NEXAFS spectra of the hyphal apexes clearly showing presence of exudate layers around them (**Figure 1**). While precise chemical composition of the complex hyphal and exudate materials cannot be determined, in the NEXAFS spectra we identified absorption peaks that can be assigned to major chemical functional groups. In the control samples (not containing mineral particles) we were able to differentiate the mostly

proteinaceous hyphal material (represented by spectral band at (c) 288.2 eV) and the exudate layer constituting of mixtures of polysaccharides and proteins (represented by spectral bands at (c) 288.2 eV, (d) 289 eV and (e) 290 eV respectively). This was confirmed by analysis of O-PTIR spectra of the same samples. Relatively to the hyphae itself, the exudates also contain high amounts of potassium (represented by spectral bands at (f) 297 eV and (g) 300 eV). In the samples containing either quartz or goethite mineral particles we also observed higher amounts of aliphatic compounds (typically lipids) in the exudates (represented by spectral band at (b) 287.6 eV). For samples containing goethite, we specifically analyzed NEXAFS spectra at Fe(L) absorption edge and were able to show changes in iron speciation in the mineral particles that were in contact with the fungal exudates of ectomycorrhizal fungus *P. involutus*. It has been shown that *P.involutus* can reduce iron which could explain the increase of Fe(II) species in those goethite particles.

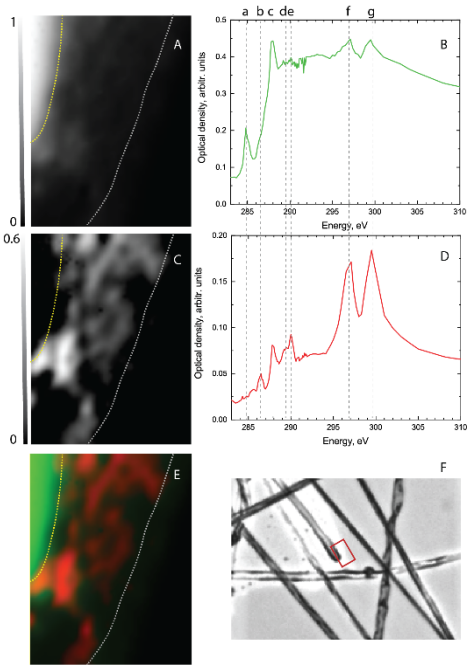

Figure 1 | NEXAFS spectra (B, D) and their respective distribution along STXM images (A, C) of fungal hypha surrounded by exudate halo (F); E shows an RGB overlay of A and C.

µXRF analysis of soil aggregates. Aggregate size distribution from our experiment shows that presence of maze (low C:N ratio) resulted in relatively higher amounts of large macroaggregates, while samples containing straw (high C:N ratio) or only microbes – small macroaggregates. Microaggregates, formed through physical agglomeration of particles, dominated the sterile samples. µXRF analysis provided snapshots of large microaggregates formed at the end of the second incubation period in the mixed REE labels soil pot (example in **Figure 2**). Here, blue color marks sand particles and red color - Sm labeled parts of soil containing maze. They constitute larger fragments of the aggregate. Nd labeled soil containing straw (in green) is distributed sporadically along the section of the aggregate. This confirms the hypothesis that bacterial-preferred N-rich organic matter forms larger fraction of microaggregate cores (Totsche Kai Uwe et al., 2017).

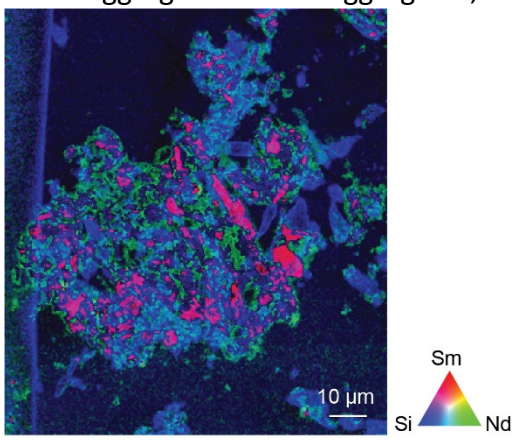

Figure 2 | RGB overlay of µXRF images recorded for Sm (red), Nd (green) and Si (blue).

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