

# **Plant-soil interactions and weathering within cryptogamic ground covers (CGCs): applying a novel multi-modal and multi-scale method via correlative imaging and focused ion beam (FIB) microscopy**

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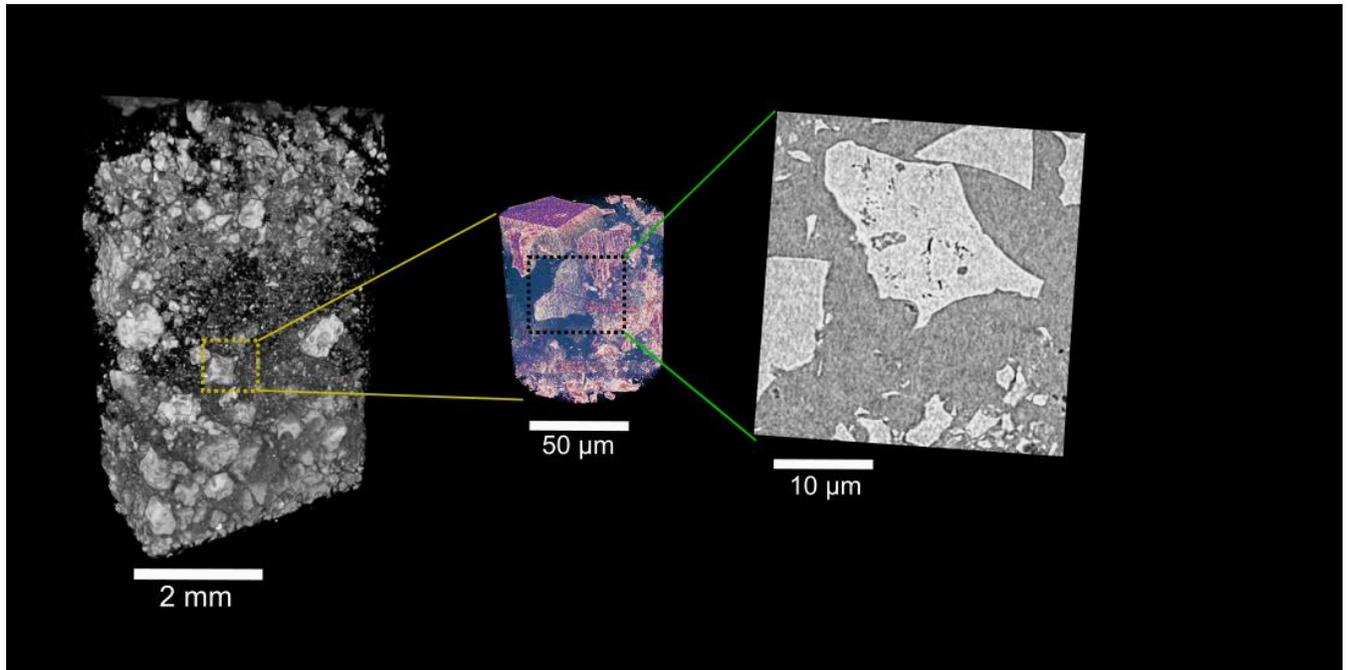
Correlative imaging provides an opportunity to understand the interactions and mechanisms involved in the structure of complex biological and geological systems by combining and utilizing information across dimensions (e.g., 2D to 4D), modes (microscopy to tomography), and scales (centimeters to nanometers).

Here, we have used the correlative potential of numerous imaging systems: optical light microscopy (LM), scanning electron microscopy (SEM), high resolution X-ray microscopy ( $\mu$ CT) and focused ion beam (FIB) microscopy to ascertain the structure and in-situ interactions present in cryptogamic ground cover (CGC) soils from Iceland. CGCs, often termed biological soil crusts, are composed of primitive early colonizing plants and organisms which include combinations of the bryophytes (mosses, liverworts, hornworts), lichens, algae, fungi, and bacteria. At the modern day they develop unique structural forms and contribute towards the stabilization of land surfaces through complex organic/substrate interactions [1]. Importantly, they are also considered modern analogues of early terrestrial plant habitats from around 450 million years ago, before the evolution of trees and herbaceous plants [2]. The expansion of a primitive terrestrial biosphere 450-400 million years ago had an influential effect on the architecture and evolution of river and sedimentary systems, and soil development, and crucially the drawdown of atmospheric CO<sub>2</sub> during organic carbon burial and the weathering process [3]. Before the evolution of expansive terrestrial biospheres, land surfaces were mostly barren (except for rare microbial crusts) meaning weathering was mainly by inorganic processes (such as hydrolysis reactions) [4]. Consequently, understanding the intricate interactions in modern CGCs can shed light on exactly how ancestors of these primitive organisms contributed to soil-forming processes, and how they develop organism/plant – soil interactions to promote biologically-mediated weathering.

Via high resolution in-situ  $\mu$ CT scanning of CGC soil cores collected from Iceland, we have identified various interactions including curious  $\sim 5 \mu\text{m}$ -wide probable biologically-mediated weathered ‘tunnels’ and pores within CGC soil grains. Successful segmentation of these ‘tunnels’ and pores has been achieved via Zeiss Intellesis machine learning segmentation to reveal complex structures and morphologies. We have utilized the correlative potential of Zeiss Zen Connect and Zeiss Atlas 5/3D software to combine 2D and 3D imaging and chemical datasets from LM, SEM and  $\mu$ CT of the soil; this has allowed targeted 4D region of interest study via FIB-SEM. The work extends the understanding of complex geological/biological systems by utilizing the potential of correlative workflows.

References:

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**Figure 1.**  $\mu$ CT volumes of soil core, displaying progressive high-resolution regions of interest. Image slice depicts grain of interest with internal tunnel and pore features.