



## **The contribution of bacterial cell wall fragments to the formation of soil organic matter - a case study from a glacier forefield**

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The contribution of microbial biomass to the formation of soil organic matter (SOM) is generally regarded to be low ( $<2\%$ ). However, recent results show that bacterial cell wall fragments frequently occur on soil mineral surfaces and accompany the microbial colonization of previously clean and sterile activated carbon surfaces after incubation in groundwater. Therefore, we hypothesized that in the initial stages of soil formation bacteria may play an important role in particulate SOM formation. For tracing the development of SOM, we analysed soil samples from a chronosequence in the forefield of a receding glacier.

Soil samples were taken from the forefield of the Damma-glacier, located in Canton Uri, Switzerland. Eight sites, spanning a soil age of 1 to 120 years, were sampled below the rooting zone. After removal of root remnants, the portion of the samples dedicated for scanning electron microscopy (SEM) analysis was immediately fixed with 2.5% glutardialdehyde solution in phosphate buffered saline; the rest was transported to the lab in sterile plastic bags for chemical analyses and phospholipid fatty acid (PLFA) extraction. Samples for SEM analysis were critically point dried, sputter coated with 10nm Au-Pd and analysed on a field-emission scanning electron microscope. For statistical analysis of the number and surface area of bacterial cell wall fragments, a  $10 \times 10$  grid was laid over scanning electron micrographs of size of  $32.5\mu\text{m} \times 32.5\mu\text{m}$  and 10 fields were randomly selected for independent examination by 3 persons. Additionally elemental analysis on selected specimens was performed by energy dispersive X-ray spectroscopy (EDX). Moreover, water contact angles as well as carbon, nitrogen and PLFA contents were analysed.

Spectroscopic analysis (SEM-EDX) of anticipated bacterial cell wall fragments gave increasing amount of carbon and lower carbon to nitrogen ratio (C:N) compared to mineral surfaces. However, the high penetration depth of the electron beam always results in an average spectrum of the anticipated cell wall fragments and the mineral surface below them, so the signal of the biomass component is attenuated. Hence, the fragments observed in scanning electron micrographs can be confidently characterised to be of biological origin. Both number and total surface area of cell wall fragments per field of view increased from younger to older soils, resulting in a complete organic coverage of the surfaces in soils older than 64 years. The overall C:N and the water contact angle have been found to increase over the sequence. Especially at the first three sites, an extremely low C:N of 6 to 10 indicates that SOM is primarily consisting of bacterial remnants, i.e. bacterial cell wall fragments. PLFA analysis revealed that in the younger soils biomarkers for bacteria dominate, while only in older samples also markers for eukaryotic microorganisms, mosses, and plants appear. Furthermore, the ratio of bacterial to fungal biomarkers increases strongly over the sequence. This further indicates the significance of bacteria for SOM establishment in developing ecosystems.

In conclusion, the initial step of SOM formation seems to be mainly controlled by bacteria and their fragments after cell death. This could be due to bacterial colonisation of previously uncovered mineral surfaces and the short generation time of bacteria compared to fungi or plants. Thereby, a complete organic coating of most mineral particles is already established 64 years after deglaciation.