WP3, D3.3, D22 An understanding of the selection and increased activity of N-fixers in glacier flour for the establishment of plants

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Introduction

As alpine glaciers retreat, ice-free land in the glacial forefield expands and experiences "greening" as vegetation slowly colonizes newly exposed and developing soils.[1-3] With increasing volumes of seasonal melt water, fine sediment known as glacial rock flour is entering downstream ecosystems in large quantities.[4] Due to its small particle size (less than 70 μm), glacial rock flour has a high surface area, which may make the material a relatively accessible microbial habitat and source of nutrients in glacial forefields. Abiotic weathering, resulting from physical and geochemical processes, and biotic weathering, resulting from the biomechanical and biochemical activity of microorganisms, plants, and other life, drive soil formation processes.[5] Microorganisms can also influence soil fertility via production of organic acids, enzymes, siderophores, and mechanical forces.[5,6] Microorganisms drive nitrogen cycling which, alongside atmospheric deposition and release of organic matter trapped in ice, will influence the concentration of nitrogen species such as NO_3, NO_2 and NH_4 in forefield soils.[7] Plants generally require nitrogen in NO_3 or NH_4 form, so the microbial ability to fix atmospheric nitrogen (N_2) into the soil and transform nitrogen pools enzymatically is indispensable for plant colonization and development of soil fertility.[8] However, our current understanding of the presence of nitrogen-fixing microbes in forefield glacial rock flours is limited to findings from diverse grain size sediments and initial soils sampled from glacial forefields, since the microbial communities that inhabit glacial rock flour specifically have yet to be reported. Research from forefields in Svalbard, Switzerland, and China offer evidence supporting the presence of diazotrophic microbial communities along chronosequences at the glacier forefields studied.[9-12] Using a strategic genomic approach, the selection and activity of diazotrophs in glacial rock flour could be determined with modern sampling and sequencing methods.

Figure and caption from Fickert (2017)[13]

Aspects of sample sites along the chronosequence in the glacier foreland of Goldbergkees (the central site out of three per level) illustrate the gradual vegetation change with time. Time since melt-out is 2 years (a), 4 years (b), 15 years (c), 25–30 years (d), 55 years (e), 85 years (f), 120 years (g), and 155 years (h).

Approach to sampling

Newly exposed glacial forefield sediments are oligotrophic environments.[9,11] The initial colonizers of glacial sediments are microorganisms that are tolerant to the relatively low nutrient content and contribute to the gradual accumulation of organic carbon and nitrogen during soil formation.[9-11] To study the potential selection for and activity of nitrogen-fixers related to plant establishment in forefields, glacial rock flour was sampled along a transect of soil development extending from the glacial terminus. Performing sampling along a chronosequence, which is typical of forefield soil development and ecological succession research, would support investigation of diazotroph communities in glacial rock flour.[14] Potential correlating variables including vegetation coverage, vegetation type, and physiochemical parameters can also be accounted for during sampling to better capture hypothesized ecological and geochemical relationships. Researchers might consider sample proximity to vegetation, considering that many symbionts detected in plant rhizobiomes are diazotrophic.[8]

Relevant molecular microbiological analyses

To study the abundance of diazotrophs in glacial rock flour, qPCR-based or shotgun metagenomic-based approaches can be pursued. Primers for qPCR targeting the nifH gene, which encodes the enzyme nitrogenase, responsible for catalyzing nitrogen fixation, are well established.[15] Quantification of nifH along a glacial forefield chronosequence has also been successfully performed, indicating the adaptability of the method for similar sediments.[12,16] To link the microbial community to its functional potential for nitrogen fixation, shotgun metagenomics may be applicable.[17] An advantage of this approach is that the functional potential for nitrogen fixation can be mapped back to specific members of the microbial community that are detected with sequencing.[18,19] This would provide evidence for which potential nitrogen-fixing taxa have been selected for, but still fails to capture activity.

To pursue analysis of nitrogen fixing activity in glacial rock flour, metatranscriptomic approaches can be applied. Metatranscriptomics (the sequencing of messenger RNA (mRNA)) can be used to identify the active portion of the soil microbial community.[20,21] The methodology involves the extraction of total RNA, followed by depletion of ribosomal RNA (rRNA) to enrich for mRNA transcripts.[20,21] These mRNA molecules can be sequenced directly or retrotranscribed into complementary DNA (cDNA).[20,21] A study on Tibetan Plateau glaciers utilized metatranscriptomes to reveal that while over 90% of glacial taxa carry the potential for nitrogen metabolism, only approximately 33% exhibit transcriptional activity, an insight that would not be available from qPCR or metagenomic methods alone.[22] Recent studies have also applied metatranscriptomics to study the immediate functional response of diazotrophs from high-arctic tundra and cryoconite aggregates.[23,24]

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