Relation of soil mineralogy and microbial communities based on micronutrient status

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Abstract
Micronutrients are trace elements which are needed by plants, animals and humans as well as microorganisms for healthy growth. The total amount and bioavailability of micronutrients is highly related to the composition as well as stability of major groups of rocks and minerals present in the soil, and can be released through various weathering mechanisms, among which bioweathering (mediated by soil microorganisms) has an important role. Meanwhile, microorganisms can also be influenced by the soil mineralogy. There is evidence that rocks and minerals with different composition support different microbial communities. It is, therefore, of interest to investigate to what extent the soil microbial communities are correlated with the mineral composition in terms of micronutrient content of soils, with focus on agriculturally important groups e.g. rhizobia and AM fungi. This objective will be achieved by setting up pot experiments with soils different in micronutrient content, amended with basaltic rockdust, and by a landscape study of soils with widely differing mineralogy.

Key Words
Bioweathering, microbial community composition, rockdust, trace elements.

Introduction
Trace elements are defined as elements that are present at low concentrations (mg/kg or less) in most soils, plants, and living organisms. Among these elements, boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are essential to the normal growth of higher plants (Alloway 2008) and cobalt (Co), Cu, Fe, iodine (I), Mo, Mn, selenium (Se) and Zn are essential to the growth and health of animals and humans (Welch 2008). These elements are collectively called micronutrients because even though some are present in large concentrations on earth, they are not required by organisms in large amounts. Micronutrient deficiencies can considerably reduce the yield as well as the nutritional quality of the crop products which are subsequently consumed by animals and/or humans (Alloway 2008), leading to nutrient deficiencies and imbalances. In addition to plants, animals and humans, microorganisms also require micronutrients. In relation to soils, microorganisms are important in the soil plant system because they are essential for the turnover and recycling of nutrients and some are important symbionts with plants that directly mediate uptake of nutrients e.g. arbuscular mycorrhiza (AM) fungi and others also help acquire nutrients e.g. by fixation of nitrogen. There is evidence of specific trace element deficiencies in rhizobia bacteria which are one of the most agriculturally important microorganisms and are known to require B, Co, Cu, Fe, Mn, Mo, Ni, Se and Zn for their survival as free-living soil saprophytes, as well as their symbiotic relationship with legumes (O'Hara *et al.* 1988). The response of rhizobia to nutrient deficiencies varies considerably between genera, species and strains (O'Hara 2001).

Whilst microorganisms have a significant effect on releasing the nutritional elements from minerals into soil environment through the mechanism of bioweathering, their community composition can also be affected by elemental constituents of different types of rocks and minerals. A literature review showed that there is evidence that mineral composition affects the structure of the associated microbial communities in some different environments (e.g. Boyd *et al.* 2007; Certini *et al.* 2004) including soil (Carson *et al.* 2009; Carson *et al.* 2007). However, all of the foregoing studies focused on the relation between microbial colonization and 'major' element constituents of different minerals, whilst trace elements (mainly micronutrients which are also needed by microorganisms) content of minerals may also be influential in this aspect.

In this project we are studying how minerals added to soils as well as differences in the inherent mineralogy of soils, may affect the microbial community composition. The study has a particular focus on agriculturally important crops and microorganisms as there is an important context in relation to adding minerals as nutrient sources in such systems. The aim is to answer the questions: how does variation in inherent mineral composition of soil explain microbial community composition, and how do soils varying from low to high availability of micronutrients (e.g. B, Mo) explain the diversity and abundance of rhizobia? Inherent mineralogical differences and associations with microbial community composition will be studied in a landscape scale study of Scottish soils using field samples of widely differing mineralogy while the addition of rockdust will be studied in pot experiments using Swedish soils.

Methods

Experimental set-up

A landscape study using the soil data and DNA archive of the National Soil Inventory of Scotland (NSIS) has been set up. In order to assess the functioning, composition and diversity of the microbial communities of soils, a set of physiological (MicroResp), biochemical (phospholipid fatty acids analysis, PLFA), and molecular (terminal restriction fragment length polymorphism, T-RFLP) techniques are used. Multivariate statistical methods will be used to analyse the data.

In two pot experiments we study if rockdust as a mineral amender improves the micronutrient content of the plants, as well as its effect on soil microbial communities using physiological, biochemical and molecular techniques. Plant biomass growth has been determined and plant samples are being analysed for micronutrient concentrations. In the first pot experiment three different soil types (sand, clay and peat) amended with two rates (high: 5 kg/m^2, low: 0.5 kg/m^2) of basaltic rockdust have been planted with a mixture of perennial ryegrass (*Lolium perenne*, L.) and red clover (*Trifolium pratense,* L.), and a treatment with silica dust used as control. The rockdust was mixed with the soils two year prior to our study and kept outdoors in a semi-natural growing area, enabling a study of more long-term effects of rockdust application. The second pot experiment was established 2009 using two soils poor in micronutrients and formed on different parent materials and a ryegrass and clover mixture. The same type and rates of rockdust and plants was used as described above.

Soil microbial methods

In MicroResp technique, substrate-induced respiration (SIR) through utilizing sole-carbon sources is used to determine and compare the physiological capacity of microbial community in contrasting soils (Leckie, 2005; Campbell *et al.* 2003). MicroResp has advantages over existing methods in that it does not rely on extraction of a soil suspension and subsequent growth of organisms (Biolog) and is a miniaturized system that is quicker and more sensitive than existing SIR methods (Lalor *et al.* 2007). Phenotypic fingerprinting of soil microbial communities can be done by the PLFA technique, which is based on the variability of fatty acids present in cell membranes of different organisms. After cell death, phospholipids degrades rapidly in soils and total PLFA content has been shown to correlate well with other measures of microbial biomass in soils (Bailey *et al.* 2002; Zelles *et al.* 1992). It has the additional advantage that the abundance of different microbial taxa can be estimated quantitatively. Molecular fingerprinting techniques are used for a rapid assessment of a microbial community, particularly for comparison or monitoring purposes. T-RFLP analysis (Singh *et al.* 2006) is based on restriction enzyme digestion of PCR-amplified DNA that has been fluorescently labelled at one end. As all molecular fingerprinting methods are likely to introduce biases during DNA extraction and PCR amplification but give a great deal more detailed information of which taxa, genus and species might be present depending on what genetic markers are used. TRFLP is being used widely due to its sensitivity and can give semi-quantitative information.

Soil mineralogical methods

In terms of mineralogical studies and sample characterisation, quantitative analysis of minerals using X-ray powder diffraction (XRPD) (Hillier 2003; Hillier, 1999) provides precise information about the mineral composition of soil. The chemical composition including the micro element concentration will be obtained by X-ray fluorescence (XRF) or total chemical dissolution and ICP-MS analyses of the digest. The partitioning of major elements between different mineral phases will be estimated by normative calculation as has been successfully demonstrated for K (Andrist-Rangel *et al.* 2006). This approach will now be further explored in relation to correlations between mineralogy and selected micronutrients of interest, e.g. Cu, Fe and Zn.

Progress in project
Two pot experiments were started in spring 2009, the first using soils that were mixed with rockdust 2007 and the second with two micronutrient deficient agricultural soils. Plants were harvested at the end of the growing season and are being analysed for micronutrients concentrations. Soils were sampled both before planting and after harvest and will be analysed using mineralogical, chemical and microbiological methods mentioned above during the winter and spring 2010. The landscape study has been started working on data sets and using DNA archive of NSIS and will be continued during spring 2010. This means that the project is in good progress and will have generated results both from the landscape study and the pot experiments that will be presented at the congress.

Conclusion
With the refined analytical methods available today, it is possible to study and get a better understanding of the relation between soil mineral composition and soil microbial communities as well as how soil microbial communities are affected by soil amendment with basaltic rockdust. This will have implications for what management approaches are options to improve micronutrient content of feed and food, e.g. within low-input agricultural systems.

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