Root-fungus symbiosis in agricultural crops selectively makes soil clays

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Abstract
In addition to the mutual association of roots and fungi, mycorrhizas benefit the general ecosystem through the production of soil clays. X-ray diffraction analyses showed that the fungus *Glomus* when inoculated onto barley, canola and alfalfa produced various types of potassium-deficient minerals (e.g., smectites) and slightly altered biotite, the K-rich mineral in the experiment. Non-inoculated plants produced mixed layer minerals, illite and left no biotite intact. The presence of K-poor clay minerals and unaltered biotite in inoculated samples implies selective K-extraction from some biotite grains to benefit plant growth and at the same time leaving unaltered biotite for future extractions. Symbiosis between fungus and plant roots (or mycorrhiza) is more efficient than roots alone in producing soil clays through the selective and efficient extraction of potassium ions from biotite

Key Words
Arbuscular mycorrhizae, agricultural crops, PCR.

Introduction

Fungal-root symbioses (or mycorrhizas) are found with an estimated 95% of all plant species (Sylvia 1998). It is known that mutual benefits results when host plants provide carbon to fungus and the latter supplies plants with nutrients and water. The symbiosis is more than beneficial to plant and fungus because synthesis of new clay minerals is often reported in root-soil interface (rhizosphere). The uptake of potassium by plants, such as ryegrass, converts soil biotite (potassium-rich mica) into vermiculite, a high-charged clay mineral (Hinsinger *et al.* 2006). The removal of magnesium from chlorite (magnesium-rich mineral) leaves behind high-charged hydroxy inter-layered clays (HIC) (Arocena and Velde 2009). Negative charges associated with surfaces of these high-charged clays and HIC are effective adsorption sites to store cations (e.g., magnesium and potassium) and improve the natural fertility of soils. Although HIC and soil vermiculites are commonly observed in many rhizosphere soils, the identity of fungi associated with the formation of new clay minerals is limited to our work in spruce and fir forest ecosystems (Glowa *et al.* 2004). In the Canadian sub-boreal forests, the symbiosis between *Piloderma* (an ectomycorrhizal fungus) and white spruce roots transformed soil mica and chlorite to HIC with subsequent improvement in soil fertility arising from increased cation exchange capacity (CEC) and exchangeable potassium and magnesium (Glowa *et al.* 2004). The removal of potassium and magnesium through uptake by white spruce trees developed some negative charges in soil biotite and chlorite, hence the synthesis of HIC and other high-charged clays in mycorrhizal soils. The objective of the study was to investigate the transformation of biotite in the rhizosphere soils of barley (*Hordeum vulgare*), alfalfa (*Medicago sativa*), and canola (*Brassica napus*) to determine the role of arbuscular mycorrhizal (AM) associations (*Glomus* sp) in the formation of expanding clays in rhizosphere soils of common crop plants.

Materials and methods

Growth chamber experiment

We cultivated three common agricultural crops (i.e. barley, canola and alfalfa) in a growth chamber. The growth medium was composed of Ottawa sand (691 g) and biotite (9 g) with the essential nutrients (except K) provided to the plants through a modified Hoagland solution. One group of seedlings (IN) were inoculated with MycoApply Seed Inoculant (Fort Myers, Florida) and maintained in a growth chamber while the second group in another growth chamber was left uninoculated (NIN). The plants were grown to 100 days and then harvested. Each treatment was replicated three times.

DNA extraction and amplification

Total DNA was extracted from root samples of non-inoculated (NIN) and inoculated (IN) alfalfa, barley and canola using a 2X CTAB (hexadecyl trimethyl ammonium bromide) protocol for plants (Hillis *et al.* 1996). DNA was extracted from the seed inoculant using the Ultraclean Soil DNA kit (MoBio, Carlsbad, CA,

USA), following the manufacturer's recommended alternative protocol for increased yield. Fungal DNA was amplified by polymerase chain reaction (PCR) using the universal primer ITS1 (White *et al.* 1990) and the AM fungal-specific primer AM1 (Helgason *et al.* 1998). DNA fragment analysis followed runs on a CEQ^{TM} 8000 automated sequencer (Beckman-Coulter Inc.).

X-ray diffraction analyses

Clay fractions were separated from rhizosphere (*i.e.*, soils clinging to roots) and non-rhizosphere (*i.e.*, > 3 mm from roots) collected from IN and NIN samples. Calcium- and K-saturated oriented clay samples were analyzed using a Bruker D8 with GADDS® x-ray diffractometer. X-ray diffractograms were subjected to deconvolution analysis (Lanson 1997) to estimate the proportion of various secondary clay minerals from the transformation of biotite.

Results and discussion

Advanced analysis (Lanson 1999) of X-ray diffraction patterns of clay minerals collected from rhizosphere soils showed that various types of low-potassium clays were produced from the potassic high temperature mineral biotite in non-inoculated (NIN) and *Glomus*-inoculated (IN) barley, canola and alfalfa (Figure 1A-F). The biotite in non-planted soil (control treatment) retained its original structure with a sharp 1.0 nm peak. Soil clay minerals produced by the alteration of biotite by NIN plants were dominated by a mixed layer (ML) mineral (mixed layering of potassic, micaceous mineral and a low K mineral, smectite), and illite (Figure 1B, D, F). *Glomus*, a mycorrhizal fungus used to improve the yield in cultivated crops (Powell 1981), when inoculated to barley, canola and alfalfa produced several types of K-deficient minerals from the initial biotite which were not present in the NIN treatments. Calcium saturation of clays in the air dried state showed 1.42 nm spacing indicating the presence of aluminum hydroxyl-compounds instead of the normal hydrated (two water layer) state (1.52 nm) common for smectite (Figure 1A, C). Mixed layering of illite and low K minerals was indicated by reflections at 1.17-12.2 nm (Figure 1A-D) which were somewhat modified by glycol treatment.

Both inoculated and NIN alfalfa produced illite (Figure 1E, F) which indicates only minor destabilization of the biotite structure due to minimal removal of K showing that plant species is an important factor in plant-soil mineral breakdown. Electrical charges on the HIC result from the removal of K in biotite, and are commonly compensated by cations (e.g., Ca^{2+}, NH_4^+, Mg^{2+}, Al-OH) in the soil system many of which are essential to maintain vigorous growth of plants and organisms. Thus, synthesis of HIC in rhizosphere improves the long term fertility of soils. In Figure 1, one can easily see that the IN samples showed mineral phases with peaks of higher basal spacing (low 2θ position) indicating stronger loss of K than in NIN samples. However, the most K-rich mineral is biotite with a peak at near 10.2 °2θ Co radiation was still present in IN samples while in the NIN samples, all biotite appeared to have been altered.

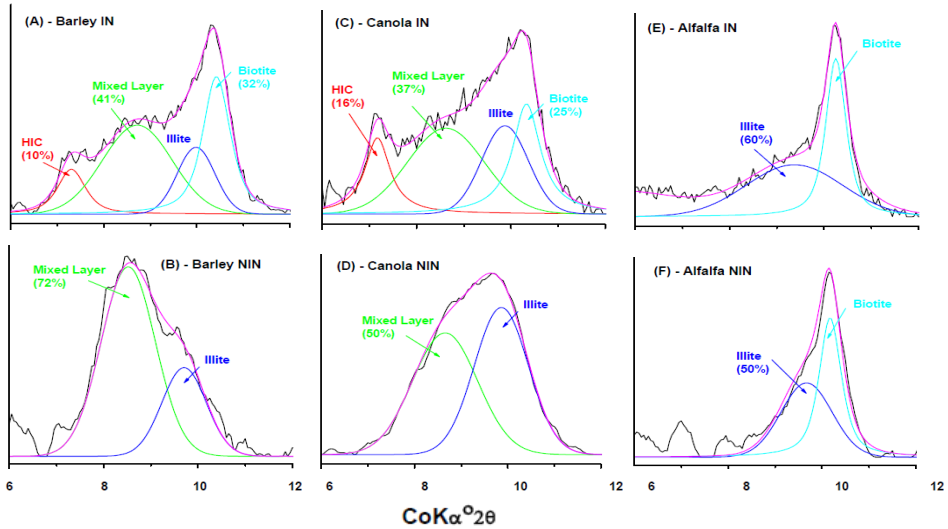

Figure 1. Biotite is transformed into several types of hydroxy-interlayered soil clays (HIC) in *Glomus*-inoculated (A, C, E) and non-inoculated rhizosphere soils of barley, canola and alfalfa (B, D, F). Position of the peaks to the left (lower angle) indicates more potassium extraction.

The presence of more K-poor minerals (*i.e*., smectites, HIC) in the IN than in NIN samples indicates a more efficient K-extraction process from individual biotite grains when *Glomus* and other organisms are present in the system (Figure 2). Overall, there were 57 fragments comprising the AM fungal communities ranging from approximately 95-700 bp in size. Of the 47 fragments in the IN group, only 7 were shared with the NIN group. Two DNA fragments were significantly associated with the IN group ($p=0.02$) whereas no fragments were specifically associated with the NIN group. In general, IN plants showed a greater diversity (i.e. richness of fragment sizes) of AM fungi than NIN plants. Differences in the functional attributes of different AM fungi colonizing a root system may increase resource acquisition and plant survival (Helgason and Fitter 2005). Most unique fragments were associated with alfalfa (IN); however, further analysis by plant could not be conducted due to small sample size (*i.e*., n=1 except for barley (NIN)).

Unaltered biotite in the IN samples suggests that there was a selective extraction of K from some biotite grains by mycorrhiza leaving others intact. In the NIN samples, no biotite is left intact and all material is altered but less in the extreme cases than in the IN samples. Thus, mycorrhiza selectively extracts K to immediately benefit plant growth and at the same time leave unaltered biotite for future extractions. Further in the NIN treatment, all biotite grains were transformed with the implication that much of the K released by root – mineral interaction can be lost from the plant growth system through leaching. It is interesting to note that the extent of alteration of the biotite grains is not only dependent on the mycorrhizal treatment, but also on the type of plant used. Overall alfalfa has less of an altering effect in both inoculated and non-inoculated experiments. Canola and barley have similar effects in the IN experiments but barley shows a greater effect (shift to lower spectral positions) than in the NIN experiment. This indicates, as one might expect, somewhat different responses of extraction efficiency depending upon plant type.

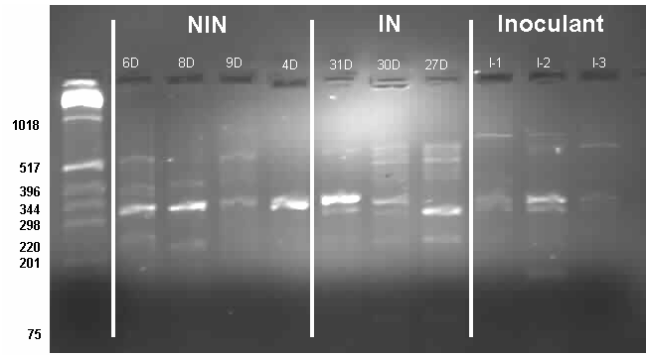

Figure 2. High resolution gel with 1 kb DNA ladder (lane 1) comparing PCR products of NIN, IN and inoculant samples. (NIN: 6D-alfalfa, 8D and 9D-barley, 4D-canola; IN: 31D-alfalfa, 30D-barley, 27D-canola).

Conclusion

We can conclude that the symbiotic plant–fungus system is more efficient through the selective extraction of K in some biotite grains, conserving K resources for future use in others. Our results provide useful information to the important role of fungus-root symbiosis in the maintenance of fertility and long term productivity of agricultural (and forest) soils.

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