

# Ectomycorrhizal Basidiomycete Fungi Detected in Rhizospheres of Mixed Grass Prairie Grasses

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Ectomycorrhizal basidiomycete fungi have the ability to aggregate and stabilize soil particles improving soil quality (Caesar-TonThat and Cochran 2000). Soil aggregating tests indicated that basidiomycete fungi from the russuloid clade form water-stable aggregates of soil particles and are very effective soil stabilizers (Caesar-TonThat et al. 2001a).

Apparent changes in soil structure and quality have been observed on native rangeland pastures after a few years of being managed with the twice-over rotation grazing management strategy; watershed harvest dams fail to fill because of a decrease in water runoff, and grass plant roots grow to greater depths of around 12 to 24 inches deeper than with previous grazing management practices. The predominantly clayey soils on a McKenzie county, ND ranch changed from a rooting depth and water holding soil profile of 2 to 3 inches to an aggregated soil of 18 to 24 inches in depth after seven years of management with the twice-over rotation system. A project was conducted to determine if ectomycorrhizal fungi were the cause of the observed improvements in soil characteristics on the twice-over rotation managed pastures.

## Procedures

This project was conducted on the NDSU Dickinson Research Extension Center ranch located in Dunn county in western North Dakota, USA, at 47° 14' north latitude, 102° 50' west longitude. The region has a typical continental climate (Manske 2007). Soils are primarily Typic Haploborolls developed on sedimentary deposits. Native vegetation is the Wheatgrass-Needlegrass Type (Barker and Whitman 1988) of the mixed grass prairie.

Soil samples with grass roots were collected during the field seasons of 1999 and 2000 from sandy and silty ecological sites of pastures managed with 6.0-m seasonlong and twice-over rotation grazing management strategies. Each of the grazing treatments had two replications. Livestock on the 6.0-m seasonlong management treatment grazed one native rangeland pasture for 6.0 months (183 days) from mid May until mid November. Livestock on the twice-over

rotation management treatment followed a double rotation sequence through three native rangeland pastures for 4.5 months (135 days) from early June until mid October.

Soil samples were analyzed for the presence of ectomycorrhizal basidiomycete fungi by TheCan Caesar-TonThat PhD, Soil Microbiologist, USDA, Agricultural Research Service, Sidney, MT. The soil field cores were divided into 3 layers: layer 1, 0-1 inch (0-2.5 cm); layer 2, 1-3 inches (2.5-7.5 cm); and layer 3, 3-5 inches (7.5-12.5 cm) in depth. Roots with 1 mm diameter and greater were delicately removed from each soil layer, placed on a 2 mm sieve, and immersed in water for 5 minutes with continuous agitation. The soil adhering to the roots was defined as water stable rhizosphere soil (Caesar-TonThat et al. 2001b).

An immunological technique, Enzyme-Linked Immunosorbent Assay (ELISA), was developed for the detection and quantification of ectomycorrhizal basidiomycete fungi (Caesar-TonThat and Cochran 2000, Caesar-TonThat et al. 2001a). Polyclonal antibodies were raised against a soil aggregating basidiomycete fungus (BB1) isolated from plant residue of a cornfield in eastern Montana (Caesar-TonThat and Cochran 2000). These antibodies cross-reacted specifically with fungi from the russuloid clade of the homobasidiomycetes (Caesar-TonThat et al. 2001a). Antigens derived from rhizosphere soil were prepared in carbonate buffer (pH 9.6) and were loaded in microtiter plate wells, followed by incubation overnight at 55° C. After three washings with PBS-Tween 20 buffer, polyclonal sera were added to each well. After 90 minutes of incubation at 22° C, the plates were washed, then incubated for 60 minutes at 22° C with horseradish peroxidase-conjugated goat anti-rabbit polyspecific immunoglobulins. The substrate consisted of a solution of 3, 3', 5, 5' tetramethylbenzidine and hydrogen peroxide. Absorbance was read at the dual wavelength of 450 nm. All samples were processed in triplicate. Results were statistically analyzed using ANOVA models (Caesar-TonThat et al. 2001b).

## Results

Absorbance readings (Caesar-TonThat et al. 2001b) for the detection of antigens in the rhizosphere soil adhering to roots of mixed grass prairie grasses determined the presence of ectomycorrhizal basidiomycete fungi from the Homobasidiomycete class and the Russuloid clade. These ectomycorrhizal fungi were previously unknown from the mixed grass prairie. The absorbance values were significantly greater on the twice-over rotation treatment than on the seasonlong treatment for all 3 layers of the sandy soils and for layer 2 of the silty soils. No significant difference was detected in layers 1 and 3 of the silty soils (Caesar-TonThat et al. 2001b).

## **Discussion**

Fungi in the Homobasidiomycete class and the Russuloid clade form ectomycorrhizae; the hyphae do not enter tissue of the host plant but develop a sheath around the root (Harley and Smith 1983). Rhizosphere organism growth and activity are limited by a deficiency of carbon (energy) (Curl and Truelove 1986). Enhancement of the development of ectomycorrhizal fungi in rhizospheres of mixed grass prairie grasses occurs as a result of the beneficial effects from defoliation by grazing controlled with the twice-over rotation grazing management strategy. Partial defoliation by grazing of grass tillers with 25% to 33% of the leaf material removed during phenological growth between the three and a half new leaf stage and the flowering (anthesis) stage (early June to mid July) causes exudation of greater quantities of carbon compounds through grass plant roots into the rhizosphere stimulating organism activity (Manske 1999). Active basidiomycete fungi form water-stable aggregates in soil that are water

permeable but not water soluble by secreting large amounts of insoluble extracellular polysaccharides that have adhesive qualities (Caesar-TonThat et al. 2001b). Adhesive polysaccharides act as binding agents for soil particles, causing aggregation of soil (Caesar-TonThat 2002) that range from about the size of air rifle pellets to the size of large marbles. Increases in soil aggregation enlarges soil pore size and improves distribution and stabilization of soil particles. These improvements in soil quality cause increases in soil oxygenation, increases in water infiltration, and decreases in erodibility (Caesar-TonThat and Cochran 2000, Caesar-TonThat et al. 2001a, Caesar-TonThat et al. 2001b, Caesar-TonThat et al. 2002, Manske and Caesar-TonThat 2003). Increased soil aggregation contributes to improvement of grassland ecosystem health and productivity.

## **Importance**

Detection of ectomycorrhizal basidiomycete fungi in the rhizosphere of grass plants in the mixed grass prairie is an important scientific discovery. Very few herbaceous species are known to form ectomycorrhizae on their roots (Harley and Smith 1983). Ectomycorrhizal fungi are slow growing and are limited almost exclusively to associations with woody perennial plants (Harley and Smith 1983).

Substantiation that defoliation by grazing controlled with the twice-over rotation grazing management strategy beneficially stimulates activity of ectomycorrhizal fungi that results in increased soil aggregation and thereby improving the quality of soil is a significant development for biologically effective grazing management of grassland ecosystems.

## **Acknowledgment**

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