

---

---

# Proliferation of Mycorrhizal Fungal Species on Organic Matter Improves Nitrogen Nutrition in Russian Wildrye

Atul-Nayyar<sup>1,2</sup>, Chantal Hamel<sup>1,2</sup>, Keith Hanson<sup>1</sup> and Jim Germida<sup>2</sup>

<sup>1</sup> Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, SK S9H 3X2

<sup>2</sup> Department of Soil Science, University of Saskatchewan, SK, S7N 5A8

---

---

**Key words:** Arbuscular mycorrhizal fungi, organic matter, decomposition, N mobilization, plant nutrition, biomass.

## Abstract

Arbuscular mycorrhizal fungi (AMF) facilitate plant growth by aiding nutrient movement to plants especially under low fertility conditions. Arbuscular mycorrhizal fungi generally takes N as  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . We hypothesized that AMF enhance soil organic matter decomposition through their stimulating influence on soil microorganisms and help in the transport of nitrogen to plants. Hyphal chambers (HC) containing labelled  $^{15}\text{N}$  organic matter (wheat root and shoot) were inserted in pots. Russian wild rye was inoculated or not with three AM fungal species (*G. intraradices*, *G. claroideum*, *G. clarum*) and was grown for six months. The amount of total C retained in the HC was lower in the presence of AMF hyphae as compared to control and substrate C:N ratio was lower indicating that decomposition was faster in AMF colonized systems. The faster rate of decomposition in HC in presence of AMF was concurrent with change in microbial community structure. Higher N uptake and greater plant biomass were measured in AMF treated Russian wildrye as compared to control. Results of the present investigation suggest that presence of AMF hastens organic matter decomposition, thus enhancing soil N fertility and plant growth.

## Introduction

Arbuscular mycorrhizal fungi are obligate biotrophs that depend on their plant partner for carbon supply and in return provide nutrients to plants. Most of the literature on the role of arbuscular mycorrhiza in plant growth and nutrition has been related to uptake of immobile nutrient such as P (Li et al., 2006). However, with the use of  $^{15}\text{N}$  tracer techniques it has been shown that AM fungi can also uptake inorganic N from soil and contribute to plant N nutrition (Johansen et al., 1994; Mader et al., 2000) but their role in residue decomposition and N mobilization is unclear. The objective of this study was to clarify the role of AMF in organic matter decomposition and N transport to plants.

## Material and Methods

### Experimental design

The pot experiment was set up in a greenhouse at the Semiarid Prairie Agricultural Research Centre in Swift Current, Saskatchewan, Canada. Each pot had a small hyphal compartment (HC) and a root compartment. The root compartment was a plastic pot and contained 6 kg of soil. The HC were made from PVC material and contained 4g of  $^{15}\text{N}$ -labelled organic matter (wheat residue, 38.44% atom) and pasteurised soil. Four Russian wildrye seedlings initially grown in growth cabinet were transplanted in pots. Plants were maintained at day/ night temperature of  $22^{\circ}\text{C}/18^{\circ}\text{C}$  with a photoperiod of 16h. Plants were watered as needed. The experiment was carried out for a period of 24 wk. At harvest shoots were cut near the soil surface and dried at  $40^{\circ}\text{C}$  until constant weight, and their dry biomass was recorded. The root samples were thoroughly washed with distilled water to remove the adhering soil, dried at  $40^{\circ}\text{C}$  and weighed. Root sub samples were thoroughly washed with distilled water, stained (Vierheilig et al., 1998) and analyzed for AMF root colonization (Giovannetti and Mosse, 1980). Soil from each pot was passed through 2mm sieve and analysed for hyphal lengths (Newman, 1966). The HC were carefully removed from the pots and the mixture of organic matter and soil in HC was used to analyse fatty acid methyl esters (FAME) as described by Hamel et al (2006). The mixture of soil and organic matter was also analysed for  $^{15}\text{N}$  content by element analyser Carlo Erba NA1500 coupled with mass spectrometry (Optima, V.G. Isothch).

The significance of treatment effects on soil and plant variables were assessed by ANOVA using jmp 3.2.6, (SAS Institute, Cary, USA) and means were compared using fisher's LSD test at  $\alpha=0.05$ . Differences in soil microbial community composition was determined using backward stepwise discriminant analysis (Legendre and Legendre, 1998) using Systat v. 10 (Point Richmond, USA).

## Results

### Mycorrhizal root colonization

The roots of uninoculated plants were not colonized. In the inoculated treatments, all the three mycorrhizal fungal species successfully colonized Russian wild rye plant roots. AMF root colonization was highest for *G. clarum* while *G. claroideum* infected Russian wild rye to a lesser extent than the other two species (Table 1).

### Hyphal length density

The hyphal densities for all the treatments are shown in Table 1. Note that the background values of hyphal densities from uninoculated plants was not subtracted from values in AMF pots. The external hyphae of all the three AMF species developed extensively and the hyphal densities were higher than those for uninoculated control. The hyphal densities for the *G. claroideum* and *G. intraradices* species was significantly higher as compared to *G. clarum*.

### Total C and C:N ratio

The amount of total C in HC was lower in AMF treatments as compared to control and there were no differences among AMF species. A similar trend was observed for  $^{15}\text{N}$  in HC.

The ratio of carbon to nitrogen in the HC of all the three species did not differ significantly among themselves but it was significantly lower than the control treatment (Table 1).

### Soil microbial community structure

Mycorrhizal fungal species modified the microbial community structure inside the HC as revealed by soil PLFA profiles (Fig 2). The backward stepwise discriminant analysis as grouped by fungal species showed variability in microbial community due to 11 PLFAs. However, there was no change in total active biomass (data not shown).

### $^{15}\text{N}$ plant content

The  $^{15}\text{N}$  concentration in AMF treated plants was significantly higher as compared to control treatment (Fig 1). The  $^{15}\text{N}$  content of shoots was 26%, 31.7% and 25.2% and that of roots 10.8%, 11.6% and 8.4% greater for the plants treated with *G. claroideum*, *G. clarum* and *G. intraradices* respectively as compared to untreated control. However, there was no significant difference in the  $^{15}\text{N}$  content of shoots and roots of mycorrhizal plants.

### Plant biomass

Shoot and root dry biomass varied among different treatments. Russian wild rye in symbiosis with *G. clarum* and *G. intraradices* produced 16% and 24% higher shoot biomass as compared to control while shoot biomass for *G. claroideum* was at par with control (Fig 1). A similar trend was observed in root dry mass with 45% and 35% higher biomass in *G. clarum* and *G. intraradices* respectively as compared to control (Fig 1).

Table 1. Total C and C:N ratio in hyphal chambers, AM root colonization, hyphal density in different treatments

AMF Species	Total C (HC §)	C:N ratio	AMF root colonization (%)	Hyphal density (mm g <sup>-1</sup> soil)
Control	1.56 <sup>a</sup>	12.31 <sup>a</sup>	0.00 <sup>c</sup>	16.57 <sup>c</sup>
<i>G. claroideum</i>	1.25 <sup>a</sup>	9.80 <sup>b</sup>	18.80 <sup>b</sup>	22.74 <sup>a</sup>
<i>G. clarum</i>	1.05 <sup>b</sup>	9.58 <sup>b</sup>	25.60 <sup>a</sup>	21.92 <sup>b</sup>
<i>G. intraradices</i>	1.13 <sup>a</sup>	9.84 <sup>b</sup>	21.30 <sup>a,b</sup>	23.31 <sup>a</sup>
P value	0.07**	0.02	0.0001	0.0001

§ = hyphal chambers

Means (n=5) followed by different letters in the same column are significantly different

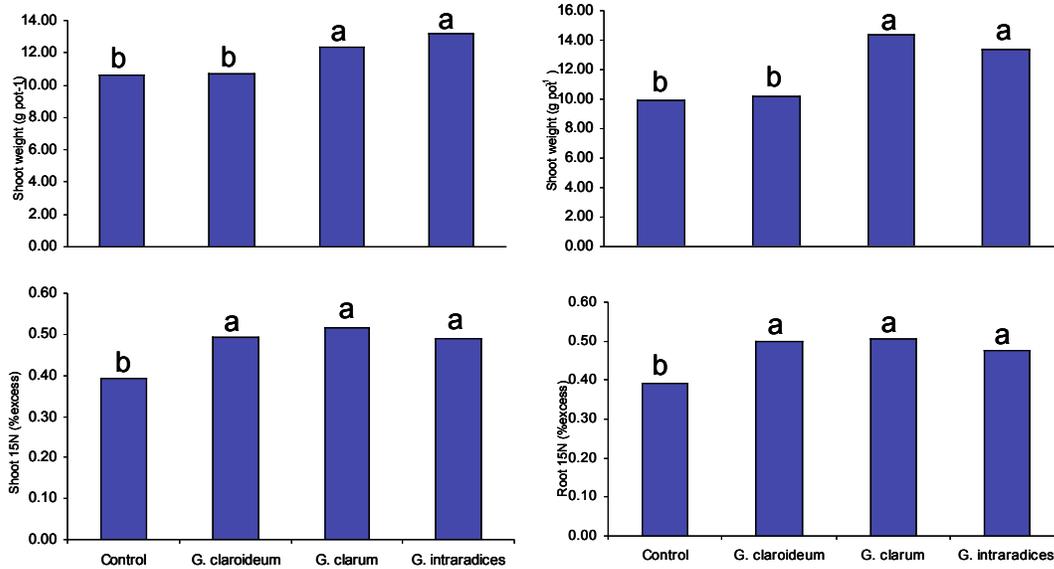


Figure 1. Root and shoot biomass and 15N content in AMF treated Russian wildrye

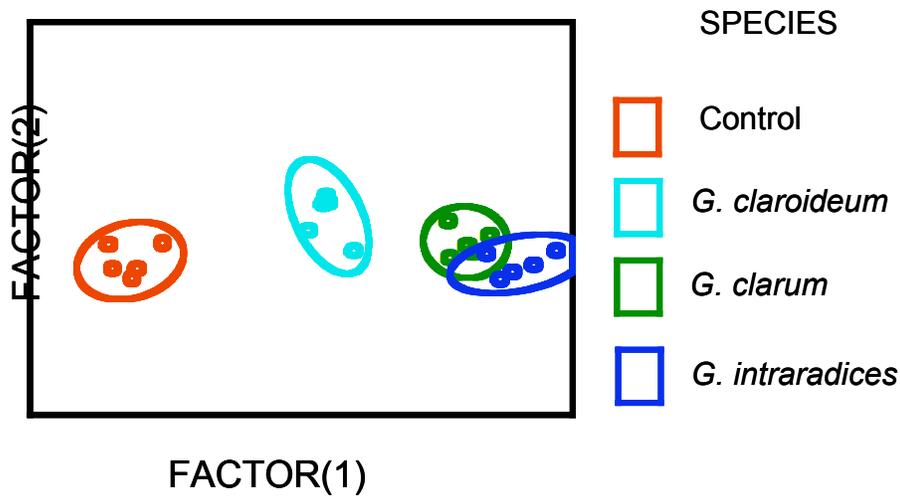


Figure 2. Soil microbial community composition as influenced by AMF treatments

### Discussion

Our results suggest that the presence of AMF enhances organic matter decomposition and mobilizes N from the decomposing organic matter to the host plant. The hyphae of all the three AMF species proliferated extensively in the presence of organic matter in the HC. The lower amount of total C retained and lower C:N ratio of the substrate in HC in presence of AMF hyphae indicated that AMF hastens soil organic matter decomposition. Based on the higher <sup>13</sup>C retention in their control chambers, Hodge et al (2001) also reported that faster residue decomposition was due to the presence of AMF hyphae. As compare to their study, we tested the role of different species of AMF in organic matter

decomposition. We report here that all the species tested could accelerate organic matter decomposition.

The faster decomposition of organic matter in HC in the presence of AMF hyphae could be due to change in microbial community composition brought about by the hyphae. The increased microbial activity in HC in the presence of fungal hyphae could be due to increased availability of energy-rich C compounds derived from the host plant (Nakano et al., 2001; Zak et al., 2000). The C drawn from plant by AMF hyphae is distributed in soil (Johnson et al., 2002) leading to differences in microbial population in mycorrhizal systems (Ravnskov et al., 1999).

During the process of decomposition, microorganisms draw energy by breaking C bonds and release CO<sub>2</sub> through respiration. In the process C:N ratio of soil organic matter decreases. The nitrogen in excess of the microbial demand is released into soil in mineral form. In our study we report higher C loss in HC in the presence of AMF hyphae as compared to control. Hasten soil organic matter mineralization and is further supported by lower C:N ratio in mycorrhizal treatments. Mycorrhizal fungi were able to scavenge the N released from organic matter decomposition and transport it to host plants. The benefit of increased <sup>15</sup>N capture from organic matter was apparent in terms of greater plant biomass. Both root and shoot biomass was higher in AM plants as compared to control. Enrichment of plants with N from organic matter by AMF has significant implications in understanding ecosystem functioning and N cycling in soils.

Results obtained from the study suggest that presence of AM fungi facilitates organic matter decomposition by modifying the soil microbial community structure. It is of significant importance in that arbuscular mycorrhizas may play an important role in plant N uptake in soils where decomposing organic residues is an important N source. AM fungi reach out and capture nitrogen released during decomposition of organic residues and transfer it to plants possibly improving effective cycling of N in the soil-plant system and reducing environmental N losses.

#### **References:**

- Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84:489-500.
- Hamel, C., K. Hanson, F. Selles, A.F. Cruz, R. Lemke, B. McConkey, and R. Zentner. 2006. Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotations of the Canadian prairie. *Soil Biology and Biochemistry* 38:2104-2116.
- Hodge, A., C.D. Campbell, and A.H. Fitter. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature (London)*. 413:297-299.
- Johansen, A., I. Jakobsen, and E.S. Jensen. 1994. Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. *Plant and Soil* 160:1-9.

- Johnson, D., J.R. Leake, N. Ostle, P. Ineson, and D.J. Read. 2002. In situ  $^{13}\text{CO}_2$  pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist* 153:327-334.
- Legendre, P., and L. Legendre. 1998. *Developments in Environmental Modelling* 20., 2nd English Ed. ed. Amsterdam: Elsevier. Numerical Ecology:853.
- Li, H., S.E. Smith, R.E. Holloway, Y. Zhu, and F.A. Smith. 2006. Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. *New Phytologist* 172:536-543.
- Mader, P., H. Vierheilig, R. Streitwolf-Engel, T. Boller, B. Frey, P. Christie, and A. Wiemken. 2000. Transport of N-15 from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytologist* 146:155-161.
- Nakano, A., K. Takahashi, and M. Kimura. 2001. Effect of host shoot clipping on carbon and nitrogen sources for arbuscular mycorrhizal fungi. *Mycorrhiza* 10:287-293.
- Newman, E.I. 1966. A method for estimating total length of root in a sample. *Journal of Applied Ecology* 3:139-145.
- Ravnskov, S., O.L.E. Nybroe, and I. Jakobsen. 1999. Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF57 in rhizosphere and hyphosphere soil. *New Phytologist* 142:113-122.
- Vierheilig, H., A.P. Coughlan, U. Wyss, and Y. Piche. 1998. Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. *Appl. Environ. Microbiol.* 64:5004-5007.
- Zak, D.R., K.S. Pregitzer, J.S. King, and W.E. Holmes. 2000. Elevated atmospheric  $\text{CO}_2$ , fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist* 147:201-222.