

Community structure of arbuscular mycorrhizal fungi in roots of subsequent soybean after cultivation of various winter crops



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1. Introduction

Arbuscular mycorrhizal (AM) fungi improve the uptake of phosphate from soil through symbiotic associations with plant roots. The introduction of winter crops has been shown to increase nutrient uptake and yield of subsequent crops, and may result from increased AM fungal biomass in the soil. However, there is little information regarding the effects of different winter crops on AM fungal communities of subsequent crop roots. The present study evaluated the impact of various winter crops on AM fungal community structure in the roots of subsequent soybean.

2. Materials & Methods

1) A two year field experiment was conducted at Nihon University in Kanagawa, Japan. Replicated plots of winter sown wheat, spring sown wheat, canola and fallow were established in 2007, and wheat, red clover, canola and fallow were established in 2008. After the winter crop, soybean was sown in all plots (Table 1) in the andosol.

Table 1. Cultivation summary of cropping systems.

2007-2008		2008-2009	
Winter crops	Summer crops	Winter crops	Summer crops
Winter wheat	Soybean	Wheat	Soybean
Spring wheat	Soybean	Red clover	Soybean
Canola	Soybean	Canola	Soybean
Fallow (No weeds)	Soybean	Fallow (No weeds)	Soybean

2) Root samples of soybean crops were also taken at full-flowering stages for AM colonization and DNA analysis

3) The community structure of indigenous AM fungi was characterized on the basis of the large subunit ribosomal DNA (LSU rDNA) allowing the identification of phylotype.

4) DNA was extracted directly from dried crop roots. A nested PCR reaction (First PCR: Universal primer LR1/FLR2 (Trouvelot et al., 1999), second PCR: Glomeromycota specific primer FLR3/FLR4) (Gollet et al., 2004) was used to select for AMF specific LSU rDNA. Twenty-four AMF libraries of LSU rDNA genes were established.

5) In this study, about 90 sequence samples were analyzed in each clone library. The rarefaction curves on the basis of analyzed sequence numbers in each clone library almost reached a plateau (Data is not shown).

3. Results

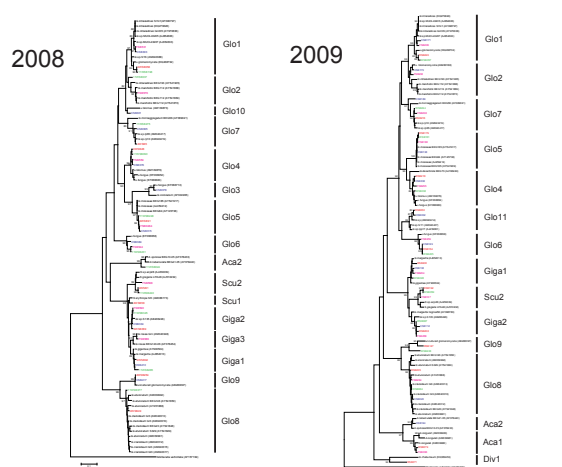


Fig. 1 Neighbor-joining tree of partial LSU rDNA sequences from AM fungi, isolated from soybean root samples, including known AM fungal sequences from DDBJ database for comparison. Bootstrap values (only values > 80 are shown) were estimated from 1,000 replicates. Representative sequences from roots are incorporated. Each individual sequenced sample is labeled with a prefix (In 2008, 11WSB, 4WSB, CSB and FSB denote the soybean roots of winter wheat, spring wheat, canola and fallow plots, respectively. In 2009, WSB, RSB, CSB and FSB denote the soybean roots of wheat, red clover, canola and fallow plots, respectively) followed by sample number.

1) Phylogenetic analysis revealed that 19 AM fungal phylotypes including eleven *Glomus*, three *Gigaspora*, two *Scutellospora*, two *Acaulospora*, one *Diversispora* were found in the roots of soybean.

Table 2 Detected phylotype in the roots of soybean in 2008

Phylotype	2008			
	Winter wheat	Spring wheat	Canola	Fallow
Glo1	+	+	+	+
Glo2	+			+
Glo3			+	+
Glo4	+	+	+	+
Glo5	+	+	+	+
Glo6	+	+	+	+
Glo7	+	+	+	+
Glo8		+	+	+
Glo9		+	+	+
Glo10			+	
Scu1		+		
Scu2	+	+	+	+
Giga1	+	+	+	+
Giga2	+	+	+	+
Giga3	+			+
Aca2	+			

Table 2 Detected phylotype in the roots of soybean in 2009

Phylotype	2009			
	Wheat	Red clover	Canola	Fallow
Glo1	+	+	+	+
Glo2		+	+	+
Glo4	+	+	+	+
Glo5	+	+	+	+
Glo6	+	+	+	+
Glo7	+	+	+	+
Glo8	+	+	+	+
Glo9	+	+	+	+
Glo11			+	
Scu2		+		
Giga1	+	+	+	+
Giga2	+	+	+	+
Aca1		+		+
Aca2			+	
Div1		+		

2) The number of phylotype in soybean roots was not difference among various winter crops, but *Glomus* was dominant in roots of soybean regardless of winter crops.

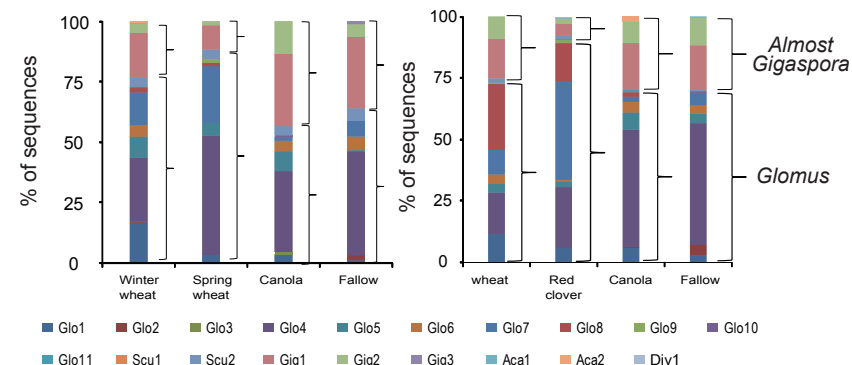


Fig.2. Bar plot showing the relative abundance of the different phylotype observed in the soybean roots from each plot, in 2008 and 2009.

3) In general, *Glomus* was dominant in the roots in 2008 and 2009. The community of canola and fallow was similar tendency both year.

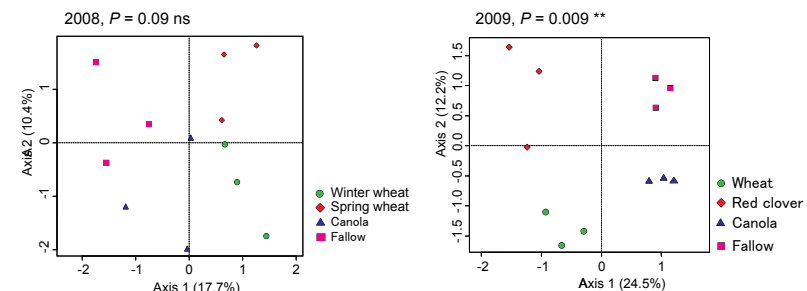


Fig. 3. Redundancy analysis (RDA) plot of AM fungal communities in the roots of soybean after the cultivation of various winter crops in 2008 and 2009. Percentage of total explained variance by first two axes are given in parentheses. Effects of winter crops on the AM fungal community structure were tested in RDA with Monte Carlo 1000 permutation tests.

4) In RDA, the AM fungal communities in the roots of soybean was not significantly difference in 2008 ($P = 0.09$ ns) but was significantly difference in 2009 ($P = 0.009$ **) by Monte Carlo permutation test.

There was significantly difference relationship between various winter crops and AM fungal communities of the roots in 2008 (Winter wheat: ns, spring wheat: $r^2 = 0.604$, $P < 0.03^*$, canola: ns, fallow: $r^2 = 0.623$, $P = 0.01^*$). In 2008, there was also significantly difference between various winter crops and the AM fungal communities of the roots (Wheat: $r^2 = 0.694$, $P = 0.003^{**}$, red clover: $r^2 = 0.804$, $P < 0.001^{***}$, canola: ns, fallow: $r^2 = 0.560$, $P = 0.01^*$).

4. Conclusion

In the present study, choice of winter crop has the potential to change the community structure of AM fungal communities in the roots of soybean.

We will continue to examine the impacts of crop rotation on AM fungal community and their dynamics to determine if a correlation exists between the crop growth and specific AMF community.