Dynamics of Community Structures of Arbuscular Mycorrhizal Fungi in the Different Winter Cropping Managements

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INTRODUCTION

The introduction of the preceding and winter crops of the symbiotic relationship with arbuscular mycorrhizal (AM) fungi has been shown to increase nutrients and yield of subsequent crops through the hyphal extension and increasing of AM fungal density in the soil. However, there is little information regarding the dynamics and functions of AM fungal community in the different winter cropping systems.

The present study evaluated 1) whether the community structure of AM fungi in the rhizosphere soil is affected by the difference of winter cropping managements and 2) if the change of the AM fungal community impacts the growth of subsequent soybean plants.

MATERIALS AND METHODS

This experiment is a two year field plot study of 9m^{-2} at the affiliated field with Nihon University, in Kanagawa preference of Japan. Three replications of four winter cropping plots (Table 1) in the andosol.

Five soil samples per plot were taken before and after the cultivation of winter crops. The samples were bulked into one composite sample (Table 2).

Root samples of winter and summer crops were also taken at intermediate stages for AM colonization and DNA analysis (Table 2).

Table 1. Cultivation summary of winter cropping systems.

	1st year			2nd year	
Plots	Winter crops	Summer crops	Plots	Winter crops	Summer crops
4W	Fallow-wheat (Sowing wheat on 5 April 2008)	Soybean	R	Red clover (Sowing on 31 October 2008)	Soybean
11W	wheat (Sowing on 19 November 2007)		W	Wheat (Sowing on 31 October 2008)	
11C	Canola (Sowing on 19 November 2007)		C	Canola (Sowing on 31 October 2008)	
F	Fallow (No weeds)		F	Fallow (No weeds)	

Table 2. Determination of these factors in this study.

Experiment	Evaluation
Soil chemical properties	soil pH, Available P, Total P N, C & EC dynamics
Spore density	Spore density dynamics
Root colonization	Persent of root colonized by AMF
Community analysis	Detected OTUs of AM fungi by molecular techniques
Plant N & P	Plant growth parameters

This experiment was used molecular techniques to assess the influence of four different winter cropping managements on community structure of AM fungi, both rhizosphere soil and dried crop roots.

The community structure of indigenous AM fungi was characterized on the basis of the large subunit ribosomal DNA (LSU rDNA) allowing the identification of operational taxonomic units (OTUs).

DNA was extracted directly from both rhizosphere soil and dried crop roots. A nested PCR reaction (First PCR: Universal primer LR1/FLR2, second PCR: Glomeromycota specific primer FLR3/FLR4) was used to select for AMF specific LSU rDNA.

RESULTS

AM fungal spore density

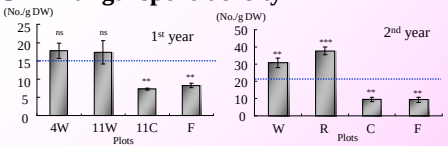

Figure 1. AMF spore density after the winter cropping managements. Vertical bars indicate ± S.E. of the mean (n= 4). ····· shows the AMF fungal spore density after the cultivation of the soybean plants. ns, not significant,. **, *** significant at 1%, 0.1% level.

The graphs in Figure 1 show the AM fungal spore density after the cultivation of host crops was higher than that of canola and fallow throughout the two year field plot study.

This result appears that the cultivation of mycorrhizal-crops as winter crops is effective means to maintain or increase the AM fungal spore density in the soil.

Community analysis of AM fungi

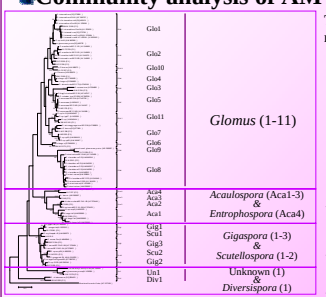

Figure. 2. Neighbor-joining tree of partial LSU rDNA sequences from AMF, isolated from the each winter crop roots and soil samples, including known AMF sequences from DDBJ database for comparison. Bootstrap values (only values > 75 are shown) were estimated from 1,000 replicates.

Table 3. Dynamics of AM fungal community in the different winter cropping managements.

	1st year									2nd year								
	After the soybean Sampling day	Sampling day						After the soybean Sampling day	Sampling day							
OTUs	18-Nov-07	8-May-08				10-Jun-08		30-Oct-08	4-May-09				2-Jun-09			
	Soil	Root		Soil				Soil	Root		Soil					
		4W	11W	11C	4WS	11WS	11CS	FS		R	W	C	RS	WS	CS	FS
Glo1		+	+		+	+				+	+		+	+		
Glo2	+	+	+			+	+		+							
Glo3																
Glo4	+	+	+	+	+	+	+		+	+	+		+	+	+	
Glo5		+	+	+		+	+				+			+	+	
Glo6	+	+	+		+	+	+		+	+	+		+	+	+	
Glo7	+	+	+		+	+	+		+	+	+	+	+	+	+	
Glo8		+	+	+	+	+	+		+	+	+		+	+	+	
Glo9	+	+	+		+	+	+		+				+	+	+	
Glo10							+									
Glo11		+			+					+					+	
Scu1																
Scu2	+	+		+		+	+			+					+	
Giga1	+	+								+					+	
Giga2	+	+													+	
Giga3	+															
Aca1		+		+		+										
Aca2			+				+									
Aca3										+						
Aca4	+															
Div1		+				+										
Un1	+	+				+	+									
Average OTUs		8.7	7.0	2.0	5.7	5.7	4.0	3.7		5.7	4.3	0.3	3.3	3.7	5.7	6.7

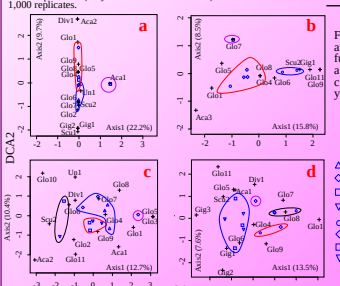

Figure. 3. Detrended correspondence analysis (DCA) ordination of AM fungal OTUs from the roots and soil. a: Roots in 1st year, b: Soil in 1st year, c: Roots in 2nd year, d: Soil in 2nd year.

In this two year field plot study, a total of 22 OTUs of AM fungi were detected in the roots and soil (Figure. 2).

Table 3 shows the detected OTUs of the AM fungal community in each three replicates data compiled into one. The average OTUs in the roots of wheat and red clover were higher than that of canola for the two year. But, the average OTUs in the soil were not different tendency in the 1st year and 2nd year.

➢*Scutellospora* and *Gigaspora* was detected in the roots of spring wheat (4W), but *Glomus* was only detected and dominant in the roots of winter wheat (11W and W).

In Figure. 3, the DCA ordination shows pattern of the AM fungal community in the roots and rhizosphere of the different winter cropping managements. The results using cluster analysis shows that there are three or four groups in the roots and soil. Especially, the AM fungal community of non-mycorrhizal cropping plot such as canola and fallow in 2nd year clustered same groups.

The effects of AM fungal community after the winter cropping managements on the growth of the subsequent soybean

Table 4. The correlation coefficients between the detected OTUs (Roots & soil after the winter cropping) and the growth of the subsequent soybean (full flowering stage, R2).

	1st year	2nd year
Above plant biomass (g m^{-2})	0.198 ns	-0.154 ns
Plant P concentration (mg g^{-1})	0.262 ns	-0.228 ns
Plant P contents (mg m^{-2})	0.198 ns	-0.162 ns
Plant N concentration (%)	0.227 ns	-0.057 ns
Plant N contents (g m^{-2})	0.197 ns	-0.138 ns
AM colonization ratio (%)	-0.221 ns	0.039 ns
Number of OTUs in the soybean roots	0.102 ns	-*

ns: not significant. *- shows it is still analyzing.

Table 4 shows the correlation coefficients between the detected OTUs (Roots & soil after the winter cropping) and the growth of the subsequent soybean (R2 stage). In this study, there were no correlation relationships between the detected OTUs and the growth of the subsequent .

The difference of the AM fungal community in the different winter cropping managements did not impact the growth parameters of the subsequent soybeans.

CONCLUSIONS

The different winter cropping managements resulted in:

➢In the 1st year, the AM fungal community of the wheat roots was different by the different sowing season day with same wheat (Table 3).

➢Although the multiple AM fungi such as *Glomus*, *Gigaspora*, *Scutellospora* and *Acaulospora* were detected in the roots of red clover, but the OTUs detected from the roots of wheat was only *Glomus* in the 2nd year (Table 3).

➢The overall community across our plots does not appear to be changing dramatically based on the presence or absence of the detected OTUs (Figure. 3 and Table 3). That is, one potential explanation for this result is that the AM fungal community shift slowly with the crop rotation such as the introduction of the winter crops .

TO BE CONTINUED IN 3rd YEAR

➢Continue to examine the impacts of crop rotation on AM fungal community and their dynamics to determine if a correlation exists between the summer soybean N and P uptake and specific AMF community.

➢Experiment to be repeated in 2009 to 2010.