Effect of Fallow on Arbuscular Mycorrhizal Fungi under Maize Cropping System

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Abstract Fallow had been reported to decline survival of arbuscular mycorrhizal (AM) spore. The effect of fallow on AM fungi under maize cropping system was studied to evaluate its effects on population of AM fungal species in community, AM spore abundance and biodiversity. A pot experiment was undertaken over two successive crops. The crop 1 was maize planting in all pots for establishing a uniform AM community. The crop 2 was maize planting and leaving pot soil as fallow. At the start of the crop 2, the AM community in both topsoil and subsoil was composed of 12 species in 4 genera: 2 species of *Acaulospora,* 2 species of *Entrophosphora*, 7 species of *Glomus* and 1 species of *Scutellospora. Glomus* spp. was dominant in the AM community. The spore number of *Glomus* spp. was approximately 70 and 80% of total spore number in topsoil and subsoil, respectively. The AM fungal biodiversity was 0.94 and 0.91 in topsoil and subsoil, respectively. The AM spore abundance was approximately 1,600±50 and 750±70 spores per 100 g^{-1} of soil in topsoil and subsoil, respectively. There were no differences in AM spore abundance of all pots at the start of crop 2. The data collection at the end of the crop 2 was compared to those at the start of crop 2. The results showed that the population of AM fungal species in community, the AM spore abundance and the biodiversity in the topsoil and the subsoil did not change under maize crop. However, fallow treatment had decreased the AM spore abundance by 30% and 15% in the topsoil and the subsoil, respectively. It had greatly affected on spore number of *Glomus* spp. than the other AM genera. The AM fungal biodiversity had declined to 0.79 and 0.84 in topsoil and subsoil, respectively. Therefore, the results indicated that fallow had negative effect on AM fungal community in soil. This may be due to life cycle of AM fungi was disturbed by absence their host during fallow period.

Keywords arbuscular mycorrhizal fungi, fallow, *Glomus*, maize

INTRODUCTION

Symbiotic associations between arbuscular mycorrhizal (AM) fungi and plant roots are widespread in the natural environment. It can provide a range of benefits to the host plant, soil and environment. These include improved plant nutrient uptake (Marschner and Dell, 1994), enhanced tolerance to drought and salinity of soil (Schreiner et al., 2007), enhanced tolerance to nematode and soil-borne pathogen (Sylvia and Chellemi, 2001), and provided stability of soil structure (Bedini et al., 2009). In addition, AM extraradical hyphae itself and glomalin producing by the hyphae have highly correlated with soil organic carbon and are the major component of soil microbial biomass (Rillig et al., 2002). These are resulted in an important role of AM fungi in regulating carbon fluxes between the biosphere and the atmosphere under climate change.

Thailand is an important country in South-east Asia for production of feed maize The global feed maize demand will increase to around 50% by 2020 (FAO, 2004). This is due to increasing in consumption of red and white meats which has resulted in an increase in demand for maize as feed for livestock. Otherwise, some maize is also used industrially for ethanol production. Therefore, research on enhancing sustainable maize yield in Thailand has value more broadly in the world. However, the production of maize for animal feedstock occurs in more than 40 provinces in 3 regions: Central, North-eastern and Northern parts of Thailand. Nakhonratchasima is particularly

important production areas. On Pak Chong soil, high phosphorus (P) fixation presently limits productivity, as many farmers do not have the finances to apply rates of fertilizer required for optimum yields. Application of AM fungi might become a solution in this case. Due to AM fungi are well known to enhance the P nutrition of crop plants and the importance of AM fungi for maize production in Thailand has been discussed (Na Bhadalung et al., 2005).

However, the fallow period is another farming practice which can reduce the population of AM propagules in soil. Harinikumar and Bangyaraj (1988) and Thompson (1994) observed that low AM abundance following fallow led to reduced AM sporulation and colonization in the succeeding crop. Otherwise, fallow is widely practiced in central Thailand and maize farmers also are interested in crop rotation. Therefore, the pot experiment was undertaken to evaluate effects of fallow on populations of indigenous AM fungi.

OBJECTIVE

To evaluate effect of fallow on population of AM fungal species composition in community, AM spore abundance and biodiversity in topsoil and subsoil of Pak Chong soil series under maize cropping system in Thailand.

METHODOLOGY

An experiment was conducted in large pots using a completely randomised design with 4 replications. The treatments were maize-maize crop and maize-fallow crop. The experiment was conducted with two successive crops. In crop 1, all pots were planted with maize for 4 months. In crop 2, maize and fallow were randomly applied to 4 replicate pots for 4 months.

The soil sample belonged to the Pak Chong soil series: clay-loam, kaolinitic, isohyperthermic, Typic Paleustults (Soil survey staff, 1998). The soil was collected from field plots (14° 38´ N, 101° 19´ E; elevation 354 m above sea level; National Corn and Sorghum Research Centre, Amphur Pak Chong, Nakhonratchasima Province, Thailand) at two depths, 0-15 cm topsoil and 15-30 cm subsoil. The soil was allowed to air-dry before use. The soil chemical properties are given in Table 1. The soils before potting were analysed for soil pH, soil organic matter (OM) (Kjeldahl method), available P (Bray II method) and exchangeable K, Ca and Mg (1N NH_4OAc) using Black et al. (1965)

Table 1 Chemical property of the topsoil and subsoil

Soil layer	pH	OM	P	K	Ca	Mg
	1:1 soil:H_2O	%	mg kg^{-1}			
Topsoil	7.0±0.0	2.6±0.1	9.8±0.6	160.0±9.1	1920.0±32.7	225.0±5.0
Subsoil	6.8±0.0	1.1±0.1	4.8±0.5	57.5±4.8	1620.0±75.7	175.0±5.0

The soil was crushed with a mallet, roots removed by hand and then well mixed. Five kg coarse washed river sand was placed into glazed clay pots (32 cm diameter at the top, 25 cm diameter at the bottom and 30 cm in height) to enhance drainage and then 7 kg subsoil and 13 kg topsoil were placed, respectively. Each pot had a single, central drainage hole sealed to a U-shaped drainage pipe (1.3 cm diameter) with the free end of the pipe being slightly below the bottom of the pot to reduce air flow into the pot base. Between the crop cycles, the pots were left uncovered and exposed to rain. Vertical plastic sheets (1.5 m high) were placed between pots to prevent rain-splash.

In crop 1, all pots were cropped with maize (*Zea mays* L., cv. Suwan 4452), one plant per pot. This was undertaken to produce a uniform population of AM fungi under maize for testing agronomic practices. N fertilizer was applied to the soil surface three times at the rate of 2.52 g of urea per pot (on a soil weight basis, equivalent to 112 kg N ha^{-1}), split equally at D7, D30 and D45. This rate was used to ensure normal grain production since the volume per plant of the pot soil was about a half of that in the field. P fertilizer was applied as triple super phosphate (TSP) at the rate of 1.67 g TSP per pot (equivalent to 32.75 kg P ha^{-1}) by surface banding on one side of the plant at

D14. Zinc (Zn) fertilizer was applied at the rate of 0.38 g of Zn per pot as Zn-EDTA (equivalent to 30.4 kg Zn ha^{-1}) at D40. Other nutrients were not applied as previous studies had shown that maize growth in this soil is limited by N, P and Zn (Suwanarit et al., 1992).

In crop 2, maize and fallow were randomly applied to 4 replicate pots. In the cases with crops, one plant was grown per pot. Fertilizer was applied for maize cropping; N fertilizer was applied two times at the rate of 1.12 g urea per pot (equivalent to 50 kg N ha^{-1}), equally split at D14 and D40. P fertilizer was applied as TSP at the rate of 1.12 g TSP per pot (equivalent to 21.8 kg P ha^{-1}) by surface banding on one side of the plant at D14 and Zn fertilizer was applied at the rate of 0.38 g of Zn per pot (equivalent to 30.4 kg Zn ha^{-1}) at D40. The rates of fertilizers, lower than that used in the preliminary crop but adequate for maize, was used to minimize the difference in nutrient status in soil among cultural practices. Fallow treatment was not fertilised but was exposed to the weather (infrequent rain events).

Soil samples were collected from 0-15 cm depth for topsoil and 15-20 cm depth for subsoil using a 2 cm diameter steel auger, 3 augers per pot at the start and the end of crop 2. The holes were filled with sterilized Pak Chong soils that were stored at room temperature in laboratory before use. The soil samples were left to air-dry for determining the AM spore number. The AM spores were sorted into group using morphology and size traits and identified using the INVAM species guide and manual for AM fungal identification (Schenck and Perez, 1988). The AM spore of each species was counted for calculating AM biodiversity using the Shannon-Wiener index (Pielou, 1975).

All data were checked for normal distribution by Levene's test. ANOVA was used to determine effect of treatments. Duncan's Multiple Range Test at $P<0.05$ was used for post hoc testing.

RESULTS

The AM community in the soil (both topsoil and subsoil) at the start of crop 2 was composed of 12 species in 4 genera: 2 species of *Acaulospora*, 2 species of *Entrophosphora*, 7 species of *Glomus* and 1 species of *Scutellospora*. The 12 AM species were described in Table 2.

At the start of crop 2, there were 1,637 and 765 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. *Glomus* spp. was dominant. It contributed 73 and 78% of the total spore number in topsoil and subsoil, respectively. The spore of *Glomus* spp. was 1199 and 599 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The spore of *Acaulospora* spp. was 232 and 94 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The spore of *Entrophospora* spp. was 188 and 56 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The spore of *Scutellospora* sp. was 18 and 16 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The AM spore number of each species was shown in Fig. 1. The AM fungal biodiversity was 0.94 and 0.91 in topsoil and subsoil, respectively.

At the end of crop 2, total spore number, spore number of each AM species and AM biodiversity did not change under maize-maize treatment. However, under maize-fallow treatment, there were 1166 and 649 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. Comparing to the AM spore number at the start of crop 2, fallow treatment had decreased the AM spore abundance by 30% and 15% in the topsoil and the subsoil, respectively. It had greatly affected on spore number of *Glomus* spp. than the other AM genera. The spore of *Glomus* spp. was 838 and 455 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The spore of *Acaulospora* spp. was 190 and 140 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The spore of *Entrophospora* spp. was 128 and 42 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The spore of *Scutellospora* sp. was 10 and 12 AM fungal spores 100 g^{-1} in topsoil and subsoil, respectively. The AM spore number of each species was shown in Fig. 2. The AM fungal biodiversity of fallow treatment had declined to 0.79 and 0.84 in topsoil and subsoil, respectively.

Table 2 Characteristics of the AM fungal taxa and their codes

AM fungal species	Spore characteristic
Acaulospora sp. 1 (A1)	Globose shape, 76-112.5 μm, white to orange, soporiferous succule (80-100 μm). Two layers of spore wall, combined thickness 3-7 μm.
Acaulospora sp. 2 (A2)	Globose shape, 100-120 μm, yellow to orange, soporiferous succule (120-130 μm) and fine spines present. Two layers of spore wall, combined thickness 5-10 μm.
Entrophospora sp. 1(E1)	Globose shape, 165-208 μm, orange to dark orange, hyaline outer layer, and hyaline, subglobose soporiferous succule (180-220 μm) and thick spines present. Three layers of spore wall, combined thickness 15-17.5 μm.
Entrophospora sp. 2 (E2)	Globose shape, 60-80 μm, hyaline colour and hyaline subglobose soporiferous succule (60-80 μm). Three layers of spore wall, combined thickness 2-5 μm.
Glomus sp. 1 (G1)	Globose shape, 80-125 μm, white to cream colour, single chlamydospore. Four to five layers of spore wall, combined thickness 4-8 μm.
Glomus sp. 2 (G2)	Sporocarp formation without peridium (200-1500 x 200-2000 μm), globose (80-140 μm) or subglobose (80-96 x 120-140 μm) chlamydospore, pale yellow, forms spores in roots. Three layers of spore wall, combined thickness 4-5 μm.
Glomus sp. 3 (G3)	Sporocarp formation without peridium (200-800 x 200-1000 μm), globose (60-120 μm) or subglobose (40-112 x 96-188 μm) chlamydospore, pale yellow to yellow, forms spores in roots. Two to three layers of spore wall, combined thickness 2-4 μm.
Glomus sp. 4 (G4)	Sporocarp formation without peridium (1-3 mm), globose (80-100 μm) or subglobose (60-100 x 90-140 μm) chlamydospore, yellow to orange. Three layers of spore wall, combined thickness 3-5 μm.
Glomus sp. 5 (G5)	Sporocarp without peridium, globose chlamydospore (92-112 μm), white to cream. Three spore wall layers, thickness 5-7 μm.
Glomus sp. 6 (G6)	Globose shape, 120-300 μm, pale yellow to yellow, recurved septum. Two layers of spore wall, combined thickness 4-5 μm.
Glomus sp. 7 (G7)	Globose shape, 124-180, yellow-brown to dark orange-brown, shiny and containing lipid content. Two to three layers of spore wall, combined thickness 4-8 μm.
Scutellospora sp. (S)	Globose shape, 168-240 μm, white to cream colour, germination shield and auxillary cell. Five layers of spore wall, combined thickness 4-8 μm.

DISCUSSION

Fallow had negative effect on AM fungal community in soil. The fallow period reduced AM fungal spore number in the topsoil by 30%. This finding is consistent with many previous studies that have been undertaken in containers or in the field. For example, in a field study (acid to neutral soil), one year of bare fallow treatment reduced AM density by 80% compared to continuous maize cropping for a range of sites in Iowa, USA (Troeh and Loynachan, 2003). Similarly, in Queensland, Australia, viable propagules of AM fungi declined in cracking clay soils during long periods of fallow (>1 year) resulting in poor root colonization and symbiotic effectiveness of subsequent crops (Thompson, 1994). Ryan and Angus (2003) observed that the percentage of root colonization of wheat and field pea grown after fallow in South-Eastern Australia was depressed compared to those grown after clover, an AM host. One possibility for the reduction in AM populations during fallow is a lack of host plant roots necessary to maintain viable spore populations for the next crop. Alternatively, Pattinson and McGee (1997) suggested that periodic wetting and drying of soil during fallow periods in agro-ecosystems may also reduce AM fungal propagules during the fallow period.

Fallow affected the proportion of species composition in the AM community. *Glomus* species were more sensitive to fallow than species of other AM genera. In general, AM fungal spores are able to germinate in the absence of host-derived signals. Soil edaphic conditions, such as moisture, temperature and pH, promote AM spores from a dormant to a germinating state, but they are unable

to complete their life cycle without their host plant (Giovannetti, 2000). Therefore, the decrease in spore number in fallow treatment was likely the consequence of failure to establish a functional symbiosis with a host plant during the fallow period.
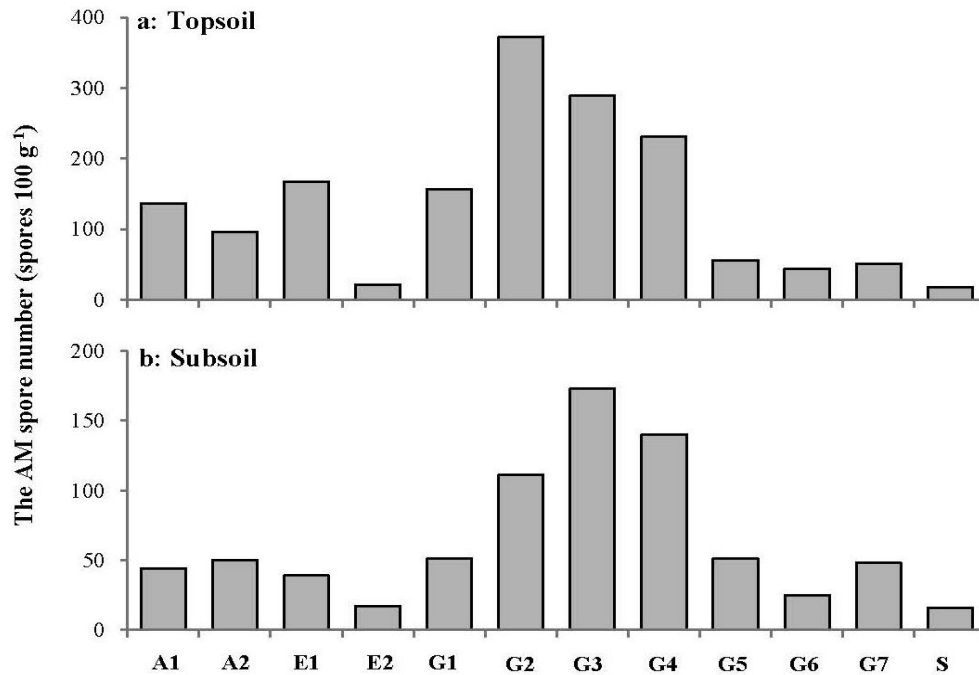

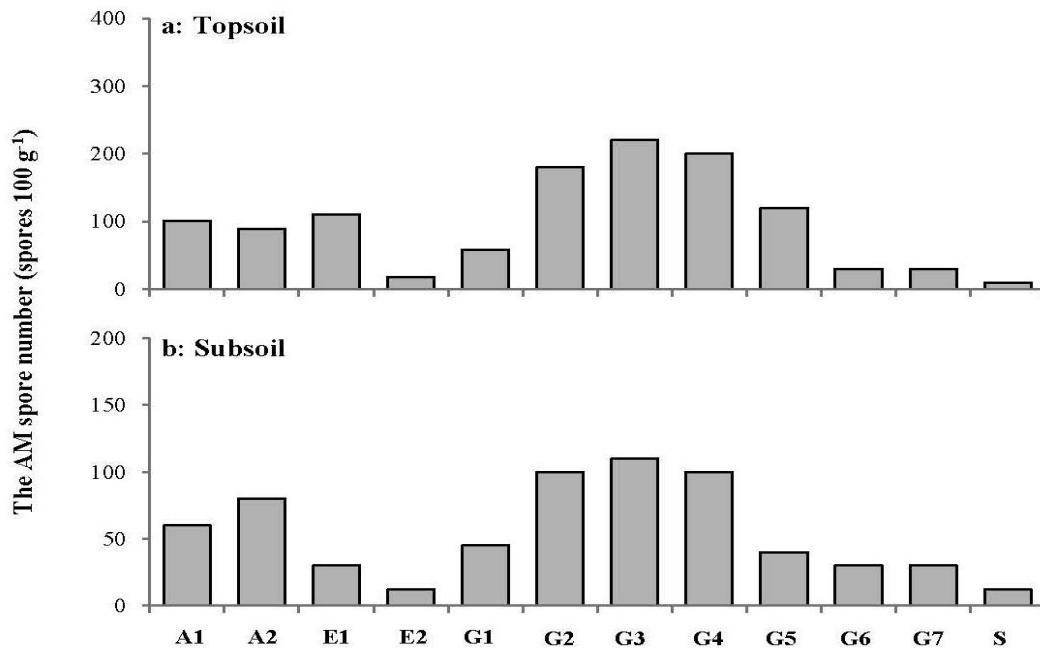**Fig. 1 AM spore number in topsoil (a) and topsoil (b) at start of crop 2**

Fig. 2 AM spore number of fallow treatment in topsoil (a) and topsoil (b) at end of crop 2

CONCLUSION

Fallow had negative effect on the proportion of species composition in the AM community. *Glomus* species were more sensitive to fallow than species of other AM genera.

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