

Short Communication:

Abundance of Arbuscular Mycorrhiza associated with corn planted with traditional and more modern farming systems in Kupang, East Nusa Tenggara, Indonesia

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Abstract. *Setyawan. 2017. Short Communication: Abundance of Arbuscular Mycorrhizal Fungal spores associated with corn planted with traditional and more modern farming systems at East Nusa Tenggara, Indonesia. Biodiversitas 18: 887-892.* Mycorrhizal fungi play an important role in improving plant productivity and soil conservation. One factor that may have an impact on the population of mycorrhizal fungi in the soil is the land management. The study aimed to investigate the impact of land management on the spore density of arbuscular mycorrhiza (AM) associated with corn, and was undertaken at 6 sites in Kupang District of East Nusa Tenggara Province, Indonesia. Two farming systems (traditional and more modern) were chosen for the study, and three locations were selected to represent each farming system practiced. Representative soil samples were taken from each location/farming system for AM observation and soil analysis. The results showed that spore density of AM fungi was higher in soil taken from corn plantation with traditional practice than that found in soil taken from corn plantation with more modern practice, indicating a negative impact of the more modern farming practice on the population of AM fungi. The research should be extended to further investigate the diversity and communities of AM fungi at different time and location.

Keywords: Arbuscular mycorrhizal fungi, farming practice, land management

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi, an obligate symbiotic mutualism between beneficial soil fungi and higher plants, are ecologically important. Arbuscular mycorrhiza absorb mineral nutrients, and water from the soil and deliver them their host plants in exchange for carbohydrate (Smith and Read 2008), may benefit to their host by enhancing plant resistance to pathogens (Song et al. 2015) or abiotic stresses, such as drought (Porcel and Ruiz-Lozano 2004), salinity (Ashraf et al. 2009), and heavy metals (Gohre and Paszkowski 2006). More importantly, AM may play a vital role to improve soil structure in agricultural soils that is critical for the sustainability of the land (Jeffries et al. 2003). Although the beneficial effects of AM on plant performance and soil health are important for the sustainable management of agriculture, less attention has been given to the impact of agricultural activities in particular land management may have on the abundance of AM in the field.

The occurrence of mycorrhiza (population, diversity, and distribution) is closely related to environmental conditions including soil (Sieverding 1991). Soil conditions, in particular, are constantly changing either natural or due to human influence. The change in the soil condition by a human through agricultural activities such as

the intensity of tillage, agrochemical inputs (fertilizers and pesticides), and crop rotation can affect the development and structure of the AM fungal community (Brundrett 1991, Sieverding 1991). Tillage may have detrimental effect on the survival of AM fungal propagules (Kabir 2005) and the use of inorganic fertilizers, especially P in large amount for long period could have a negative impact on the population and the effectiveness of mycorrhiza (Ezawa et al. 2000; Joner 2000; Rubio et al. 2003).

In East Nusa Tenggara, there are two types of farming systems commonly practiced by farmers when growing corn, namely traditional practice, and more modern practice. In the traditional practice, farmers cultivate the soils once a year with a hoe, and do not apply any agrochemicals, while in the more modern practice; farmers use the tractor for cultivating the soil and agrochemicals (inorganic fertilizers, pesticides) are routinely applied. There are many studies regarding the relationship between land management and AM (Galves et al. 2001; Oehl et al. 2003; Kabir 2005), however, up to now, no such study has been undertaken in the region. Therefore, this was the first study to explore indigenous AM fungi in semi-arid calcareous land in East Nusa Tenggara aimed to investigate the impact of local land management systems on the abundance of AM fungi in the soil

MATERIALS AND METHODS

Study area

East Nusa Tenggara, Indonesia is a semi-arid region with average annual rainfall ranging between 1250-1500 mm per year. In Kupang District particularly, the soils are calcareous and commonly less fertile.

Soil sampling

Soil samplings were conducted on corn plantation where either traditional or more modern farming are practiced. In the traditional farming practice, corn is grown once a year, soils are ploughed with a hoe before planting, and no agrochemical input has been used during growing season. In the more modern practice, corn is grown 1 to 2 times a year, chemical fertilizers (N, P, and K) are routinely applied at recommended doses when planting. Furadan (insecticide) is commonly applied and other pesticides are used when it is necessary. The soils are cultivated with hand tractor before planting. The term of more modern is used instead of modern just to emphasize that the land use is less intensive (only 1-2 times per year) due to unavailability of the irrigation system to support the farming system (planting mainly depends on rain as water source).

Soil samples were collected at the end of rainy season (March). In each type of farming practice (traditional or

more modern farming), soils samples were taken at three different locations (each type of farming practice consisted of three different locations; 6 locations in total). Before sampling the soil, the initial survey and interviews were conducted to obtain representative corn plantation for each type of farming practice. At each location, five soil samples were diagonally taken. Soil samples were collected around the plant at a depth of 0-20 cm. Soil samples taken were partly used for the analysis of AM fungal spore density, and the rest were bulked for soil physical and chemical properties analysis. Sampling position was plotted using GPS coordinates, and other nearby vegetation than corn was recorded.

AM fungal spore density

Spores of AM fungi were extracted from 100 g soil sub-samples using the wet-sieving and sucrose methods (Tommerup 1988; Brundertt et al. 1996). The sizes of sieves used were 750, 250, 150 and 45 μm . The AM fungal spore was isolated and then counted manually under a compound microscope (Leica Galen III). Broken spores were not included in the isolation process. Spore density was expressed as a number of spores found per 100 g soil samples. The spores were grouped into morphotypes based on some criteria including color, size, the surface of the spore, and subtending hyphae.

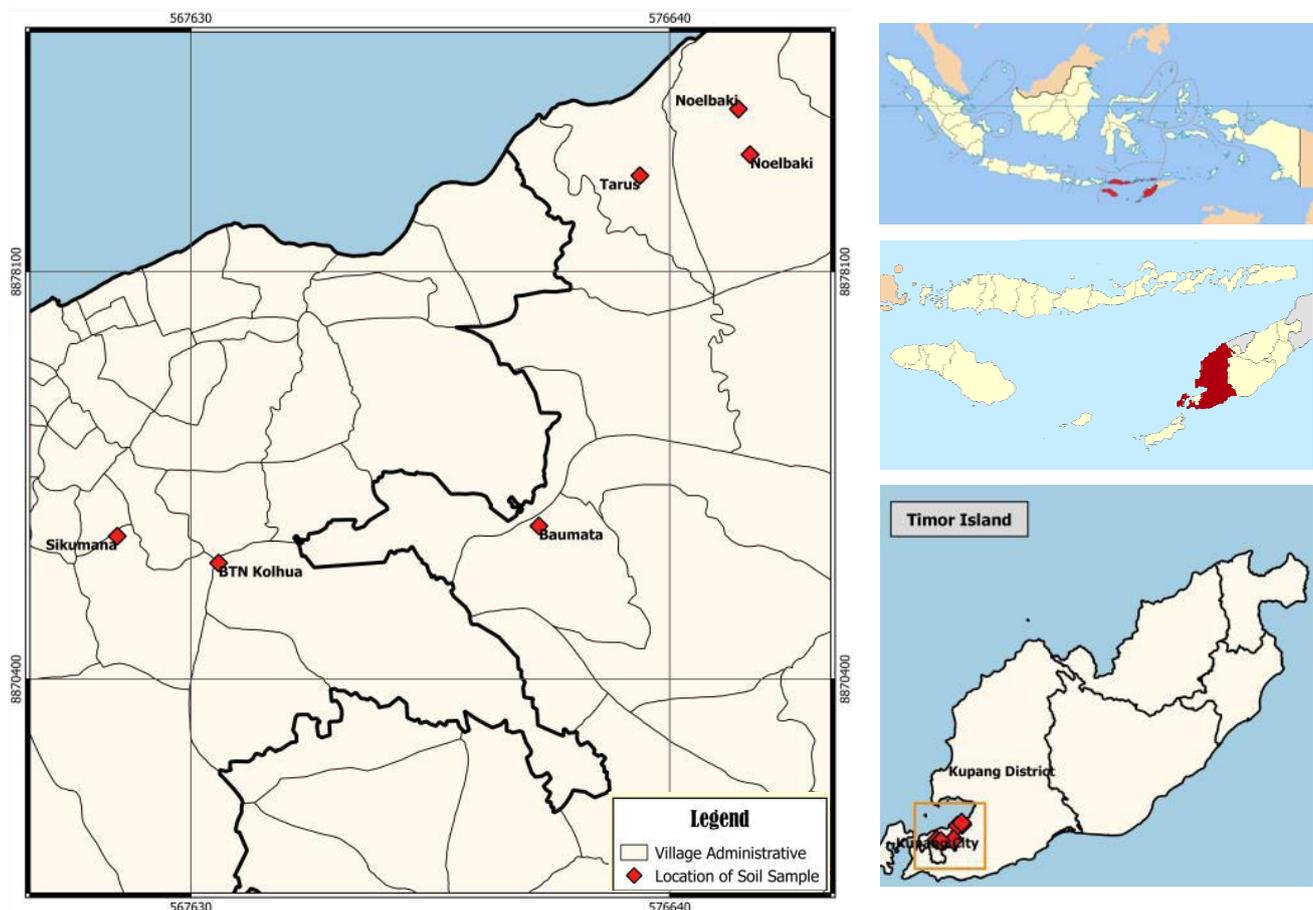


Figure 1. Study site at 6 locations in Kupang District, East Nusa Tenggara, Indonesia

Table 1. Soil chemical properties at corn plantation with traditional and more modern farming practices

Soil variable	Value		Criteria*)	
	Traditional	More modern	Traditional	More modern
Organic C (%)	2.5	1.76	Moderate	Low
Total N	0.35	0.27	Moderate	Moderate
Available P (mg. kg ⁻¹)	84.28	60.59	Very high	Very high
Exchangeable K (Cmol.kg ⁻¹)	1.2	1.1	Very high	Very high
Cation exchange capacity (CEC) (Cmol.kg ⁻¹)	38.3	38	Very high	Very high
pH	7.3	7.4	Neutral	Neutral

Note: *) Indonesian Center for Agricultural Soil Resources Research and Development (ICALRD) (2009)

Soil analysis

Soil properties were analyzed including pH (H₂O); total nitrogen (Kjedhal method); available Phosphorus (P) (Olsen method); organic Carbon (C; lost on ignition method); exchangeable potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) (saturation of 1 N NH₄ Acetate pH 7); texture (three fractions pipet method). Soil analysis was conducted at the Laboratory of Soil Chemistry of Agricultural Faculty of Nusa Cendana University, Kupang East Nusa Tenggara.

Statistical analysis

Spore density for each location was an average of five replicates, while spore density of each farming system was an average of 15 replicates (5 samples x 3 locations). Data of spore density were log (x+1) transformed before analyzing. The data were analyzed using Nested (Hierarchical) Analysis of Variance where the locations of the study were nested within the farming system practice factor.

RESULTS AND DISCUSSION

Soil properties

The soil pH was neutral, very high in P and K, very high in exchangeable cations, and moderate total N content. Soil organic-C was moderate at corn plantation with traditional farming practice, while it was low at corn plantation with more modern farming practice. Soil textures at both corn plantations with traditional and more modern practices were dominated by sandy loam (Tabel 1).

Effect of land management on AM fungal spore density

Nested analyses showed that at traditional farming practice, the spore density of AM fungi was not significantly different between the three locations where the soils were sampled. The averages of AM fungi spore density of the 3 locations were 3.1, 3.05 and 3.4 spores per 100 g soil, respectively [(data were log (X+1) transformed)]. Similarly, at corn plantation with more modern practice, the nested analysis also showed that the AM fungal spore density was not significantly different

between the three locations where the soil samples were taken. The average of spore density in location 1, 2 and 3 were 1.67, 1.54 and 1.58 spores per 100 g soil, respectively [(data were log (X+1) transformed)].

On the contrary, when comparing the spore density of AM fungi between the two types of farming system (traditional and more modern farming practices) the result of nested analysis showed that the spore density of AM fungi in soil samples taken from corn plantation with traditional farming practice was significantly higher ($P < 0.05$) than the spore density of AM fungi found in soil samples taken from corn plantation with more modern farming practice. The spore density of AM fungi in soils sampled from corn plantation with traditional practice was 1.91 [(data were log (X+1) transformed)] spores per 100 g soil compared with 1.6 spores [(data were log (X+1) transformed)] per 100 g soil found in soil sample taken from corn plantation with more modern practice (Figure 1).

Type of spore found on both traditional and more modern was similar (6 morphotypes) except one morphotype was only found in traditional practices. Examples of AM spore morphotypes found are illustrated in Figure 2.

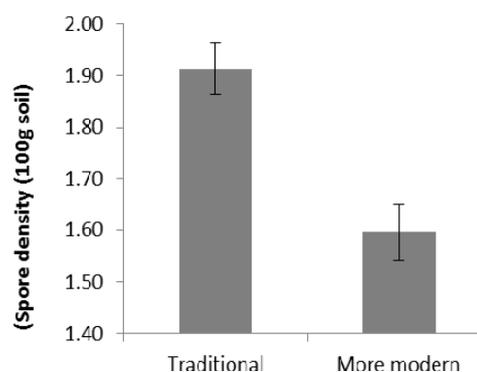


Figure 1. Spore density of AM fungi in soil sampled from corn plantation with traditional practice and more modern farming practice. Values are means (n=15) \pm SE. Data were log (X+1) transformed.



Figure 2. Examples of AM spore morphotypes found in the study. A. Morphotype 1 (white, round, smooth, 118 μm) and morphotype 2: (light yellow, round, smooth, 47 μm); B. Morphotype 3 (dark brown, round, smooth, 152 μm); C. Morphotype 4 (light brown, round, rough surface, 118 μm). Bar = 20 μm

Discussion

The soil properties at both farming practices were not much different; excluding organic C. Availability of P in both farming systems was very high, that was possibly due to soil pH. The solubility of P is strongly influenced by the pH of the soil. Phosphorus will be highly soluble when the soil pH is around neutral with the optimum availability occurs at the pH 7.2 (Brady and Weil 2002). In addition to pH, the high availability of P in the soils might be related to the input of P either through fertilization (more modern practice) or through the release of P from mineralization of organic matters (traditional practice). Exchangeable K and CEC of soils of both types of farming practices are high. These could be contributed from the parent material. As the parent material of the soil at the island of Timor is mainly calcareous, it is very likely that the mineral of soils contains a high amount of base cations. Besides, the base cations are unlikely leached due to low rainfall. The total N were moderate at both traditional and more modern farming systems, whereas organic-C was moderate at the traditional system but low at the more modern farming system. Nitrogen and organic-C soil can be affected by some factors such as the input of organic matter to the soil, intensity of land use, type of crops/vegetation. Decomposition of organic matter is generally faster when the land is more often cultivated.

It seemed that only soil organic-C was quite sensitive to explain the differences in soil chemical properties between the two farming system practiced. The soil organic-C on corn plantation with more modern practice was lower than that was observed on corn plantation with more modern practice, and this might be due to both removing plant residues at harvest and the intensity of land use. Organic carbon is an important soil factor that can influence the physical, chemical and biological properties of the soil (Brady and Weil 2002). The growth of soil microorganisms is generally stimulated by the addition of organic matter, and some studies have reported about the positive effect of organic matter on the growth of external mycelium of AM fungi (Vaidya et al. 2007; Hammer et al. 2011). Accordingly, the organic matter could alter the soil condition that may benefit AM fungi. A greater soil organic matter in soil could provide a more suitable physical

growing space that may benefit the growth of AM. For instance, improved soil aggregation and soil porosity can decrease the mechanical resistance to growth of AM hyphae in the soil (Rillig and Steinberg 2002). In addition, soil chemical components released during the decomposition process of organic matter and the secondary metabolites produced by microorganisms involved in organic matter decomposition is also considered to influence the growth of AM fungal mycelia (Gryndler et al. 2009).

This study clearly showed that local farming system practice has an impact on AM fungi spore density. An agricultural system with less soil disturbance and low input of agrochemical has more AM fungal population than an agricultural system that was more frequently disturbed and received more input of agrochemicals. The impact of agricultural activity (fertilizer, pesticide, tillage) on the occurrence and affectivity of AM fungi have been widely reported. Fertilization, in particular, is an important factor influencing AM fungal sporulation. The sporulation of AM fungi could be reduced when the soil conditions cannot promote plant growth such as extreme low or high soil fertility and imbalance nutrient supply especially N and P (Baath and Spoke 1989). The use of inorganic fertilizers, especially P in large numbers in the long term could have a negative impact on the population and the effectiveness of AM fungi (Ezawa et al. 2000; Joner 2000; Rubio et al. 2003). Fertilization could also change AM fungal communities' composition (Johnson et al. 1993), and the long-term fertilization could cause decreases in AM total spore numbers and variation in species diversity, although the impact on the AM fungi might depend on sampling time (Bhadalung et al. 2005). The impact of inorganic fertilizer have on AM biodiversity, however, might be different among the species due to differences in sensitivity of the species to disturbance or the tolerance to the amount of inorganic fertilizer applied (Bhadalung et al. 2005). The application of P fertilizer commonly could decrease the amount of soluble carbohydrate content in root exudates, however, since the species of AM fungi might differ in their requirement of soluble carbohydrate (Johnson, 1993), the response of the species to the application of inorganic fertilizer could be variable.

In addition to fertilizer, the lower AM fungal spore density in more modern corn farming practice could be due to some other possibilities. For instance, the frequency of tillage and land use at corn plantation with more modern farming practice is more intensive than those at corn plantation with traditional practice. As a consequent, soils are more often disturbed resulting in increased negative impact on AM communities. Some studies indicated that tillage could negatively affect AM fungal propagules and mycorrhizal colonization including the destruction of external hyphae network, loss of propagules from top soil layer and acceleration of root decomposition (Jasper et al. 1989; Kabir 2005; Schalamuk and Cabello 2010). Beside tillage, the use of other chemicals particularly pesticides could also adversely affect mycorrhiza. The use of pesticides such as those contained benomyl materials could reduce the formation of AM fungi and could inhibit the infection of AM fungi on some plants/herbs that in turn could decrease the absorption of P (Entry et al. 2002).

Not many AM fungal spore morphotypes obtained in this study, ranging from 6 to 7 morphotypes at more modern and traditional practices, respectively. It is not known whether the low morphotypes observed is due to low diversity of indigenous AM fungi in the location of study or due to time for soil samplings. The study was the first study to explore indigenous AM fungi in semi-arid calcareous land in East Nusa Tenggara, therefore no data of AM fungal diversity is available for comparison. Furthermore, the soil samples were collected at the end of the rainy season that possibly could influence the sporulation of certain types of AM, particularly those types which are sensitive to high moisture.

From all the AM fungal morphotypes observed, *Glomus* sp. was the most dominant AM fungal species found in this study. *Glomus* sp. has been reported as the most common species and most widely spread of AM fungi than the other types (Gai et al. 2006), and was the dominant group of AM fungi especially on land that was not often cultivated (Schalamuk and Cabello 2010). One type of *Gigasporae* genus was found at traditional farming practice but in very low amount (3 spores).

At traditional farming practice, the spore density of AM fungi was not significantly different between the three locations where the soils were sampled. The same result was also observed at more modern farming practice. These results were not surprising as the locations for soil sampling were purposively selected through initial survey and interviews to ascertain the history of land use in order to minimize the variation between the locations. Furthermore, the soil properties between the locations where the soil was sampled were not much different.

This study was based on soil sampling conducting once at rainy season. Since the sporulation of AM fungi could be dynamic in spatial and time, future study for soil samplings and root infection observation need to be undertaken at different time and location in order to get more representative information on the impact of agricultural activities on the population and communities of AM fungi in these two farming systems.

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REFERENCES

- Ashraf M, Sonmeza O, Aydemira S, Tunac AL, Cullua MA. 2009. The influence of arbuscular mycorrhizal colonization on key growth parameters and fruit yield of pepper plants grown at high salinity Cengiz Kayaa. *Sci Hort* 121: 1-6
- Baath E, Spokes J. 1989. The effect of added nitrogen and phosphorus on mycorrhizal growth response and infection in *Allium schoenoprasum*. *Can J Bot* 67: 3227-3232
- Bhadalung NaN, Suwanarit A, Dell B, Nopamornbodi O, Thamchaipenet A, Rungchuang R. 2005. Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. *Plant Soil* 270: 371-382
- Brady N, Weil R. 2002. *The Nature and Properties of Soils*. 13th eds. Pearson Education, Inc. Upper Saddle River, New Jersey.
- Brundrett M. 1991. Mycorrhizas in natural ecosystems. *Adv Ecol Res* 21: 171-313
- Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with Mycorrhiza in Forestry and Agriculture. Australian Centre for International Agricultural Research, Canberra, Australia
- Entry JA, Rygielwicz PT, Watrid, LS, Donnelly PK. 2002. Influence of adverse soil conditions on the formation and function of *Arbuscular mycorrhizas*. *Adv Environ Res* 7: 123-138
- Ezawa T, Yamamoto K, Yoshida S. 2000. Species composition and spore density of indigenous vesicular-arbuscular mycorrhizal fungi under different conditions of P-fertility as revealed by soybean trap culture. *Soil Sci Plant Nutr* 46: 291-297.
- Gai, JP, Christie P, Feng G, Li XL. 2006. Twenty years of research on community composition and species distribution of arbuscular mycorrhizal fungi in China; a review. *Mycorrhiza* 16: 229-239
- Galves L, Douds DD, Drinkwater LE, Wagoner P. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil* 228: 299-308
- Göhre V, Paszkowski EU. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* 223: 1115-1122
- Gryndler M, Hrselova H, Cajthaml T, Havrankova M, Rezacova V, Gryndlerova H, Larsen J. 2009. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth. *Mycorrhiza* 19: 255-266.
- Hammer EC, Nashr H, Wallander H. 2011. Effects of different organic materials and mineral nutrients on arbuscular mycorrhizal fungal growth in a Mediterranean saline dryland. *Soil Biol Biochem* 43: 2332-2337
- Indonesian Soil Research Institute 2009. *Chemical Analysis of Soil, Plant, Water and Fertilizer (In Indonesia)*. Indonesian Agency for Agricultural Research and Development
- Jasper DA, Abbot LK, Robson AD. 1989. Soil disturbance reduces the infectivity of external hyphal of vesicular-arbuscular mycorrhizal fungi. *New Phytol* 112: 93-99
- Jeffries S, Gianinnazi S, Perroto S, Turnau K, Barea JM. 2003. Contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil* 37: 1-16
- Joner EJ. 2000. The effect of long-term fertilization with organic or inorganic fertilizers on mycorrhiza-mediated phosphorous uptake in subterranean clover. *Biol Fert Soils* 32: 435-440.
- Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecol Appl* 3: 749-757.
- Kabir Z. 2005. Tillage or no tillage impact on mycorrhizal. *Can J Plant Sci* 85: 23-29
- Oehl F, Sieverding E, Ineichen K, Mader P, Boller T, Wiemkun A. 2003. Impact of land use intensity on the species diversity of arbuscular

- mycorrhizal fungi in agroecosystems of Central Europe. *Appl Environ Microbiol* 69: 2816-2824
- Porcel R, Ruiz-Lozano JM. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *J Exp Bot* 55: 1743-1750
- Rillig MC, Steinberg PD. 2002. Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification. *Soil Biol Biochem* 34: 1371-1374
- Rubio R, Borie F, Schalchli C, Castillo C, Azcón R. 2003. Occurrence and effect of arbuscular mycorrhizal propagules in wheat as affected by the source and amount of phosphorus fertilizer and fungal inoculation. *Appl Soil Ecol* 23: 245-255
- Schalamuk S, Cabello M. 2010. Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: possible effect on Glomeromycota diversity. *Mycologia* 102: 261-268
- Sieverding E. 1991. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Federal Republic of Germany
- Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. Elsevier, New York, USA
- Song Y, Chen D, Lu K, Sun Z, Zeng R. 2015. Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus. *Front Plant Sci*. DOI.org/10.3389/fpls
- Tommerup IC. 1988. The vesicular-arbuscular mycorrhizas. *Adv Plant Pathol* 6: 81-89
- Vaidya GS, Shrestha K, Khadge BR, Johnson NC, Wallander H. 2008. Organic matter stimulates bacteria and arbuscular mycorrhizal fungi in *Bauhinia purpurea* and *Leucaena diversifolia* plantations on eroded slopes in Nepal. *Restor Ecol* 16: 79-87