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Diversity of arbuscular mycorrhiza and maize yield in Cajeput agroforestry system with different fertilizer management

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Abstract

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This research was conducted with the aims: the first, to study diversity of arbuscular mycorrhiza in maize rhizosphere with different fertilizer management in Cajeput (*Melaleuca leucadendron* LINN.) agroforestry system in Ponorogo, East Java, Indonesia; the second, to detect the correlation between diversity of arbuscular mycorrhiza with soil fertility (nutrient content of the soil) and maize yield. Fertilizer management was comprised to conventional management (CM), universal management (UM), and alternative managements (AM1, AM2, and AM3). Plot of CM used low inorganic fertilizer and chicken manure. Plot of UM and AM3 used medium inorganic fertilizer and chicken manure. Plot of AM1 used medium inorganic fertilizer and high chicken manure. Plot of AM2 used high inorganic fertilizer. Specially plot of AM2 used maize straw and AM3 used maize straw plus intensive tillage. Results of the research showed that diversity index value of arbuscular mycorrhiza in the plot of AM2 was highest and in the plot of AM1 was lowest. There were four genera of arbuscular mycorrhiza identified: *Glomus, Acaulospora, Gigaspora* and *Scutellospora*. The dominant species in all fertilizer management was *Glomus contrictum. Glomus minutum* was found only in the plot of CM, *Scutellospora colospora* in the plot of UM, and *Acaulospora favoeta* in the plot of AM3. Infection of arbuscular mycorrhiza in roots maize ranged from 34.62% to 44.77% and spore density ranged from115 to 195 spore/100g of soil in five fertilizer management. The highest infection of arbuscular mycorrhiza and spore density were in the plot of AM2, but the highest maize yield was in the plot of AM1. Diversity index value of arbuscular mycorrhiza (species richness) has negative correlation with nutrient content of the soil and maize yield.

Keywords: arbuscular mycorrhiza; Cajeput agroforestry system; diversity; fertilizer management; maize yield

Introduction

One part of soil microbial communities in rhizosphere of plants is arbuscular mycorrhiza which established mutualistic symbioses with plants. Arbuscular mycorrhiza was beneficial to plant because it increases nutrient and water uptake (Cahyani, 2009), resistance to environmental stress (Khalil and Yousef, 2014), resistance to diseases (Kamal et al., 2014), and improves soil quality (Cardoso et al., 2006). Because arbuscular mycorrhiza is beneficial for plants, it is very important to maintain stability of the ecosystem (Jeffries et al., 2003).

Arbuscular mycorrhiza is very compatible on maize rhizosphere, so infection of arbuscular mycorrhiza affects soil fertility (Prasetya and Anderson, 2011). Soil fertility in Cajeput agroforestry system was low, so fertilizer management is necessary, such as the application of inorganic fertilizers, chicken manure, returning the crop waste, and soil tillage. Fertilizer management not only affects plants yield, but also affects diversity of arbuscular mycorrhiza (Qin et al., 2015).

Community of arbuscular mycorrhiza in rhizosphere of maize in monoculture system showed high diversity in difference management system. Diversity of arbuscular mycorrhiza depends on soil tillage (Yang et al., 2012), fertilizer management (Chen et al., 2014), and soil fertility (Jeffries et al., 2003). Diversity of arbuscular mycorrhiza in rizosphere of maize in Cajeput agroforesty system has not been identified. Therefore, this research was necessary for the basis of sustainable agroforestry systems management.

Materials and Methods

Location and spacing

This research was conducted by observation on Cajeput agroforestry system in Ponorogo (7°52′ S, 111°35′ E, altitude 265 m) with different fertilizer management of maize: conventional management (CM), universal management (UM) and alternative management (AM1, AM2, and AM3).

Tabel 1
Fertilizer management of maize

	Inorganic fertilizer	Chicken manure	Maize straw
CM	Low	Low	-
UM	Medium	Medium	-
AM1	Medium	High	-
AM2	High	-	+
AM3	Medium	Medium	+

CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3
Inorganic fertilizer (Low: 360 kg Urea/ha + 200 kg NPK 15-15-15/ha; Medium: 650 kg Urea/ha + 330 kg NPK 15-15-15/ha; High: 730 kg Urea/ha + 430 kg NPK 15-15-15/ha); *Chicken manure* (Low: 2 t/ha; Medium: 3.3 t/ha; High: 4 t/ha)

Minimum tillage is used in CM, UM, AM1 and AM2, whereas intensive tillage is used in AM3. Cajeput is planted with spacing 6 x 1 m in 2007. Cajeput trees are pruned each year in May – July. Maize is grown between cajeput with spacing rows 0.6 x 0.5 m. Maize is planted in December and harvested in April. In each fertilizer management three sample plots of 10 x 6 m were established.

Sampling and analysis

Soil samples were taken randomly from maize rhizosphere in Cajeput agroforestry systems. Five sub sample of soil was taken from maize rhizosphere of three replicate with five different fertilizer management each. Each sub sample comprised of 1 kg soil, which was put into a polybag and kept in room temperature before processing soil analysis. Soil samples obtained from the composite sub sample.

Chemical analysis of the soil was done on soil samples, which had been finely pounded by < 2 mm in size. Analysis on pH of the soil was done using water as the solvent (1:5). Organic C analysis was done using the method by Walkley and Black (1934). Available P was done using the method by Olsen et al. (1954). Total N analysis was done using the method by Kjeldah (Nelson and Sommers, 1973).

Mycorrhizal spores were isolated by wet sieving and decanting methods (Gerdemann and Nicolson, 1963; Walker et al., 1982). The identification is based on the morphological description provided by the international collection of vesicular arbuscular mycorrhizal fungi (*http:// invam.caf.wvu.edu*) and originally published species descriptions. Infection of arbuscular mycorrhiza in cajeput root was detected by the technique of Phillips and Hayman (1970).

Plant samples for N, P, K analysis were taken from the top part of the plant. They were dried in oven at 80^{o}C, and then crushed and kept before tissue analysis. N level analysis was done using Kjeldah method, P analysis was done using spectrophotometer, and K was done using flamephotometer.

Statistical analysis

Diversity of arbuscular mycorrhiza was determined from spore density, relative abundance, species richness, and Shannon-Wiener index (Jewfa et al., 2006). Species richness is estimated by Margalef index (D_{Mg}), D_{Mg} = S – 1/ln N, where S = numbers of species, N = total spores. Shannon-Wiener index is counted by equation: H′ = -∑pi (ln pi), where *pi* is number of spores for each identified species.

Data analysis was done using one-way analysis of variance (ANOVA) by SPSS program. LSD analysis (Student's t test of least significant difference) was used to compare the treatments. Pearson correlation coefficient was used to determine the relationship between diversity with soil fertility and maize yield. Regression analysis was calculated to assess the significance of the relationship between diversity with soil fertility and maize yield.

Results

Nutrient content and pH of the soil

The highest total N and available P of soil were found in the plot of AM1 and they were lowest in the plot of CM (Table 2). The highest exchangeable K of soil was found in the plot of UM, organic C of soil in the plot of AM2, and pH of soil in the plot of AM3. The lowest exchangeable K and pH of soil were found in the plot of UM and organic C of soil was found in the plot of AM3.

Table 2
Nutrient content and pH of the soil

	Total N (%)		Available P (ppm)		Exchangable K (me)		Organic C (%)		pH	
CM	0.18±0.02	a	8.67±0.48	a	0.33±0.04	a	1.82±0.11	a	5.65±0.09	a
UM	0.20±0.01	a	12.80±1.44	b	0.46±0.03	c	2.09±0.25	abc	6.03±0.70	b
AM1	0.23±0.01	b	14.15±0.32	c	0.44±0.01	bc	2.24±0.23	bc	6.04±0.15	b
AM2	0.19±0.02	a	10.75±0.91	b	0.40±0.03	bc	2.39±0.31	c	5.92±0.11	b
AM3	0.20±0.01	a	14.04±0.67	c	0.42±0.04	bc	1.68±0.23	ab	6.30±0.14	c

Data followed by the same letter in the same column show insignificant difference ($P < 0.05$)
CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3

Spore density and relative abundance

Spore density ranged from 115 to 195 spore/100 g of soil. Spore density was significantly lower in the plot of UM than in the plot of AM2, but it was higher than in the plot of AM3. There were four genera of arbuscular mycorrhiza, which comprised of thirteen species. The identified species were *Glomus contrictum, Glomus claroidem, Glomus minutum, Glomus etunicatum, Glomus ambisporum, Glomus diserticola, Acaulospora capsicula, Acaulospoa tuberculata, Acaulospora leavis, Acaulospora favoeta, Gigaspora rosea, Gigaspora margarita, Scutellospora colospora.* The most dominant species was *Glomus contrictum* (18-28%) and *Glomus claroidem* (13-26%) (Table 3). *Glomus minutum* was found only in the plot of CM, *Scutellospora colospora* in the plot of UM, and *Acaulospora favoeta* in the plot of AM3.

Diversity and infection of arbuscular mycorrhiza

Infection of arbuscular mycorrhiza ranged from 34.62 to 44.77%. The highest infection of arbuscular mycorrhiza was in the plot of AM2 and the lowest was in the plot of UM. Infection of arbuscular mycorrhiza in the plot of AM2 was higher than AM1 and AM2. The highest diversity index value of arbuscular mycorrhiza (species richness) was observed in plot of AM2, but Shannon-Wiener index was highest in the plot of AM3 (Table 4).

Nutrient concentration of plant and maize yield

Nitrogen (N) concentration of plant was highest in the plot of AM2, while P and K concentrations were highest in the plot of AM1. Nutrient concentration (N, P, K) were lowest in the plot of UM. Maize yield in the plot of CM was higher than the other four plots. Maize yield was lowest in the plot of CM (Table 5).

Table 3
Spore density (spore/100 g of soil) and relative abundance (RA) of spore of arbuscular mycorrhiza (%)

	CM	UM	AM1	AM2	AM3
Spore density	119±20.8 ab	146±28.9 b	134±14.2 ab	195±28.2 c	115±8.5 a
Relative abundance					
G. claroidem	16.50±1.5 b	14.42±2.9 b	13.94±1.7 b	26.28±3.1 b	13.46±1.7 a
G. etunicatum	1.44±0.8 a	2.40±1.6 a	2.24±0.7 a	5.28±3.2 a	2.40±0.5 a
G. ambisporum	6.09±1.2 a	8.97±4.9 a	4.73±2.5 a	14.40±6.5 b	2.08±0.6 a
G. diserticola	1.60±1.5 a	1.92±0.8 a	2.56±0.3 a	2.08±1.5 a	2.08±0.6 a
G. contrictum	20.83±3.7 ab	30.93±4.5 c	28.52±6.7 bc	28.04±4.1 bc	18.91±1.8 a
G. minutum	0.64±0.7	-	-	-	-
A. capsicula	3.20±1.9 a	2.24±1.1 a	3.44±0.5 a	5.77±2.5 a	2.40±0.5 a
A. tuberculata	2.56±0.7 a	2.88±2.9 a	4.16±4.0 a	3.85±2.9 a	2.24±1.0 a
A. foveata	-	-	-	-	2.24±0.7
A. leavis	-	-	-	1.28±1.0	0.64±0.3
Gi. rosea	3.85±0.5 a	5.77±0.9 a	5.13±0.7 a	4.65±1.1 a	5.77±2.3 a
Gi. margarita	0.48±0.5	-	-	1.76±0.7	-
S. colospora	-	0.80±0.7	-	-	-

Data followed by the same letter in the same row show no significant difference ($P < 0.05$)
CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3, G: Glomus, A: Acaulospora, Gi: Gigaspora, S: Scutellospora

Table 4
Shannon-Wiener index, species richness and infection level

	Shannon Wiener index	Species richness	Infection level (%)
CM	1.71 ± 0.08 ab	9.79 ± 0.01 ab	34.62 ± 4.43 a
UM	1.62 ± 0.11 a	8.80 ± 0.01 a	40.50 ± 4.10 bc
AM1	1.61 ± 0.15 a	7.79 ± 0.01 a	36.34 ± 2.24 ab
AM2	1.79 ± 0.11 b	9.81 ± 0.01 b	44.77 ± 1.93 c
AM3	1.85 ± 0.06 b	9.79 ± 0.00 b	36.04 ± 1.75 a

Data followed by the same letter in the same column show no significant difference ($P < 0.05$)
CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3

Relationships between diversity of arbuscular mycorrhiza and nutrient content of the soil

Diversity index value of arbuscular mycorrhiza (species richness) was negatively associated with total N, available P, and exchangeable K of the soil (Table 6). Higher content of total N reduced diversity of arbuscular mycorrhiza ($Y = -43X + 17.79$; $R^2 = 0.809$). Higher content of available P in the soil reduced diversity of arbuscular mycorrhiza ($Y = -0.221X + 11.86$; $R^2 = 0.335$). Higher level of exchangable K reduced diversity of arbuscular mycorrhiza ($Y = -1.064X + 11.09$; $R^2 = 0.623$).

Relationships between diversity of arbuscular mycorrhiza and maize yield

Maize yield was negatively correlated with diversity index value of arbuscular mycorrhiza (species richness) (Table 7). Higher maize yield reduced the diversity of arbuscular mycorrhiza ($Y = -1.064X + 16.09$; $R^2 = 0.623$). Therefore, the increased maize yield was not followed by soil health improvement. Such condition was unfavorable for sustainable agriculture.

Dicussion

Diversity of arbuscular mycorrhiza

Thirteen species were found in maize rhizosphere in Cajeput agroforestry system. There were four genera iden-

Table 5
Concentration of nitrogen (N), phosphorus (P), potassium (K) of plant and maize yield

	N (%)		P (%)		K (%)		Maize yield (t/ha)	
CM	2.63±0.08	a	0.22±0.01	a	1.10±0.02	a	5.59±0.31	a
UM	2.67±0.06	a	0.23±0.02	a	1.22±0.02	a	6.61±0.32	b
AM1	3.16±0.25	b	0.24±0.01	a	1.35±0.06	b	7.36±0.11	c
AM2	3.64±0.06	c	0.23±0.02	a	1.31±0.03	b	6.72±0.56	b
AM3	3.33±0.11	c	0.22±0.02	a	1.33±0.06	b	6.12±0.60	ab

Data followed by the same letter in the same column show no significant difference ($P < 0.05$)
CM: Conventional management, UM: Universal management, AM1: Alternative management 1, AM2: Alternative management 2, AM3: Alternative management 3

Table 6
Coefficient of correlation by Pearson (r) between arbuscular mycorrhiza with nutrient content of the soil and pH

	Total N	Available P	Exchanged K	Organic C	pH
Infection level	-0.179	-0.050	0.173	0.580*	0.022
Spore density	-0.041	-0.186	0.101	0.473	-0.044
H	-0.162	-0.133	-0.276	-0.175	0.345
SR	-0.749*	-0.526*	-0.529*	-0.341	-0.162

* coefficients of correlation show significant difference

Table 7
Coefficient of correlation by Pearson (r) between diversity of arbuscular mycorrhiza with nutrient concentration of plant and maize yield

	N	P	K	Maize yield	pH
Species richness	0.268	-0.287	-0.336	-0.706**	0.022
Infection level	0.238	-0.142	0.271	0.217	-0.044
Shannon-Wiener index	0.595	-0.436	0.206	-0.213	0.345
Spore density	0.198	-0.095	0.238	0.401	-0.162

** coefficients of correlation show significant difference tified: *Glomus, Acaulospora, Gigaspora* and *Scutellospora*. The identified species were: *G. contrictum, G. claroidem, G. minutum, G. etunicatum, G. ambisporum, G. diserticola, A. capsicula, A. tuberculata, A. leavis, A. favoeta, Gi. rosea, Gi. margarita,* and *S. colospora.* The number of identified arbuscular mycorrhiza species in this study was higher than those isolated by Sousa et al. (2013) from rhizosphere of maize in Gliricidia agroforestry system in the state of Paraina (Brazil) and by Jefwa et al. (2006) from maize rhizosphere in Sesbania agroforestry system in Southerm Malawi. The most dominant species in this study were *G. contrictum* (18-28%) and *G. claroidem* (13-26%). *G. minutum* was found only in the plot of CM, *S. colospora* in the plot of UM, and *A. favoeta* in the plot of AM3. The results were similarly in those of Gliricidia agroforestry system. The dominant genera were *Glomus* and than *Acalouspora.* They related to smaller size of the spores so that they would be easier to spread through water flows that permeate the soil pores (Sousa et al., 2013).

Spore density ranged from 115 to 195 spore/100 g of soil, it was higher than that found in maize rhizosphere (78 spore/100 g of soil) at Gliricidia agroforestry system (Sousa et al., 2013). It was higher also than the results in the study by Emmanuel et al. (2012) in maize rhizosphere in the maize/Centrosema system (27 spores/100 g soil) and sole maize (17 spores/100 g soil) in a Typic Ustropept and Eutric Cambisol in the Northern Guinea Savannah of Nigeria. But spore density in this research were lower than in rhizosphere of maize as reported by Harinikumar et al. (1990) in the intercrop maize/soybean (788 spore/100 g of soil) and with sole maize (605 spore/100 g of soil). This fact indicated that spore density is affected by soil properties, host species and intercropping system. It supported the result of research by Mohammad et al. (2003) that soil properties affected spore distribution.

The highest diversity index value of arbuscular mycorrhiza (species richness) was observed in plot of AM2. Application of synthetic fertilizer plus maize straw increased the diversity index value of arbuscular mycorrhiza. Returning the crop remains (straw) increased diversity of arbuscular mycorrhiza (Alquacil et al., 2014). On the other hand, diversity of arbuscular mycorrhiza has reduced with the implementation of intensive soil management (Yang et al., 2012).

Correlation between diversity of arbuscular mycorrhiza with soil fertility and maize yield

Arbuscular mycorrhiza was a soil microorganism that has a diversity to show ecological specific. One of the factors affecting diversity of arbuscular mycorrhiza was soil properties. This study showed that diversity index value of arbuscular mycorrhiza (species richness) was negatively associated with total N, available P and exchanged K of the soil. Negative correlation between diversity arbuscular mycorrhiza and available P was also obtained by Wang et al. (2011) at long term fertilization in the Chinese Academy of Science, and by Alquacil et al. (2014) at tropical savanna forage system. Negative correlation between species richness mycorrhiza and exchanged K was also found by Leski et al. (2010). Diversity of arbuscular mycorrhiza also affected N content of the soil, so that excessive N application have negative impact on arbuscular mycorrhiza (Tian et al., 2013). Furthermore, arbuscular mycorriza communities were depended on application of N fertilizer. Diversity of arbuscular mycorrhiza will decreased with increasing N fertilizer (Wang et al., 2015).

Maize yield was negatively correlated with diversity of arbuscular mycorrhiza. It showed that soil health in Cajeput agroforestry system was less favorable. Healthy soil can be achieved if the balance was found among physical, chemical, and biological properties of the soil (Abbott and Murphy, 2007). Healthy soil can be formed from positive correlation between biological and chemical properties of the soil. The presence of mycorrhiza can be used as soil fertility indicator (Syibli et al., 2013). Therefore, fertilizer management in Cajeput agroforestry system was hoped orientation to sustainable system management.

Conclusions

Diversity of arbuscular mycorrhiza in rhizosphere of maize in Cajeput agroforestry system were affected by fertilizer management and consisted of thirteen species, which belonged to four genera. *Glomus contrictum* was indicated as the dominant species. *Glomus minutum* was found only in the plot of CM, *Scutellospora colospora* in the plot of UM, and *Acaulospora favoeta* in the plot of AM3. The highest diversity of arbuscular mycorrhiza, spore density and infection of arbuscular mycorrhiza were found in the plot of AM2, but the highest maize yield was found in the plot of AM1. Diversity of arbuscular mycorrhiza was negatively correlated with soil fertility and maize yield.

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