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Research Article

Effects of biochar amendment and arbuscular mycorrhizal fungi inoculation on availability of soil phosphorus and growth of maize

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Abstract: A glasshouse experiment was conducted to study the interactive effects of biochar amendment and arbuscular mycorrhizal fungi (AMF) inoculation on phosphorus uptake by maize (*Zea mayze* L.) grown on a calcareous soil of Kupang, East Nusa Tenggara. The biochar was made of cow dung. Twelve treatment combinations (three biochars levels of 0, 5 and 7.5 g/kg of soil, and four AMF inoculation levels of 0, 5, 10 and 15 spores / kg of soil) were arranged in a completely randomized block design with three replicates. Results of the study showed that at 8 weeks after transplanting, the biochar and mycorrhizal treatments increased the availability soil phosphorus and phosphorus uptake by maize. Application 4.5 and 7.5 g biochar/kg of soil combined with inoculation of 10-15 AMF spores / kg of soil provided to high value of phosphorus uptake by maize. Application of biochar alone, however, did not significantly improve maize growth and phosphorus uptake by maize.

Keywords: *arbuscular mycorrhizal fungi, biochar, calcareous soil, maize, phosphorus uptake.*

Introduction

Dry climate conditions with a short rainy period and a long dry season, and the relatively high average annual air temperature are several factors that limit crop performance in East Nusa Tenggara of Indonesia. Soils in the area are characterized by low phosphorus availability due to binding of Ca to P that limits crop growth and yield. Fertility of the soils can easily be improved by application of inorganic fertilizers or fresh organic matters. However, most farmers cannot afford to purchase commercial inorganic fertilizers because of their low-income conditions. Dry climatic conditions of the area limits availability of fresh organic materials such plant residues and farm-yard manures. In addition, decomposition of fresh organic materials contributes to carbon dioxide emission (Lehman et al., 2003). One of possible alternatives to improve fertility of the soil, particularly soil phosphorus availability, is inoculation of arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhizal fungi are known to form mutualistic relationships with more than 80% of plants (Ulrich et al., 2002). This mutualistic relationship can provide nutrients to the host plant in exchange for carbohydrates provided by the host plant for the fungi (Javaid, 2009). Plants inoculated with AMF are generally more efficient in acquisition of nutrients which leads to an improved plant growth (Oseni et al., 2010). Colonization of roots by AMF has also been shown to enhance plant tolerance to various plant growth inhabitation factors, and thus improving crop growth and productivity (Javaid and Riaz, 2008). Arbuscular mycorrhizal fungi functionality can be improved by addition of soil amendment (Warnock et al., 2010). One of soil amendments is biochar that is known to be able to reduce the soil carbon released because biochar is resistant to weathering and difficult to decompose (Verheijen et al., 2009). Biochar can serve as refuge for AMF hyphae and protect them from fungal grazers (Warnock et al., 2007), thus enhancing plant host-fungus symbiosis. Ishii and Kadoya (1994) argued that additions of biochar altered soil physico-chemical characteristics, leading to increased soil nutrient availability and enhanced mycorrhizal root colonization. Akiyama et al. (2005) showed that some of the compounds secreted by plant roots (e.g. CO_2, flavonoids, sesquiterpenes and strigolactones) can increase the branching of mycorrhizal hyphae and is expected to increase P uptake in maize to support the growth and productivity of maize crop. In addition Biochar can also be a good habitat for mycorrhizal growth. Yamato et al. (2006) revealed that biochar can lead to changes in physical and chemical properties of the soil and resulted in increased nutrient availability in the soil and increase plant root colonization by mycorrhizal fungi. According to Lehmann et al. (2003), biochar addition can improve plant productivity directly as a result of its nutrient content and release characteristics, or indirectly, through improved nutrient retention. Since AMF and biochar can both improve crop performance, there is an increasing interest in understanding their potential synergisms in increasing phosphorus availability of calcareous soils and phosphorus uptake by maize to improve overall maize crop performance. The objective of this study was to investigate the effects of biochar amendment and arbuscular mycorrhizal fungal inoculation on phosphorus availability phosphorus and growth of maize on a calcareous soil of East Nusa Tenggara, Indonesia.

Materials and Methods

The study was conducted in December 2012 - May 2013 at a glasshouse of the Faculty of Agriculture Nusa Cendana University, Kupang. Top soil (20 cm depth) of an Alfisol collected from Oebelo Kupang was used for the study. The soil is a predominantly sandy loam with 18%N,10% total P, 15.5 mg P_2O_5/kg, 0.59 me K/100g, 26.71 me Ca/100 g, and pH of 7.02. The mycorrhizal spore propagules of the site were less than 1 /kg soil. The soil was not fumigated prior to experimentation.

Twelve treatment combinations (three biochars levels of 0, 5 and 7.5 g/kg of soil, and four AMF inoculation levels of 0, 5, 10 and 15 spores / kg of soil) (Table 1) were arranged in a completely randomized block design with three replicates. For each treatment, three pre-germinated maize seeds were planted into a plastic pot containing 10 kg or air-dried soil. The maize was grown for 8 weeks. Each pot received basal fertilizers of 7.5 mg N/kg, 7.5 mg P_2O_5/kg, 7.5 mg K_2O/kg and 5 mg S/kg (supplied as Phonska). Each pot also received 2.5 kg ground cow dung /kg. The cow dung contains 4.5 mg P_2O_5/kg. The addition of fertilizer and manure made the initial content of P_2O_5 in the soil was 27.5 mg P_2O_5/kg. During the experiment, water was regularly supplied to ensure that water did not limit plant growth. Biochar was produced at the Faculty of Agriculture, Nusa Cendana University of Kupang, from cow dung. The equipment used for preparing biochar was a simple pyrolysis drum having 55 cm high, 45 cm in diameter, and 20 cm diameter of an opening / closing hole. Prior to heating, cow dung was air dried, cleaned, and sieved to get particles size of 1.5 mm - 2.3 mm (8-10 mesh) (Sparkes and Stoutjesdijk, 2011). The pyrolysis temperature was maintained at 450ºC for 1 hour. Plant height was measured at 2, 4, 6 and 8 weeks after planting. At harvest (8 weeks), maize dry weight, total soil P, available soil P, P uptake by maize and the growing amount of spore per 50 g of soil were measured at 2, 4, 6 and 8 weeks after planting. Data obtained were subjected to analysis of variance using Minitab followed by 5% least significance different test.

Table 1. Treatments

Code	Description
B0M0	no biochar, no mycorrhiza (control)
B1M0	5 g biochar/kg, no mycorrhiza
B2M0	7.5 g biochar/kg, no mycorrhiza
B0M1	no biochar, 5 mycorrhiza spore/kg
B1M1	5 g biochar/kg, 5 mycorrhiza spore/kg
B2M1	7.5 g biochar/kg, 5 mycorrhiza spores/kg
B0M2	no biochar, 10 mycorrhiza spore/kg
B1M2	5 g biochar/kg, 10 mycorrhiza spore/kg
B2M2	7.5 g biochar/kg, 10 mycorrhiza spores/kg
B0M3	0 g biochar/kg, 15 mycorrhiza spore/kg
B1M3	5 g biochar/kg, 15 mycorrhiza spore/kg
B2M3	7.5 g biochar/kg, 15 mycorrhiza spores/kg

Results and Discussion

Plant height

At two weeks, maize growth, as measured by plant height, generally reflected the availability of soil P. Growth was vigorous during 6 and 8 weeks in all treatments. However, the interaction of AMF inoculation × biochar amendment was not significant. In this study, biochar had no positive effect on plant growth with or without AMF inoculation. Similarly, Graber et al. (2010) did not find any effect of biochar on the number of flowers or fruit yield of tomato grown in a soilless medium. However, Kimetu et al. (2008) observed increased yield in maize following three repeated applications of 7 t/ha of biochar over two growing seasons in Kenyan soils cropped to maize for up to 100 years. Even with 20 t/ha biochar applied, Major et al. (2009) found only a significant yield response in maize in the subsequent cropping year. Despite the clear evidence that increased yield is usually observed in subsequent years, some authors found positive results in the first year. For instance, in cherry tomato, Hossain et al. (2010) reported a 20% yield increase with combined biochar and fertilizer. In their studies, they used a low pH chomosol with 10 t/ha of biochar applied. The absence of a clear plant growth increase in this study could partly be attributed to the soil used (basic), number of growing seasons and application frequency. The greatest plant height was observed for AMF inoculation of 50 spores / pot (M1) (Table 1). However, the plant height of treatments with no AMF inoculation were only significantly different ($p < 0.05$) at 6 weeks, whereas at 8 weeks the plant height of treatments with no AMF inoculation were only significantly different from those of AMF inoculation of 150 spores / pot (M3). This non significant effect of AMF inoculation was probably due environmental adaptability problem of AM inoculants that were originated from Java and/or due to the present of indigenous AMF in the soil. Kuyper et al. (2004) argued that environmental factors influence the development of AMF and colonization of the fungi to the roots.

Table 1. Height of maize at 2, 4, 6 and 8 weeks

Mycorrhiza treatment	Plant height (cm)			
	2 weeks	4 weeks	6 weeks	8 weeks
M0 (0 spores / kg of soil)	24.59 a	37.39 a	55.51 a	66.89 a
M1 (5 spores / kg of soil)	28.10 a	37.93 a	68.18 c	72.57 a
M2 (10 spores / kg of soil)	27.71 a	35.91 a	62.58 b	70.50 a
M3 (15 spores / kg of soil)	28.82 a	38.88 a	63.25 bc	71.84 a

Total soil P

Biochar amendment and AMF inoculation significantly affected total soil P content at week 8 (Table 2). This significant increase of total soil P indicated the improved balance of soluble and adsorbed P (Nursyamsi and Setyorini, 2009). The significant effect of biochar and its interaction with AMF on the increase total soil P was probably related to the supply of high surface area provided by biochar (400-800 m^2 /g of soil) which created a better habitat for the development and activities of soil microorganisms, which in turn increased the availability of soil P (Fiscer and Glaser, 2012).

Available soil P

Availability of soil P significantly decreased from 30 to 4 weeks after planting but no significant decrease was observed at weeks 6 and 8 (Figure 1). Application of 5 g biochar/kg of soil (treatment B1) significantly decreased availability of soil P at 4 weeks. At 6 weeks, however, application of 5 g biochar/kg significantly increased P availability in the soil. The highest increase of soil available P was observed for the B2M2 treatment (7.5 g biochar/kg and 10 AMF spores/kg) at 8 weeks. However, this value did not significantly different from that of treatment B2M0 (7.5 g biochar/kg with no application of mycchoriza. Total P in soils inoculated with AMF with no biochar tended to decline until week 8. Results of statistical analysis showed significant differences at the level of 5% between treatments. At 2 weeks, the lowest value of available P was found in the control (B0M0) and B0M3 treatments. Treatments of B1M0 and B2M0 gave high available P but there were not significantly different from all other treatments. At 4, 6 and 8 weeks, B2M2 treatment reached the highest content of available P. The increase of soil available P due to inoculation of AMF and biochar amendment indicated that biochar provided benefits of donations adsorption complex for cations and anions needed for plant growth (Major et al., 2010). The non significant increase in available soil P was probably related to the relatively high pH of the soil studied. Van Zwieten et al. (2010) suggested that biochar would be beneficial and potential for plant growth in acid soils where the elements Al becomes the limiting factor of plant growth but not necessarily beneficial in calcareous soils.

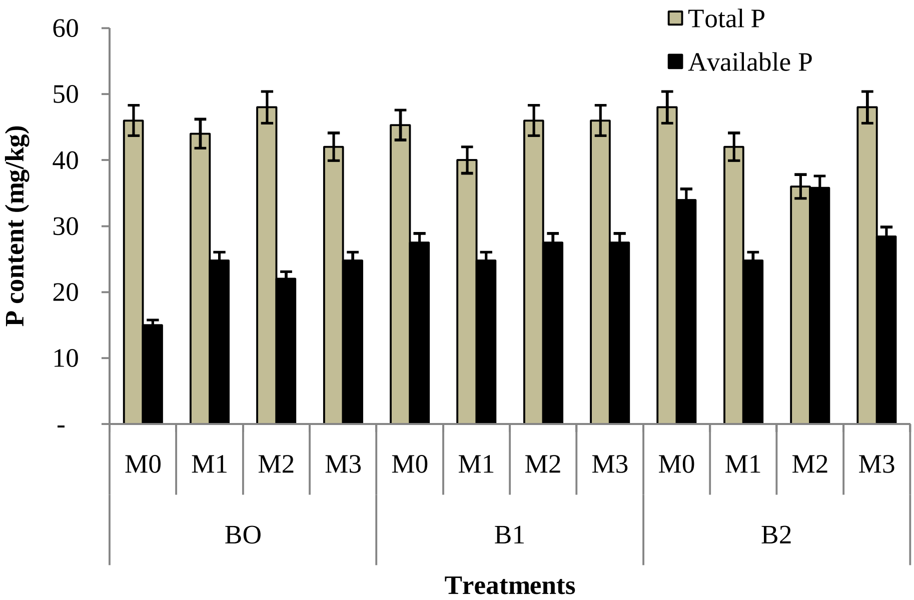

Figure 1. Total and available soil P as affected by biochar amendment and AMF inoculation. *) see Table 1

Uptake of P by maize

Uptake of P by maize was improved by biochar amendment and AMF inoculation (Figure 2). Compared to the control (B0M0), biochar amendment and AMF inoculation increased P uptake by maize from 3.6% (B0M3) to 62.7% (B2M2). This indicates that a combined application of biochar and AMF improved availability of soil P and uptake of P by maize which in turn improved plant yield. Saranya et al. (2011) reported that biochar treatment with standard 25 t/ha can increase P uptake in maize more than 15 mg / kg and did not differ significantly with biochar treatment of 10 t/ha and 15 t/ha. According to Rondon et al. (2007), application of biochar to 30 g/kg soil can increase P uptake up to 7 mg/pot on legumes. According to Pamuna et al. (2013), AMF can increase P uptake in maize to 300 kg P/ha as well as to increase the availability of P in the soil.

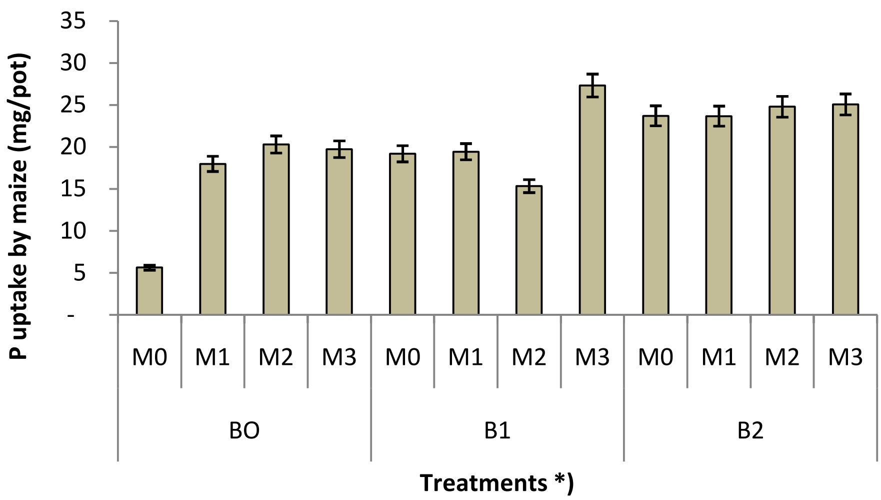

Figure 2. Uptake of P by maize as affected by biochar amendment and AMF inoculation. *) see Table 1

Mycorrhizal spore

There was a good response of mycorrhiza to the AMF inoculation 15 spores/kg of soil (Figure 3). The decline in number of spores in the early planting period was expected because the inoculated AMF that were originated from outside of the study area had not yet been able to make adjustment to the existing environmental factors, Kuyper et al. (2004) suggested that environmental factors influence the development of sufficient and colonization of mycorrhizal fungi to the roots, especially in the different conditions between the native habitat mycorrhizal growth/development of the host plant.

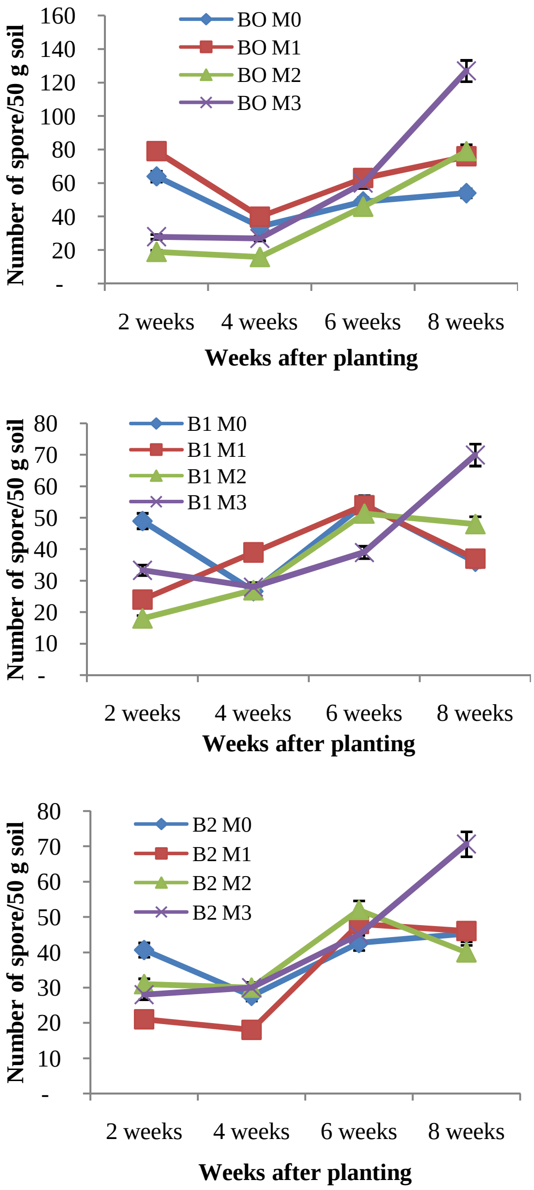

Figure 3. Number mycchorizal spores due to application of biochar *) see Table 1

Baon et al. (2008) identified that changes in soil temperature greatly influenced the development of mycorrhiza, in which the interaction between the development of mycorrhiza and soil temperature will affect P uptake by the host plant. The types of mycorrhiza identified in this study in were *Glomus sp*, *Gigaspora*, *Scutellospora* and *Acaulospora*. The most varied types of spores were found in the B2M0 treatment (7.5 g biochar/ kg of soil with no AMF inoculation). The types of mycorrhizal spores found in this study were similar to those reported by Haerida and Karmadibrata (2002) who identified the origin of mycorrhizal fungi spores on sweet maize plant in Java.

Conclusion

Combined application of biochar ranging from 5 to 75 kg biochar (equivalent to 10-15 t biochar/ha) and inoculation of mychhoriza arbuscular spores ranging from 5-15 spores/kg (equivalent to 50-15 mycchoriza arbuscular spores/ha) improved availability of soil P and uptake of P by maize which in turn improved plant yield.

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