

Influence of Long-Term Fertilization on Spore Density and Colonization of Arbuscular Mycorrhizal Fungi in a Brown Soil

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Abstract. This study aims to explore changes of long-term fertilization on spore density and colonization of AMF (Arbuscular mycorrhizal fungi) under a 38-y long-term fertilization in a brown soil. Soil samples (0-20 cm, 20-40 cm, 40-60 cm) were taken from the six treatments of the long-term fertilization trial in October 2016: no fertilizer (CK), N₁ (mineral nitrogen fertilizer), N₁P (mineral nitrogen and phosphate fertilizer), N₁PK (mineral nitrogen, phosphate and potassic fertilizer), pig manure (M₂), M₂N₁P (pig manure, mineral nitrogen and phosphate fertilizer). Spores were isolated from soils by wet sieving and sucrose density gradient centrifugation; mycorrhizal colonization levels were determined by the gridline intersect. The spore density was highest in the topsoils (0-20 cm), and was decreased with increasing of soil depth in each treatment. The spore density of M₂N₁P treatment was significantly higher than that of other treatments in each soil layer. Application of inorganic fertilizer (especially inorganic with organic fertilizer) can greatly improve the level of colonization. Our results suggested that long-term fertilization significantly affects spore density and colonization of AMF, however, spore density is not related to colonization rate.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread in most terrestrial ecosystems where they form mutualistic associations with the majority of plants to facilitate nutrient uptake from the soil via an extensive extra radical mycelium [1]. AMF transports mineral elements, water to the root for the plant to absorption and utilization; meanwhile, it obtains carbohydrates of growth and reproduction by extensive mycelium from the plant. In addition, it can improve the soil environment in the rhizosphere [2, 3], activate mineral nutrients in soil [4] and promote plant uptake of nutrients; and also enhance disease resistance of crop [5, 6], increase crop yield and improve product quality. The spores are the main propagule of AMF. Many studies have shown that soil fertility, spore amount will be reduced; high phosphorus will inhibit the production of mycorrhizae and mycelium branching and elongation [7, 8]. Tawarayama believes that the main reason is the Change of the crop root exudates components. However, there are also a few reports that AMF infection has little or no correlation with soil fertility [9, 10].



Long-term fertilization test artificially created different soil fertility conditions, in this experiment, the soil spore density and mycorrhizal infection were investigated to explore the effects of long-term fertilization on them.

2. Materials and methods

2.1. Sampling

The study was conducted in the semi-humid region of Shenhe district, Shenyang (40°48'N and 123°33'E) of Liaoning Province, China. The field experiment was conducted in a well-drained field under a rotation of maize-maize-soybean. The crop growing season started in April and ended in September. In this study, a randomized block design was adopted in the experimental field and six treatments with four replicates each were chosen as follows: no fertilizer (CK), N₁ (mineral nitrogen fertilizer), N₁P (mineral nitrogen and phosphate fertilizer), N₁PK (mineral nitrogen, phosphate and potassic fertilizer), pig manure (M₂), M₂N₁P (pig manure, mineral nitrogen and phosphate fertilizer). The mineral fertilizers were applied in the form of urea, calcium superphosphate and potassium sulphate. Basic chemical properties of experimental soil in 1979 and the application rates of fertilizer are described by Luo et al. [11].

Soil samples were collected from 0-20 cm, 20-40 cm and 40-60 cm soil at the ripening stage before harvesting. Maize roots were collected on the same day.

2.2. Spore density

Spores were isolated from soils by wet sieving and sucrose density gradient centrifugation and some modifications are as follows: briefly, 50 g of soil were passed through sieves of two-tier (0.125 mm and 0.038 mm) and thoroughly washed with distilled water. Transferring the filter residue to 50 mL centrifugation tubes, centrifuging at 3000 ×g for 3 min and gently reversing the suspension. Adding 60 mL of a 50% sugar solution centrifugation tubes and centrifuging at 1800 ×g for 2 min. The suspension was passed through sieves of 0.038 mm pore diameter and rinsed sieves with distilled water for several minutes to remove residual sucrose solution. The spore mixture in the sieve was filtered to the wet filter paper through the Buchner funnel [12]. Finally, spores were identified by microscope.

2.3. Root colonization

Mycorrhizal colonization levels were determined by the gridline intersect [13]. After clearing the roots in 2.5% KOH solution (wt/vol) and by staining then with 0.05% trypan blue [14]. The gridline intersect method of Tennant was defined for estimating the total root length [15].

2.4. Statistical analysis

The charts were made by Microsoft Office Excel 2010 and Variance Analysis and Linear Regression Analysis adopt SPSS 19.00.

3. Results and discussion

3.1. The effect of different fertilization on AMF spore density

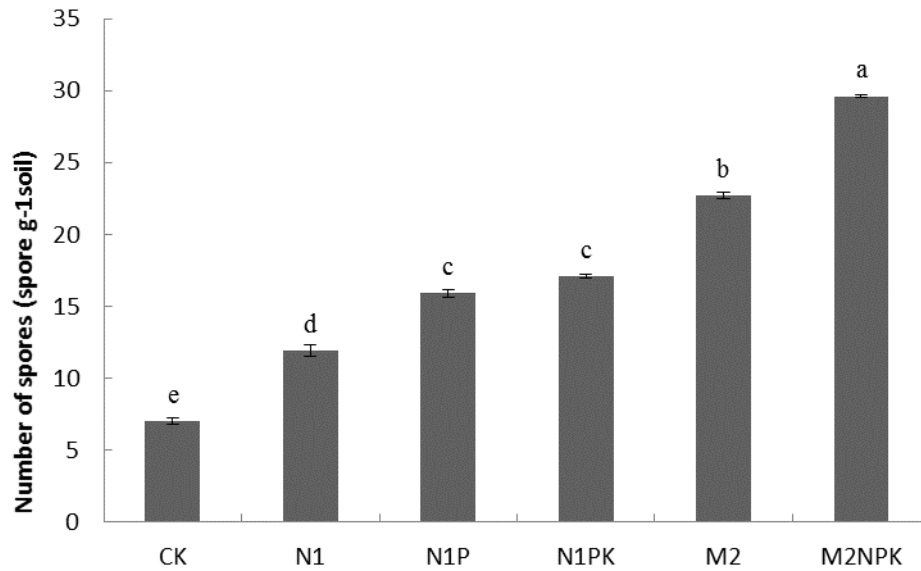


Figure 1. The spore density of different fertilization treatments (0-20cm soil)

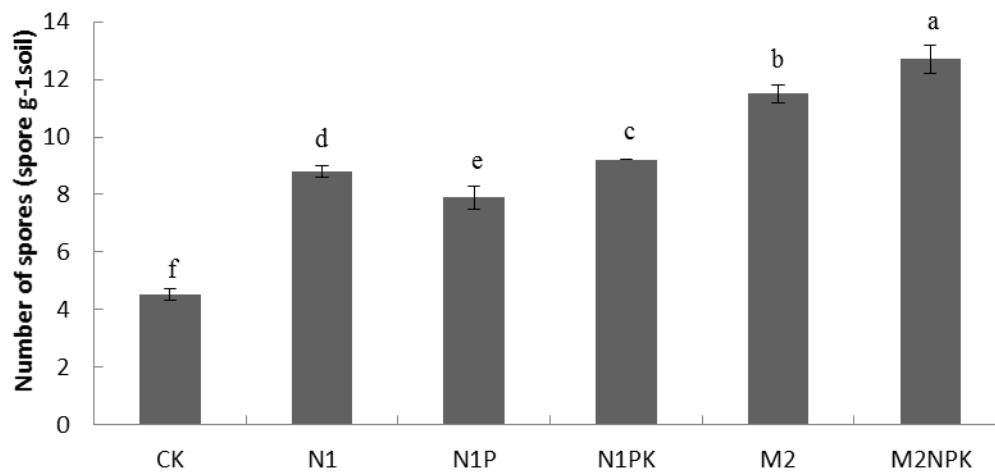


Figure 2. The spore density of different fertilization treatments (20-40cm soil)

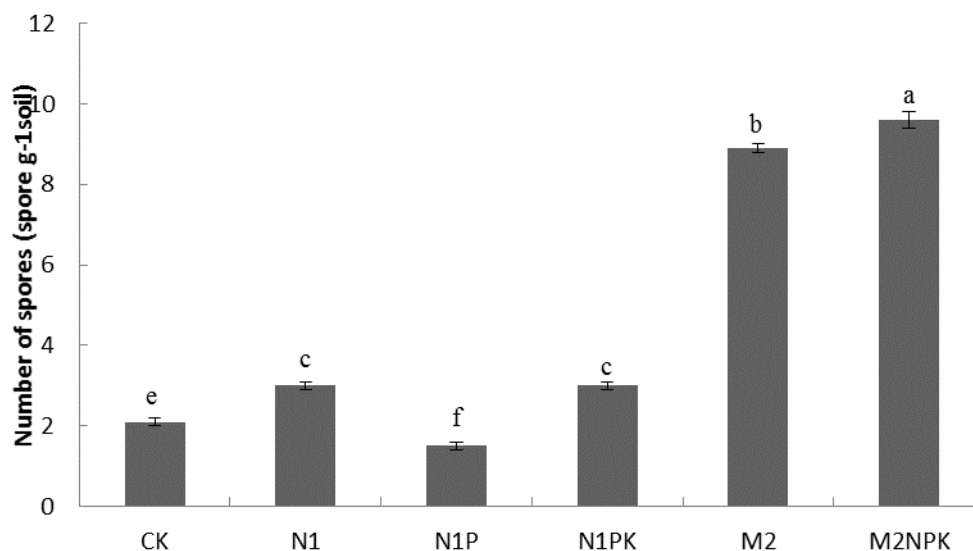


Figure 3. The spore density of different fertilization treatments (40-60cm soil)

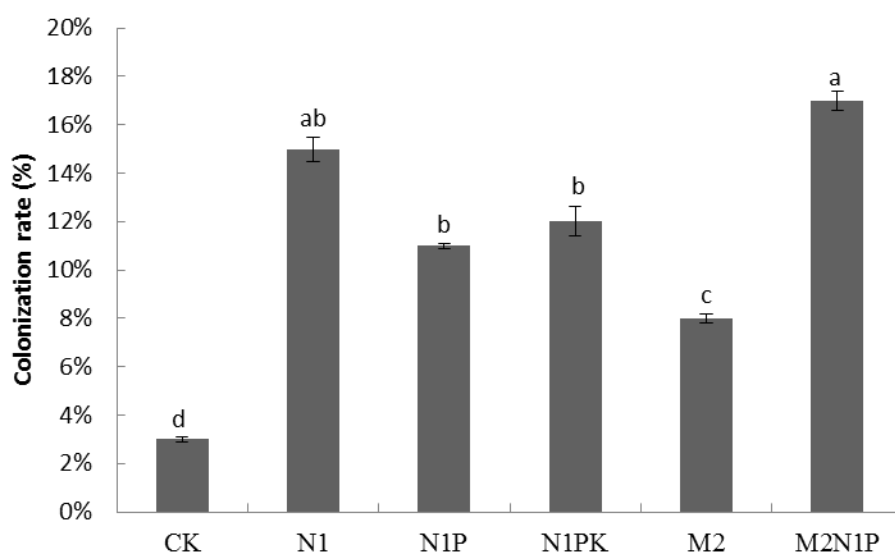


Figure 4. The colonization rate of AMF in different fertilization treatments

The spore density in different fertilization treatments and soil depths were shown in Fig.1-3. It was highest in the topsoil layer (0-20 cm), and was decreased with increasing of soil depth in each treatment. The spores density of M₂N₁P treatment was significantly higher than that of other treatments in each soil layer.

The spore density was significantly different in 0-20 cm soil of different fertilization treatments (Fig.1). The spore density of M₂N₁P treatment was the highest (29.6 spores g⁻¹), while that of CK treatment was the lowest (7 spores g⁻¹) respectively. The result indicated that the application of organic fertilizer with inorganic fertilizer is helpful to increase the spore density. The spores density was 4-14 spores g⁻¹ in 20-40cm soil of different fertilization treatments, and it was still the lowest in CK treatment (Fig.2). The spores density was 1.5-12 spores g⁻¹ in 40-60cm soil of different fertilization treatments (Fig.3), the spore density of organic fertilizer treatments was significantly higher than that of other treatments.

After 38-y long-term fertilization, there was significant different in spore density of different fertilizer treatments. Fertilizer influenced the composition of fungus species, the diversity of the mycorrhizal ecosystem and the spatial distribution of spores in soil. Generally speaking, the spore density was highest in the topsoils, and decreased with increasing of soil depth in all treatments. The spores density of M_2N_1P treatment was significantly higher than that of other treatments in each soil layer.

3.2. The effect of different fertilization on colonization of AMF

The results of maize root colonization rate were shown in Fig.4. It is reflected by the structure of hypha, carbuncular and vesicle. In general, the colonization level of AMF refers to the total percentage of colonization [16]. The colonization level of AMF was a significant difference in different fertilization treatments. The total colonization rate was low in maize maturity period. It can be seen from Fig.4 that the colonization rate of AMF was the highest in M_2NP treatment, indicating that the organic fertilizer with inorganic fertilizer can greatly improve colonization. The colonization rates of CK treatment and M_2 treatment were significantly lower than other treatments, which indicate that application of chemical fertilizer can increasing colonization of AMF.

AMF are widespread in agro ecological system. Various agricultural practice will influence the growing and colonization of AMF. There was a significant difference in the level of colonization between different fertilization treatments in this experiment. Organic fertilizer with inorganic fertilizer were the highest rate of colonization in maize root. Paradoxically, some studies have shown that increasing nitrogen fertilizer can reduce fungal colonization [17], while some studies have also shown that increasing nitrogen fertilizer can enhance fungal colonization [18]. It was found that the application of nitrogen fertilizer only increased the number of mycelia rings in cells, but it had no effects on the carbuncular, vesicles and hyphae [19]. Maize roots infection level was significantly different between CK and N treatment in this experiment. The supply of soil phosphorus increases the phosphorus level in plants but causes the decrease of mycorrhizal colonization. After nitrogen fertilizer with phosphate fertilizer was used in this experiment, the mycorrhizal colonization rate was reduced. Organic fertilizer with inorganic fertilizer can increase the rate of infection, which may be due to the high growth of maize roots and more susceptible to infection.

3.3. Linear Regression Analysis

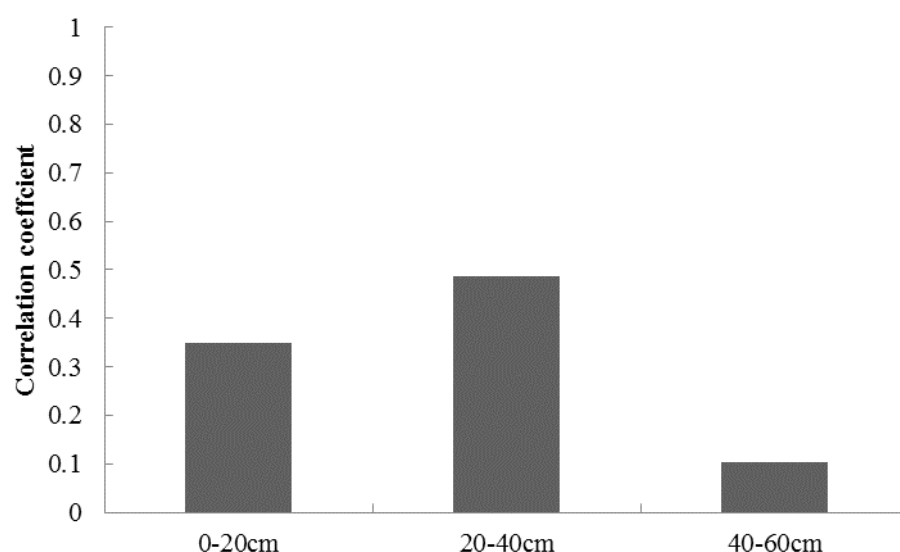


Figure 5. The correlation between the colonization rate and spore density in different soil depths ($p=0.05$)

Linear Regression Analysis was performed on the colonization rate and spore density of each layer of soils. The correlation coefficients between colonization rate and spore density was 34.9% (0-20 cm), 48.7% (20-40 cm) and 10.4% (40-60cm), respectively ($p=0.05$). The result indicated that the colonization rate of AMF was not related to the spore density.

4. Conclusion

After 38-y long-term fertilization, there was significant different in spore density and colonization rate, application of organic fertilizer with inorganic fertilizer had more influence on spore density and colonization rate of AMF. However, spore density is not related to colonization rate under long-term fertilization. Thus our future study should focus on factors influence colonization rate of AMF.

Acknowledgments

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