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Fertilizing reclamation of arbuscular mycorrhizal fungi on coal mine complex substrate

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Abstract

A series of pot culture experiments are carried out to evaluate the effects of inoculation of arbuscular mycorrhizal fungi (AMF) Glomus versifomae (G.v) B1 and Glomus mosseae (G.m) B2 in different weight ratios (A1, A2, A3) of coal mine complex substrates. The results show that the increasing rates of organic matter, total N, P, and K reach 8.86%, 5.26%, 18.66% and 3.16%. A2 is selected as the suitable covering soil and B2 (G.m) as the preponderant AMF. White clover is suitable in the coal mine reclamation. Through promoting plants roots growth and the transformation of insoluble nutrition element to available state, the inoculation of AMF increases the nutrition absorption of plants and organic content in the substrate, fertilizing the coal mine complex substrate in the coal mine reclamation. The combination of AMF and sludge is able to enhance the fertilizing of coal mine castoff.

Keywords: coal mine castoff; sludge; AM fungi; fertilize reclamation

1. Introduction

The situation that coal is the primary energy source in China will persist for a long time. In the process of coal exploitation, ore dressing and utility, a lot of coal gangue and fly ash generated and were discarded. Because coal gangue and fly ash have the characteristics of low water retentivity and low fertility, plants are difficult to grow on these substrates. However, the introduction of AMF can efficiently enhance the tolerance of plants for leanness and acidification and improve soil structure, which is benefit to recover the plants and fertilize reclamation.

Mycorrhizal fungi are a main component of the soil micro biota in most ecosystems, and it is believed that a majority of terrestrial plants are associated with some kinds of mycorrhizal fungi, with arbuscular mycorrhizal fungi (AMF) as the most common group. In this symbiotic association, host plants provide the fungi with carbohydrates and in return receive mineral nutrients [1]. The micro-organism reclamation technique of AMF has become the research focus [2-6]. The research of Daft [7-8] illuminated that the successful planting in the coal mine wasteland was inseparable with the colonization of AMF. In the taconite iron ore tailing, field inoculation with AM fungi increased plant cover and fertilizer, which forecasts the establishment of a sustainable native grass community and meets
reclamation goals [9]. Call et al [10] inoculated AMF to the pasture in degrade wasteland ecosystems and found that the N and P content increased. In this article, sludge was introduced to the mine area castoff to form coal mine complex substrate and plants were inoculated with AMF. Based on the selection of AMF and plants, the fertilizing reclamation of inoculation of AMF and the addition of sludge to coal mine complex substrate were evaluated.

2. Experimental materials and methods

2.1. Growth media

The growth medium was the compound activated substrates including fly ash (Fa), coal gangue (Cg) and sludge (Sl). The fly ash was collected from Mao village power-station, the coal gangue was from coal waste of Zhangxiaolou Mine, and the sludge was from Nanhu waste water treatment plant of China University of Mining and Technology. The coal gangues were broken up to 2 cm in diameter. The sludge was air dried to approximate 70% moisture. According to our previous study [11], three weight ratios of fly ash, coal gangue and sludge were selected in this study. The physicochemical properties of these substrates are shown in Table 1.

Table 1. The physicochemical properties of tested substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Fa:Cg:Sl</th>
<th>Organic matter content (g/kg)</th>
<th>Total N (g/kg)</th>
<th>Available P (mg/kg)</th>
<th>Available K (mg/kg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>20:50:30</td>
<td>45.23</td>
<td>4.23</td>
<td>62.96</td>
<td>216.15</td>
<td>7.42</td>
</tr>
<tr>
<td>A2</td>
<td>20:60:20</td>
<td>35.57</td>
<td>2.87</td>
<td>44.18</td>
<td>254.88</td>
<td>7.46</td>
</tr>
<tr>
<td>A3</td>
<td>20:70:10</td>
<td>27.33</td>
<td>2.38</td>
<td>34.88</td>
<td>283.96</td>
<td>7.39</td>
</tr>
</tbody>
</table>

2.2. Host plants and AM fungus

The plants used in study were white clover, rye grass and corn. The seeds of these plants were purchased from Shandong and Xuzhou. Before planting, the seeds were surface sterilized with 10% v/v hydrogen peroxide for 10 min, washed with sterile water and germinated in the dark on moist filter paper at room temperature.

Two AMF species, Glomus mosseae(G.m)B1 and Glomus versiforme(G.v)B2, were provided by Mycorrhizal Biological Laboratory of Qingdao Agriculture University.

2.3. Pot experiment

The experiment had three substrate ratios of fly ash, coal gangue and sludge. The experimental design is shown in Table 2. Single species and a mixture of both species (B3) were used as mycorrhizal fungus inocula. Each inoculated pot received 30 g of inoculum. They were sand-based mycorrhizal inocula containing abundant chopped mycorrhizal root pieces, spores and hyphae. Thus, there were three treatments (inoculated B1, B2 and B3) and one control with 3 replicate pots per treatment. A comparison without AM fungi propagates was set. The pots were randomly placed in a 3 m tall open greenhouse with natural lighting and air temperature. De-ionized water was added as required to maintain moisture content at 55% of water holding capacity by regular weighing.

Table 2. Design of substrate ratios, AMF and plants
2.4. Analysis and determination

Roots and soil were sampled after 100 days growth of plants. Before washing out roots, soil samples (around 30 g fresh weights) were collected for measuring hyphal length density. Root samples were carefully washed with deionized water to remove adhering soil particles. Sub-samples of fresh roots were collected for determination of AM colonization. Organic content was measured by the potassium bichromate titrimetric method, and total nitrogen (N) was measured by the micro-Kjeldahl procedure. Extractable P was measured by the molybdenum-antimony anti-spectrophotometric method and exchangeable potassium (K) was determined after extraction with ammonium acetate-flare photometer.

Sub-samples of fresh roots were cleared in 10% KOH and stained with Trypan blue by a modification procedure of Phillips and Hayman[12], omitting phenol from solutions and HCl from the rinse. Percentage root colonization and root length were determined by the grid-intersect method[13].

3. Results and discussions

3.1. Mycorrhizal colonization (MC)

The MC is shown in Table 3. As can be seen, the MC of A3B2C1 was highest, 66.67%, and 20–40 percent above other treatments. But the MCs of the other two treatments of A3 were lower, 26.67% and 38.33%, respectively. The MC of treatments A3 and A2 were close to each other, which were obviously beyond the range of A1. The MCs of treatments A2 were better than other treatments as a whole, and more than 40%. The AMF strains played a vital role in colonizing, and the average MC of B2 (G.m) was 13–14 percentages above that of B1 and B3, indicating G.m was suitable in coal mine reclamation.

Table 3. MC of different treatments

<table>
<thead>
<tr>
<th>Factor/level</th>
<th>C1(white clover)</th>
<th>C2(corn)</th>
<th>C3(rye grass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3 (20 : 70 : 10)</td>
<td>B3(G.v+G.m)</td>
<td>B1(G.v)</td>
<td>A3B1C3</td>
</tr>
</tbody>
</table>

The MC of A2 and A3 were higher than that of A1, which may be caused by the different phosphorus content in these substrates. The available P content must be 20-50mg/kg[11], or the growth of AMF will be restrained, then restraining the mycorrhizal effect. In our experiment, the available P content of A1, A2 and A3 were 60mg/kg, 30mg/kg and 20mg/kg, respectively. The higher available P content of A1 made the MC lower, while those of A2 and A3 between 20 and 30 mg/kg, the MC were higher. The phosphorus content was primarily from sludge, so, sludge addition should be suitable. The sludge addition of treatment A2 was suitable[11], and the mycorrhizal effects...
were pronounced. The MC of C1 were higher than C2 and C3, and the mycorrhizal effects of white clover were better than C2 (corn) and C3 (rye grass), indicating that C1 was prone to be colonized.

3.2. The influence of inoculation to the substrate organic content

The influence of inoculation to the substrate organic content is shown in Fig.1. The organic contents increased in different treatments and the organic contents of the inoculation were higher than the un-inoculation, exceeding 2.0g/kg, and the increasing rates 4.87% ~ 6.40%. The organic contents increasing of treatment A2B3C1, A2B1C2, A2B2C3, A1B2C2 and A3B2C1 were twice than the comparison, and mycorrhizal colonization rates of these five treatments were higher than 40%, indicating AMF had pronounced mycorrhizal effects in these five treatments and improved the organic contents and fertilized the coal mine complex substrate.

AMF can produce metabolites in its growth and propagation and secrete in vitro, accelerating the humification of soil and increasing the organic contents. Although mineralization of organic matter happens in the course of plants absorb nutrition, nutrition uptake of plants would gradually stabilize as the plants grow, and humification of organic matter would exceed mineralization, so the organic content would accumulate and the coal mine complex substrate would be fertilized. Organic matter is the major source of soil nutrition and the important index of the soil fertility and aging, and plays an important role in the soil structure formation and moisture and fertilizer retention. Organic content should be improved in the soil reclamation, in order to promote the soil fertility formation.

The original organic contents are between 20 and 50 g/kg, reaching the request of common planting and culturing [12]. The organic contents of A2 were higher and their mycorrhizal colonization rates were also higher, indicating the inoculation of AMF promoted the increasing of organic contents. The original nutrition influenced AMF. The results indicated that treatment A2 was the suitable substrate for coal mine reclamation.

![Fig. 1. The influence of inoculation to organic content](image-url)

3.3. The influence of inoculation to N, P, K in the substrate

The plants growth improves N utilization, and the total N in the rhizosphere declined. The influence of inoculation to total N in the substrates is shown in Fig. 2(a). The total N decrements in inoculation treatments were higher than the un-inoculation ones, indicating that the inoculation of AMF accelerated the transformation of total N to available N. The total N decrement in treatments A2 was 1.96 times higher than the un-inoculation, higher than those of 1.28 times of A1 and 1.53 times A3. Besides, as legume crop, white clover has developed root systems, and the inoculation of AMF increased legume bacteria, improving the nitrogen fixation of legume bacteria [14]. The legume bacteria were observed in our experiments obviously, indicating the existence of the legume bacteria, in order to promote white clover to absorb nitrogen.
AMF influences the available P in the substrate, showing in Fig. 2 (b). The available P decrement of inoculation treatments was higher than un-inoculation, illuminating the inoculation of AMF promoted the available P absorption of plants. The available P decrement of treatments A3B2C1, A2B2C3, A2B3C1 and A1B2C2 exceeded 7.8g/kg, reaching twice of that of the un-inoculation comparison. The MCs of these 4 treatments were high, more than 42%, the highest 66.67%, indicating that the inoculation of AMF promoted the available P absorption of plants. The higher available P change of treatment A2 indicated that the inoculation of AMF accelerated the transformation of total P to available P.

Fig. 2. (a) the influence of inoculation to N in the substrate; (b) The influence of inoculation to available P in the substrate

AMF influences the available K in the substrate, shown in Fig. 3. The available K decrement of inoculation treatments was higher than that of un-inoculation. The available K decrement of treatments A3B2C1, A2B1C2, A2B2C3, A1B2C2 and A2B3C1 were more than 2.5 times of that of the un-inoculation comparison, and the corresponding MC were between 42.86% and 66.67%. The available K decrement of different treatments was consistent with the change of available P decrement, illuminating the inoculation of AMF promoted the available K absorption of plants. The inoculation effects of A2 and A3 were better than that of A1, and the K absorption of A2 was higher than that of A3, showing better mycorrhizal effects.

Fig. 3. The influence of inoculation to available K in the substrate

The colonization of AMF connects plants roots and soil, becoming the key factor helping plants growing. So, the colonization of AMF increased the absorption of nutrition N, P, K. The nutrition and secretion increased, and more resources were offered for rhizosphere microorganism, improving the micro-ecological environment and
accumulating soil nutrition\textsuperscript{[1]}. The best performance of B2 in the absorption of N, P and K, indicating B2 was the suitable AMF in coal mine reclamation.

4. Conclusions

Based on the MC, nutrition absorption efficiency and organic content increasing in the substrate, A2 is selected as the suitable covering soil and B2 (G.m) as the preponderant AMF. White clover has developed root system, absorbs more nutrition and has better affinity with B2 (G.m), and is suitable for the coal mine reclamation.

Through promoting plants roots growth and the transformation of insoluble nutrition element to available state, the inoculation of AMF increases the nutrition absorption of plants and organic content in the substrate, fertilizing the coal mine complex substrate in the coal mine reclamation.

The addition of sludge accelerates the fertilizing reclamation. The combination of AMF and sludge is able to enhance the fertilizing of coal mine castoff.

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References