



Effect of Fertilization and Bacterial Inoculation on the Growth of Alder (*Alnus sibirica*) in Coal Mine Soil

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Authors' contributions

This work was carried out in collaboration between both authors. Author MOS conducted the study under the supervision of the author CSS. Author MOS designed the research work and performed the experiment, analyzed the data and wrote the first draft of the manuscript. Author CSS managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to evaluate the effect of fertilization and nitrogen fixing (N-fixing) bacterial inoculation on the vegetative growth of alder (*Alnus sibirica*) plant species while grown in coal mine soil. The study was conducted in a greenhouse of the Forest Science Department, Chungbuk National University, South Korea, during the period of May 2019 to July 2019. A completely randomized design (CRD) comprising of four treatments, including T₀—non-fertilized non-inoculation (control), T₁—fertilization, T₂—bacterial inoculation and T₃—fertilization along with bacterial inoculation with three replications were used in the study. The results of the study showed that maximum growth of all studied parameters of alder were observed in fertilization along with bacterial inoculation treatment (T₃) and this treatment had significant effect on the growth of these parameters as compared to control, except root dry weight and shoot/root ratio. Fertilization treatment (T₁) showed significant increase of stem height, shoot fresh and dry weight, plant dry weight, canopy spread, number of leaves, branches and nodes per plant, leaf area and leaf area index of alder in coal mine soil, as compared to control. Bacterial inoculation treatment (T₂) also had

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positive effect on the vegetative parameters of plants comparing to control, excluding root length, root dry weight, shoot/root ratio, and canopy spread. Therefore, it can be summarized that fertilizer application and bacterial inoculation to the soil have a significant role in improving the vegetative growth of alder in coal mine soil.

Keywords: Alder; fertilization; bacterial inoculation; vegetative growth; coal mine soil.

1. INTRODUCTION

Coal is one of the most important energy resources which has been widely used since long time to produce steel and cement, and to generate electricity in thermal power plants [1]. However, coal mining is also responsible for degradation of land of a huge area. The disturbances in coal mine soil properties due to mining activities reduces the land productivity. Reduced productivity of the coal mine spoil causes nutrient scarcity in the soil which can restrict the growth of plants [2,3]. Coal mining also has adverse effects on the nutrient cycle that is crucial for maintaining the sustainability of plant community and healthy ecosystem [4].

Use of quick growing N-fixing plant species in revegetation for restoring degraded coal mine soil could be an effective method by enhancing nutrient cycling, reducing soil erosion, and increasing C assimilation in the soil [5]. N is one of the most important nutrients required for plant growth and development [6]. Previous studies have shown that N has a significant role in increasing the vegetative growth of plant and can also increase photosynthesis and assimilation in plants [6,7]. Regular application of nitrogenous and other nutrient fertilizers to the soil is a good approach to maintain the sustainability and healthy growth of vegetation in coal mine land [8,9]. Application of biofertilizer or inoculation with compatible N-fixing bacteria result in efficient nodule formation, better atmospheric N-fixation, and increasing content of N, biomass and yield in plants [10]. So, bacterial inoculation can be an alternate for reducing chemical fertilizer use during plant growth. However, it is also necessary to apply decent amount of N fertilizer to enhance N fixation and to get maximum growth of plant because plant cannot achieve all its demand for N only through biological nitrogen fixation even under favorable situations [11,3].

Alder is the common name of a genus of flowering plants (*Alnus*) belonging to the family Betulaceae. *Alnus sibirica* is one of the important deciduous species of this genus [12]. Alder has

important symbiotic relationship with *Frankia alni*, an actinomycete N-fixing bacterium. This symbiotic relationship improves the fertility of the soil where this plant grows and has established the alder as an important species in ecological succession [13]. The presence of the N-fixing bacteria and accumulation of N rich leaf litter of alder also enrich the nutrient status of soil and increase the production of trees on poor quality soils [12].

There are no studies with a view to assessing the effect of fertilizer along with bacterial inoculation on the growth of alder (*Alnus sibirica*) plant species while grown in coal mine soil. Hence the main objective of this study was to evaluate the effect of fertilizer application and inoculation of frankia bacteria on the growth of alder (*Alnus sibirica*) in coal mine soil.

2. MATERIALS AND METHODS

2.1 Soil Collection and Seed Germination

Coal mine soil for growing alder seedling was collected from three different locations of an abandoned coal mine area located in the Taebaek city of South Korea. Firstly, collected coal mine spoil was air-dried, ground, and sieved (mesh size 2 mm). After that, each pot was filled with equal amount of experimental soil. The volume of each pot was 4.8 L and the shape of the pot was conical frustum. Seeds for growing alder seedling were collected from the National Forest Seed Variety Center, South Korea. After collection, seeds were washed three times with sterile water and then, planted in the plastic tray, and kept in the growth chamber for germination. Two weeks after germination, required amount of alder seedlings were transplanted in the experimental soil containing pots and seedlings were irrigated three to four times in a week.

2.2 Greenhouse Experiment Setup

The study was conducted in a greenhouse of the Forest Science Department, Chungbuk National University, South Korea, during the period of May 2019 to July 2019. Inside the greenhouse, the

environments were maintained at 26-28°C temperature, approximately 90% relative humidity, and a photoperiod of 9–12-h light/24 h. A completely randomized design (CRD) comprising of four treatments with three replications was used for the experiment. Treatments were as follows: T₀—control (non-fertilized non-inoculated), T₁—fertilization (NPK fertilizer was applied to the coal mine soil), T₂—bacterial inoculation (N-fixing frankia bacteria was inoculated on the coal mine soil), T₃—fertilization together with bacterial inoculation (both NPK fertilizer and N-fixing frankia bacteria were added on the coal mine soil). Six seedlings were used for each replication of the experiment.

2.3 Fertilizer Application and Bacterial Inoculation

NPK 20:20:20 fertilizer was applied to the seedlings by mixing with water. This fertilizer consists of 20% nitrogen (N), 20% phosphorus (P₂O₅), and 20% potassium (K₂O) macro elements. In the experiment, the rate of fertilizer application was 250 NPK kg ha⁻¹. Fertilizer was dissolved in the water at the rate of 0.5 g L⁻¹. 250 mL of dissolved fertilizer was applied to each seedling of treatment T₁ and T₃ through a broadcast irrigation system. Fertilizer was applied at 15, 30, and 45 days after germination of the plant, respectively. Fertilization was done according to the instruction of the fertilizer company.

For inoculation, strain of *Frankia alni* was collected from the microbial germplasm of National Institute of Agricultural Sciences, South Korea. The source of the strain was alder plant species, and the location of isolation was Suwon, Gyeonggi, South Korea. Frankia strain was used for inoculating alder in treatment T₂ and T₃. From the collected strain, a single colony of *Frankia* was further subcultured and subsequently maintained in liquid defined propionate medium (DPM) until the inoculation of bacterial strain. After 15 days of germination, alder plants were inoculated with 5 mL of a homogenized mycelium suspension of Frankia strain containing 3 µg/ml of total protein in 1/8 strength NH₄Cl-free Hoagland solution as described previously [14,3].

2.4 Collection of Data on Vegetative Growth Parameter

The measuring growth parameters of alder seedling were stem height, root length, fresh and dry weight of root and shoot, ratio of shoot and root dry weight, plant dry weight, number of

leaves, nodes and branches per plant, canopy spread, leaf area, and leaf area index (LAI). The stem heights (cm) and root lengths (cm) were recorded using a measuring tape. The measurement of leave, node and branch number per plant was done manually. The canopy spread (cm) was recorded from the last leaf at one side of the plant to the last leaf of other side of the same plant using a tape measure. Leaf area per plant was measured using a leaf area meter (Model LAI 3100C, LICOR, Lincoln, NE), then leaf area index (LAI) was calculated using the following equation: LAI = Leaf area per plant (cm²)/Plant ground area (cm²). Plants were separated into shoots and roots. Shoots and roots were weighted in an electronic balance to determine the fresh weight of these parts. After that, these parts were dried in an oven at 65°C for 48h and weighted again to determine the dry weight of shoot and root. Dry matter weight of alder seedling was the sum of all oven dried plant parts.

2.5 Statistical Analysis

Data were analyzed using a standard procedure for one-way analysis of variance (ANOVA) to determine the effects of different treatments. Differences between treatment means were separated by the Tukey's test at significance level $P < .05$ using GraphPad software (GraphPad Prism version 7.00, GraphPad Software, La Jolla, CA, USA).

3. RESULTS AND DISCUSSION

3.1 Effects of Fertilization and Bacterial Inoculation on the Growth of Alder (*Alnus sibirica*) in Coal Mine Soil

The mean values of stem height, root length, fresh and dry weight of shoot and root, ratio of shoot and root dry weight and plant dry weight of alder plant species at different treatments are shown in Table 1. On the other hand, Table 2 is presenting the mean values of canopy spread, number of leaves, branches and nodes per plant, leaf area, and leaf area index of alder grown in coal mine soil and the effect of different treatments on these parameters.

In the present study, fertilizer application and bacteria inoculation showed positive effect on most of the studied growth parameters of N-fixing alder species (*Alnus sibirica*) grown in coal mine soil as compared to uninoculated and unfertilized treatment. Highest mean values of

Table 1. The effect of different treatments on stem height, root length, fresh and dry weight of shoot and root, ratio of shoot and root and plant dry weight of alder seedling grown in coal mine soil (Mean±SD)

‡ Treatments	Parameters							
	Stem height (cm)	Root length (cm)	Shoot fresh weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root fresh weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Shoot/Root ratio	Plant dry weight (g plant ⁻¹)
T ₀	21.7 ± 2.85 ^c	18.7 ± 2.36 ^b	40.2 ± 2.62 ^c	10.2 ± 1.15 ^c	11.4 ± 0.66 ^b	3.8 ± 0.89 ^a	2.68 ± 0.81 ^a	14.05 ± 1.06 ^c
T ₁	32.1 ± 2.46 ^{ab}	22.5 ± 2.52 ^{ab}	56.5 ± 4.48 ^{ab}	17.0 ± 1.35 ^{ab}	14.0 ± 0.89 ^{ab}	5.0 ± 0.46 ^a	3.40 ± 0.55 ^a	22.04 ± 1.11 ^b
T ₂	28.5 ± 2.01 ^b	24.0 ± 1.45 ^{ab}	53.3 ± 2.01 ^b	16.1 ± 1.64 ^b	14.7 ± 0.70 ^a	5.4 ± 0.87 ^a	2.98 ± 0.65 ^a	21.80 ± 1.83 ^b
T ₃	36.3 ± 2.88 ^a	25.9 ± 1.78 ^a	63.1 ± 2.98 ^a	19.8 ± 1.41 ^a	15.2 ± 1.54 ^a	5.6 ± 0.87 ^a	3.53 ± 0.70 ^a	25.59 ± 1.03 ^a

‡ ^{a, b, c} indicate significant difference. Mean values (± standard deviation) in the same row followed by the different letters are significantly different from each other by the Tukey test at the 5% probability level ($P \leq .05$). T₀ = Control (No source of N was given on the soil), T₁ = Fertilization (NPK fertilizer was added on the soil), T₂ = Bacterial inoculation (N fixing frankia bacteria was inoculated on the soil), T₃ = Fertilization along with bacterial inoculation (Both NPK fertilizer and N fixing frankia bacteria were added on the soil)

Table 2. The effect of different treatments on canopy spread, number of leaves, branches and nodes per plant, leaf area and leaf area index of alder seedling grown in coal mine soil (Mean±SD)

‡ Treatments	Parameters					
	Canopy spread (cm)	Number of leaves (plant ⁻¹)	Number of branches (plant ⁻¹)	Number of nodes (plant ⁻¹)	Leaf area (cm ²)	Leaf area index (LAI)
T ₀	15.1 ± 2.72 ^c	20.8 ± 1.44 ^b	5.8 ± 0.66 ^b	37.2 ± 2.98 ^b	16.4 ± 1.66 ^c	3.05 ± 0.58 ^b
T ₁	24.2 ± 2.96 ^{ab}	32.9 ± 2.98 ^a	10.7 ± 0.44 ^a	49.8 ± 1.97 ^a	31.9 ± 1.40 ^a	6.80 ± 0.36 ^a
T ₂	20.4 ± 1.71 ^{bc}	33.7 ± 3.21 ^a	10.0 ± 1.31 ^a	51.6 ± 3.61 ^a	25.5 ± 2.89 ^b	5.60 ± 0.95 ^a
T ₃	28.6 ± 3.70 ^a	35.4 ± 2.84 ^a	11.6 ± 0.96 ^a	55.7 ± 1.85 ^a	33.3 ± 2.80 ^a	7.15 ± 0.79 ^a

‡ ^{a, b, c} indicate significant difference. Mean values (± standard deviation) in the same row followed by the different letters are significantly different from each other by the Tukey test at the 5% probability level ($P \leq .05$). T₀ = Control (No source of N was given on the soil), T₁ = Fertilization (NPK fertilizer was added on the soil), T₂ = Bacterial inoculation (N fixing frankia bacteria was inoculated on the soil), T₃ = Fertilization along with bacterial inoculation (Both NPK fertilizer and N fixing frankia bacteria were added on the soil)

all the studied vegetative growth parameters of alder were recorded in fertilization along with bacterial inoculation treatment (T_3) and lowest mean values of all these parameters were found in the control treatment where no source of nitrogen was applied to the soil. The results in Table 1 demonstrate that stem height, shoot fresh and dry weight, and plant dry weight of alder seedling increased significantly at treatment T_1 , T_2 and T_3 comparing to control treatment (T_0). Significant difference was also found in increased root length, and root fresh weight of alder at treatment T_1 as compared to control treatment. There was no significant effect of different treatments on the plant root dry weight and shoot/root ratio of this plant species. Root length and root fresh weight at treatment T_1 and root length at treatment T_2 also showed no statistical difference compared to control when applied to alder. There was no statistical difference between the treatments T_1 and T_3 for all these vegetative growth parameters, except plant dry weight. Canopy spread, number of leaves, branches and nodes per plant, leaf area, and leaf area index (LAI) of the experimental alder plant species increased significantly at treatment T_1 , T_2 and T_3 compared to control (Table 2). Number of leaves, branches and nodes per plant, and leaf area index (LAI) was statistically similar between the treatments T_1 , T_2 and T_3 as compared with each other. Treatment T_3 applied to the plant was statistically different from bacteria inoculated treatment (T_2) for canopy spread and leaf area but showed statistically similar results with fertilization treatment (T_1). Significant difference between fertilization treatment (T_1) and bacteria inoculated treatment (T_2) was observed only for leaf area of alder plant species (Table 2).

From the results of the study, it is apparently clear that supply of N sources played a significant role in the vegetative growth of alder even in degraded coal mine soil. There is evident from the previous study that N must be required for the growth and development of plant, and it can stimulate photosynthesis in plants by making more area available for photosynthesis process, and subsequently increase assimilation [6,7]. Therefore, N fertilizer and N fixation in our study showed a significant increase in the growth parameters of alder in coal mine soil. Fertilization and N fixing bacteria inoculation could supply sufficient nutrient to the soil required for improving plant growth [15,16,17]. There was no significant difference for all the growth

parameters of the study when fertilization along with bacteria inoculation was compared with the sole application of fertilizer. The reason for this could be the addition of very few N through biological N fixation in plants when frankia bacteria and fertilizer were applied together on the coal mine soil because presence of NO_3^- ions provided by N fertilizer inhibit the nodulation and N fixation in N fixing plant species [18]. Effect of bacterial inoculation was significantly higher in the study compared to control treatment because of N fixation in the plants, but in contrary, bacterial inoculation showed significantly lower mean values of some growth parameters than fertilized alder seedlings. Inadequate supply of N to the soil could be the reason behind this condition because previous studies reported that N fixation in N fixing plant species can provide only 50-60% of the demanding N in soybean and some other N fixing plants [11,19].

4. CONCLUSION

In the study, sole application of fertilizer, bacterial inoculation, and combined application of fertilizer and bacterial inoculation to the coal mine soil exhibited significant increase of most of the studied vegetative parameters of alder (*Alnus sibirica*) as compared to control. The highest mean values of all vegetative growth parameters of alder in coal mine soil were found when fertilizer was applied to the soil along with bacterial inoculation. Fertilizer application along with bacterial inoculation treatment also showed significant increase of stem height, shoot fresh and dry weight, plant dry weight, canopy spread and leaf area of alder when compared with bacterial inoculation treatment but combined application of fertilizer and bacterial inoculation showed significant difference with sole application of fertilizer only for plant dry weight of alder when grown in coal mine soil. Therefore, it can be concluded that both fertilization and biological nitrogen fixation (BNF) played a significant role in improving the vegetative growth of alder (*Alnus sibirica*) in degraded coal mine soil. Further studies can investigate the effects of fertilization and bacterial inoculation on plant nutrient content and nutrient cycling in coal mine soil by growing alder plant species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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