International Journal of Plant & Soil Science

31(4): 1-8, 2019; Article no.IJPSS.53547
ISSN: 2320-7035

Effect of Fertilization and Bacterial Inoculation on Nutrient Status in Coal Mine Soil under Alder (*Alnus sibirica*) Plantation

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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.

Article Information

DOI: 10.9734/IJPSS/2019/v31i430214
Editor(s):
(1) Dr. Abigail Ogbonna, Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria.
Reviewers:
(1) Cristiane Ramos Vieira, Cuiabá University, USA.
(2) Shaimaa Hassan Abd-Elrahman, Ain Shams University, Egypt.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53547

Short Research Article

Received 25 October 2019
Accepted 30 December 2019
Published 06 January 2020

ABSTRACT

This study was conducted to assess the effect of fertilization and nitrogen fixing (N-fixing) bacterial inoculation on the available nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), present in the coal mine soil, by growing N-fixing plant species, alder (*Alnus sibirica*). 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 T_0—non-fertilized non-inoculation (control), T_1—fertilization, T_2—bacterial inoculation and T_3—fertilization along with bacterial inoculation with three replications were used in the study. The results of the study showed that available N (NH_4^+-N and NO_3^--N) in the coal soil were increased by the applied treatments for alder, as compared to control. Apart from control, difference was also found for increasing ammonium (NH_4^+-N) between treatments T_1 and T_3 and for increasing nitrate (NO_3^--N) between

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treatments T_1 and T_2 and treatments T_2 and T_3. Available P and K in the soil also increased when NPK fertilizer was applied solely, and together with inoculation to the plants but reduced at other treatments. Therefore, it can be concluded that fertilization and biological N fixation in alder plant can improve the fertility of coal mine soil, and hence, this plant species could be a good option for the reclamation of degraded coal mine soil.

Keywords: Alder; fertilization; nitrogen fixation; available nitrogen; coal mine soil.

1. INTRODUCTION

Coal is one of the vital energy resources which has been widely used since long time to generate electricity in thermal power plants, and to produce steel and cement [1]. However, coal mining is also responsible for land degradation of a vast area. The disturbances in coal mine soil properties due to mining activities reduces the productivity of land. Reduced productivity of the coal mine spoil causes nutrient scarcity in the soil which can restrict the growth of plants [2]. Coal mining also has adverse effects on the nutrient cycles that is important for maintaining a sustainable ecosystem. Without nutrient cycling, nutrients will be immobilized or restrained, and as a result, plant regeneration will be limited in the coal mine spoil [3].

N cycle re-establishment is very important to regulate the plant regeneration, but often it become difficult to establish in degraded coal mine land as N is found to be a limited nutrient in coal mine spoils [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. Symbiotic association between N-fixing plant species and N-fixing bacteria can increase the soil fertility through the formation of root nodule and fast decomposable nutrient-rich leaf litter which influence N accumulation and organic matter development in the soil. Minimizing the ratio of C/N in soil and consequently, help in the process of N mineralization and cycling of nutrients, essentially required for degraded soil reclamation [5]. Another alternative approach to maintain the sustainability and healthy growth of vegetation in coal mine land can be regular application of N and other nutrient fertilizers to the soil [6,7].

Alder is the common name of a genus of flowering plants (*Alnus*) belonging to the birch family Betulaceae. The genus comprises about 35 species [8] of monoecious trees and shrubs, among which *Alnus sibirica* is one of the important deciduous species. Alder is known for its important symbiotic relationship with *Frankia alni*, an actinomycete nitrogen-fixing bacterium. This symbiotic relationship improves the fertility of the soil where this plant grows and has establish the alder as an important species in ecological succession. 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.

There is scanty of information regarding the effect of N-fixing species on the available N of degraded coal mine soil through the application of fertilizer and bacterial inoculation. Therefore, our present study aimed to evaluate the performance of N-fixing tree species, alder (*Alnus sibirica*), in improving the available N and other soil nutrients 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 piedmont soil containing plastic tray, and kept in the growth chamber for germination. Two weeks after germination, alder seedlings were transplanted in the experimental soil containing pots (one seedling per pot) 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

Table 1. Physical and chemical characteristics of the experimental coal mine soil

		Nutrient level in the soil							
Texture	**pH**	**P_2O_5 (mg kg^{-1})**	**K (cmol (+) kg^{-1})**	**Ca (cmol (+) kg^{-1})**	**Mg (cmol (+) kg^{-1})**	**NH_4 (mg L^{-1})**	**NO_3 (mg L^{-1})**	**Total N (%)**	**EC (dS m^{-1})**
Clay loam	5.7	6	0.4	7.5	2.3	1.09	4.36	0.12	0.01

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: T_0—control (non-fertilized non-inoculated), T_1—fertilization (NPK fertilizer was applied to the coal mine soil), T_2—bacterial inoculation (N-fixing frankia bacteria was inoculated on the coal mine soil), T_3—fertilization together with bacterial inoculation (both NPK fertilizer and N-fixing frankia bacteria were added on the coal mine soil). Soil sample from 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 (P_2O_5), and 20% potassium (K_2O) macronutrients. 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 T_1 and T_3 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 T_2 and T_3. From the collected strain, a single colony of *Frankia* was further sub cultured 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 NH_4Cl-free Hoagland solution as described previously [9].

2.4 Soil Sampling and Analysis

The experimental coal mine soil was analyzed to determine the physical and chemical properties of the soil (Table 1) before plantation of alder plant species. Before analysis, soil samples were dried at 60°C for 48 h and thrashed in a 2 mm sieve. The concentration of available N (NH_4^+ and NO_3^-), P, K, Ca, Mg, pH and electrical conductivity (EC) in the experimental coal mine soil were determined.

After plantation of alder, soil analysis was done for determining the effect of fertilization and bacterial inoculation on the change of pH, available N, P, K, Ca and Mg in the coal mine soil. Soil sampling for soil analysis was done after 60 days of inoculation. Soil samples were collected from the base of root of each pot. Each soil sample was divided into two parts. One part of the divided samples was for the determination of available N (NH_4^+ and NO_3^-) which was done by using stream distillation according to the Kjeldahl method, as described by AOAC [10]. The other part of the soil samples was for the measurement of soil pH, available P, K, Ca, and Mg. The concentration of available P was determined by Bray P1 method [11]. Available K was determined with a flame photometer. Available Ca and Mg were determined titrimetrically by the ethylenediaminetetraacetic acid (EDTA) method. All the soil samples were sent to the Soil Science Department of Agricultural Research and Extension Services, Chungcheongbuk-do, South Korea, for analyzing the soil parameters.

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 NH_4^+-N and NO_3^--N in Coal Mine Soil

The increased concentration of NH_4^+-N in the coal mine soil, at different treatments for alder, were shown in Fig. 1A. The results revealed that NH_4^+-N increased (P ≤ .001) in the soil at different treatments, as compared to control (T_0). The increasing rate of NH_4^+-N in the coal mine soil was highest when fertilizer was applied on the soil along with bacterial inoculation (Fig. 1A) because N fertilizer application and N-fixation help in the accumulation of NH_4^+-N in soil profile even after used by plants [12]. Increase of NH_4^+-N in the soil due to the inoculation of N-fixing frankia bacteria, illustrate the significance of N-fixing plants in enhancing NH_4^+-N in coal mining soil through biological nitrogen fixation (BNF). NH_4^+-N increased in a little amount at non-fertilized non-inoculated treatment without application of additional N, despite of using NH_4^+-N by developing plants for their growth, might be due to the mineralization of organic N present in the soil. Shin et al. [13] reported increase of NH_4^+-N in the coal mine soil after 60 days of inoculation and fertilization, applied to shrub lespedeza and soybean. Cakmakci et al. [14] also showed the same result in the soil of barley plant.

In our study, the concentration of NO_3^--N in the coal mine soil also increased ($P \leq .001$) as a result of fertilization and bacterial inoculation into alder seedling, compared to control (Fig. 1B) and NO_3^--N was observed highest when both fertilization and bacterial inoculation were applied to plants. NO_3^- is formed in the soil by organic N mineralization and oxidation of NH_4^+ produced from supplied fertilizer and N fixation, and this NO_3^- form of N is available for plant use [15]. Application of N-fertilizer is a general practice to minimize the N deficiency and increase the fertility of degraded soils. It has been reported that when symbiotic N fixation cannot provide enough N for gaining maximum soil fertility, then N fertilizer can be applied to the N-poor soils to improve soil fertility [15]. The increasing concentration of NO_3^--N was lower at bacteria inoculated treatment compared to the fertilization and fertilization along with bacterial inoculation treatments (Fig. 1B). The reason behind this might be the formation of less active and smaller nodules on the roots of plant because the presence of soil NO_3^- inhibits nodulation and N fixation by N-fixing bacteria. It has been documented that development of nodule, and nitrogenase activity are decreased by the presence of soil nitrate ions [15]. Therefore, inoculated treatment may add only a small concentration of NO_3^--N to the soil through nodulation because of weak nodule formation. A previous study showed increase of NO_3^--N in the coal mine soil through bacterial inoculation and fertilization when applied to soybean and shrub lespedeza plant [13].

3.2 Effects of Fertilization and Bacterial Inoculation on pH, P, K, Ca and Mg in Coal Mine Soil

The effect of different treatments on soil pH and other available nutrients (P, K, Ca, and Mg) in the coal mine soil by growing alder are recorded in Table 2. The mean pH value of the studied

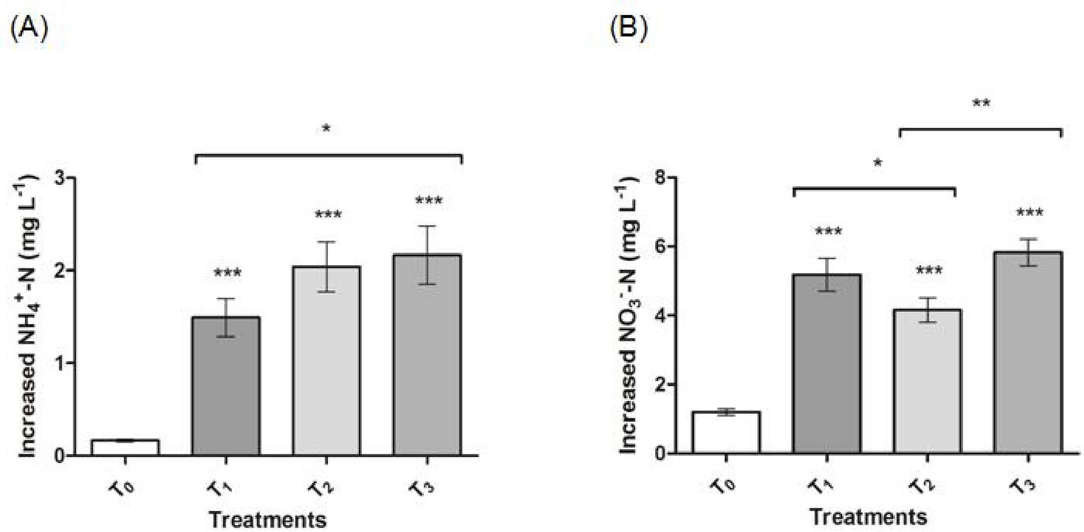

Fig. 1. Effect of different treatments on the concentration of (A) NH_4^+-N and (B) NO_3^--N in coal mine soil by growing Alder

T_0 = Control (No source of N was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (N fixing frankia bacteria was inoculated on the soil), T_3 = 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 pH and different available nutrients in coal mine soil by growing alder seedling (Mean±SD)

Measured soil pH and available nutrients					
‡ Treatments	Soil pH	P (mg/kg)	K (cmol (+)/kg)	Ca (cmol (+)/kg)	Mg (cmol (+)/kg)
T_0	6.9 ± 0.06 [b]	4.5 ± 0.44 [b]	0.25 ± 0.05 [b]	6.1 ± 0.46 [a]	1.80 ± 0.10 [a]
T_1	7.1 ± 0.06 [a]	24.6 ± 2.40 [a]	0.54 ± 0.05 [a]	5.1 ± 0.66 [b]	1.53 ± 0.06 [b]
T_2	7.0 ± 0.06[ab]	3.9 ± 0.82 [b]	0.27 ± 0.04 [b]	5.6 ± 0.53[ab]	1.60 ± 0.10[ab]
T_3	7.1 ± 0.00 [a]	25.8 ± 2.12 [a]	0.49 ± 0.07 [a]	5.0 ± 0.36 [b]	1.47 ± 0.06 [b]

‡ [a, b] 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 ≤ .05). T_0 = Control (No source of N was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (N fixing frankia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (Both NPK fertilizer and N fixing frankia bacteria were added on the soil)

coal mine soil was slightly acidic (Table 1). The soil pH was found to increase at different treatments (Table 2), and the coal mine soil become neutralized after the study. Soil acidity can be neutralized with anything that supplies alkalinity. The base cations are the sources of alkalinity, principally Ca or Mg [16]. Generally, the pH of rhizocylinder area increased by NO_3^--N and decreased by NH_4^+-N. The pH of rhizosphere region affected by N source by three mechanisms: (i) different reactions of nitrification and denitrification; (ii) H^+ absorption and discharge by roots in response to the absorption of NO_3 and NH_4; and (iii) displacement of H^+/OH^- uptake on the solid phase [17]. So, fertilizer can change the pH of soil apart from changing the NO_3^- and NH_4^+ content in soil [18].

The concentration of available P and K in the coal mine soil after growing alder was found high ($P \le .001$) when NPK fertilizer was applied to the plants (Table 2). Generally, NPK fertilizer is expected to increase the concentration of P and K in the soil [19]. On the other hand, soil P and K decreased in non-fertilized and control treatments of alder. Application of N can increase soil acidity which may induce decreased solubility of P compounds, and finally, result in a declined P concentration in soil [20]. Usually, NH_4^+ enhance the absorption of available P and NO_3^- enhance the absorption of available K by plants. K cation do not have competition with NH_4^+ for absorption, rather, they increase assimilation of NH_4^+ in plants [21].

After growing alder in the coal mine soil, concentration of Ca and Mg cations in the soil decreased (P = .025 for Ca; P = .005 for Mg) at different studied treatments compared to control (Table 2). Usually, Ca and Mg uptake by plats is also enhanced by soil nitrate due to the interaction of anion–cation. Cakmak et al. [19] reported that imbalance between the input and output of exchangeable Ca and leaching of Ca that happened by nitrification processes and soil acidification, may result in the decreased concentration of Ca cations. Mg cations in the studied coal mine soil also showed decreasing tendency comparing to control (Table 2) might be because of the similar condition. Although Ca requirement of plant for its growth and metabolism is low, but it has an important role in maintaining the balance of other nutrients, including N [20].

4. CONCLUSION

Fertilized and inoculated treatments of the study, applied to soil after planting of alder species, exhibited increase of NH_4^+-N and NO_3^--N concentration in the experimental coal mine soil compared to control. The highest concentration of both NH_4^+-N and NO_3^--N was found at fertilization along with bacterial inoculation treatment. There was also increase in the available P and K in coal mining soil by the application of NPK fertilizer along with bacterial inoculation to the plant. Therefore, it can be concluded that plantation of alder for improving the available N of coal mine soil could be a good option because of its N fixation ability, and alder could also assist in restoring the nutrient cycling in the declined coal mine soil by increasing available N. 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.

ACKNOWLEDGEMENTS

We would like to express our special thanks to the Soil Science Department of Agricultural

Research and Extension Services, Chungcheongbuk-do, South Korea for helping us in the analysis of soil sample.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53547