sustainability

MDPI

Article

Evaluating the Effect of Bacterial Inoculation and Fertilization on the Soil Nutrient Status of Coal Mine Soil by Growing Soybean (*Glycine max*) and Shrub Lespedeza (*Lespedeza bicolor*)

Chang-Seob Shin [1], Md. Omar Sharif [1,2,*] and Hwa-Yong Lee [1]

1 Department of Forest Science, College of Agriculture, Life and Environmental Sciences, Chungbuk National University, Cheongju 28644, Korea; sinna@chungbuk.ac.kr (C.-S.S.); hoasis82@chungbuk.ac.kr (H.-Y.L.)
2 Department of Agroforestry and Environmental Science, Sylhet Agricultural University, Sylhet 3100, Bangladesh
* Correspondence: sharif.aes@sau.ac.bd; Tel.: +82-104-389-3236

Received: 29 October 2018; Accepted: 11 December 2018; Published: 15 December 2018

Abstract: Revegetation with fast-growing N-fixing leguminous plants can be an alternate for reclamation of degraded coal mining areas. Selection of appropriate plant species is an important factor in deciding the success in the remediation of mine spoil. Thus, this study was carried out in greenhouse conditions to evaluate the effect of two N-fixing leguminous plant seedlings, soybean (*Glycine max*) and shrub lespedeza (*Lespedeza bicolor*), on the available N and other soil nutrients (P, K, Ca, and Mg) of the experimental coal mine soil. Four treatments, including T_0—non-fertilized non-inoculation (control), T_1—fertilization, T_2—bacterial inoculation, and T_3—combination of fertilization and bacterial inoculation with three replications were applied to both plants. Concentration of NH_4^+-N and NO_3^--N increased significantly in the soil at different treatments for both soybean and shrub lespedeza, as compared to control, but apart from control no significant difference was observed between other treatments of increased NH_4^+-N for soybean and increased NO_3^--N for shrub lespedeza. The highest number of nodules and dry weight of nodule per plant (g) was recorded 5.73 and 1.8, respectively in soybean, and 7.77 and 2.76, respectively, in shrub lespedeza with bacteria-inoculated treatment (T_2), whereas fertilized treatment (T_1) produced the lowest number of nodule and dry weight of nodule in both plants. Increasing of available P and K was significantly high when NPK fertilizer was applied to the plants but decreased at other treatments. Therefore, it can be concluded that soybean and shrub lespedeza have a significant role in changing soil nutrient status in coal mining soil through fertilizer application and biological N fixation.

Keywords: soybean; shrub lespedeza; N fixation; available N; coal mine soil

1. Introduction

In spite of significant progress in renewable resources, socioeconomic activities of our present society still rely on fossil fuels. Coal is one of the important energy sources for cement, steel, and thermal power plants [1]. However, coal mining leads to the elimination of topsoil and vegetation cover, discarding of mine fire, overburden materials, etc.; this kind of activities cause adverse effects on the physical, chemical, and biological properties of the environment, threatening biodiversity and natural resources [2]. Along with severe land deterioration, coal mining often had detrimental effect on ecosystems [3]. Coal mining spoils are not convenient for both microbial growth and plant because of unfavorable pH, low organic matter content, and drought arising from oxygen deficiency or course texture caused by compaction [4]. The other restraining aspects for revegetation of coal mine spoil may be acidity, salinity, enhanced rate of erosion, and poor water-holding capacity [5]. In addition to soil physical characteristics, inadequate supply of nutrient in coal mine spoil is also an important factor of restricting plant growth. The nutrient cycling maintains the sustainability of all plant community. Nutrients will be restrained or lost without cycling, and consequently, the plant community will not be able to regenerate [6].

Natural succession of plant is very slow on coal mine land. N is a major restricting nutrient on mine spoils and regular application of N fertilizer may be required to maintain endurance and healthy growth of vegetation [7,8]. An alternate method might be to introduce different N-fixing species of legume family. N-fixing species have a great effect on soil fertility through creation of quickly decomposable nutrient-rich litter and change of fine roots and nodules. A self-sustaining ecosystem can be developed through the mineralization of N-rich litter from these species which allow substantial transfer to companion species and subsequent cycling [9]. The association between legume species and symbiotic N-fixing bacteria can improve the potentiality of legume species for use in revegetation programs. Their association stimulates increased accumulation of N, decreasing the ratio of soil C/N, which helps mineralization and nutrient cycling, in addition to developing soil organic matter, an essential condition for reclamation of degraded soils [2].

N-fixing plants discard N from the air, converting it to organic forms by which the soil become enrich. Microorganisms transform the organic form of N into ammonium (NH_4^+). Specific microbes in the soil oxidize ammonium N (NH_4^+) first into nitrite N (NO_2^-), and then into nitrate N (NO_3^-) during their use of ammonium N for energy under certain conditions. This process is referred to as nitrification. Plants can use ammonium N (NH_4^+) and nitrate N (NO_3^-) for their growth. After nitrification, some of available NO_3^--N is taken in by plants, and rest of the NO_3^--N release into the atmosphere [10].

Soybean (*Glycine max*) is one of the most vital grain legume crop for feeding livestock and humans. It is an important source of oil and protein for a vast population residing in America and Asia continents [11]. Soybean is a strong user of N and protein content of soybean seed is about 40% [12]. Soybean can utilize atmospheric dinitrogen (N_2) through N fixation of root nodules associated with rhizobia bacteria. Combined N, such as nitrate, can be absorbed by soybean for their nutrition, either from fertilizer N or soil-mineralized N [11]. On the other hand, shrub lespedeza (*Lespedeza bicolor*), which is also known as Japanese bush clover, is a N-fixing legume plant used for wildlife habitat improvement, erosion control, and stabilization along streambanks and steep slopes. This plant can be planted on coal mine sites for restoring N to the soil because of its N-fixing ability [13].

The capability of different legumes is variable to establish symbiosis with rhizobia, with different degrees of efficacy. Furthermore, after transplanting of seedlings, rhizobia inoculants can lose effectiveness due to competition with the indigenous rhizobium population and adaptation to local conditions [14]. Nonetheless, studies have shown the ability of N-fixing plants for the reclamation of degraded areas [15]. However, there are no studies with the purpose of evaluating the role of N-fixing crop species soybean (*Glycine max*) and N-fixing tree species shrub lespedeza (*Lespedeza bicolor*) in improving the fertility status of degraded coal mine soil. Therefore, the study aims to investigate the improvement of fertility status of the coal mine soil through the application of N fertilizer and inoculation of N-fixing rhizobia bacteria into the soil by growing soybean and shrub lespedeza.

2. Materials and Methods

2.1. Soil Collection

Coal mining soil for growing plants were collected from three locations of an abandoned coal mine situated in the Taebaek city of South Korea. The soil was collected in clean plastics boxes and was taken to the greenhouse where the experiment was conducted. Coal mine soil was air-dried, ground, sieved (mesh size 2 mm), and mixed with piedmont soil in 1:1 ratio for ensuring the survival of plants in adverse conditions, because piedmont soil could improve the chemical and physical environment of the mine spoil [16]. Then, each pot of was filled with equal amount of experimental soil (mixture of coal mine soil and piedmont soil). The shape of the pot was conical frustum, and the volume of each pot was 4775.2 cm^3.

2.2. Seed Germination and Transplantation of Plant

Seeds of shrub lespedeza were obtained from the National Forest Seed Variety Center, South Korea. After collecting, seeds were washed with sterile water for three times and planted in the plastic tray for germination. Seeds were germinated in the growth chamber with 28 °C temperature and 60%–90% relative humidity. Photoperiod of 16 h light and 8 h darkness was maintained by using 400 W lamps [17]. After two weeks of germination, seedlings were transplanted into pots containing experimental soil, and all the pots were kept in the green house. In the case of soybean, seeds were collected from the Crop Science Department of Chungbuk National University. Seeds of soybean were germinated in the greenhouse following the same condition of shrub lespedeza. After one week of germination, seedlings of soybean were also transplanted into the experimental pots. Germination percentage of both plants were about 90%. During the germination period of seeds, regular watering was done as per requirement. All seedlings were irrigated three to four times in a week after transplantation.

2.3. Greenhouse Experiment Setup

The experiment was carried out in a greenhouse of the Department of Forest Science, Chungbuk National University, South Korea, during the period of June 2017 to August 2017. The conditions inside the greenhouse 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) was used, consisting of four treatments with three replications for each plant. Treatments were as follows: T_0—control (non-fertilized non-inoculated), T_1—fertilization (NPK fertilizer was added on the soil), T_2—bacterial inoculation (N-fixing rhizobia bacteria was inoculated on the soil), T_3—fertilization along with bacterial inoculation (both NPK fertilizer and nitrogen-fixing rhizobia bacteria were added on the soil). Six seedlings were used for each replication; hence, eighteen seedlings were used for each treatment.

2.4. Application of Fertilizer

NPK 20:20:20 fertilizer was applied to the plant by mixing with water. This fertilizer contains 20% nitrogen (N), 20% phosphorus (P_2O_5), and 20% potassium (K_2O) macro elements. In the study, the application rate of the fertilizer was 250 NPK kg ha^{-1}. Fertilizer was dissolved in the water at the rate of 0.5 g L^{-1}. Dissolved fertilizer (250 mL) was applied to each plant of treatment T_1 and treatment T_3 through a broadcast irrigation system. Fertilizer was applied at 20, 40, and 60 days after germination of the plant, respectively. Fertilization was done by following the instruction of the fertilizer company.

2.5. Collection of Bacterial Strain

For the purpose of inoculation, two strain of *Rhizobium* sp. were collected from the microbial germplasm of National Institute of Agricultural Sciences, South Korea. The source of first strain (KACC No: 10996) was *Glycine max*, and the location of isolation was Suwon, Gyeonggi, South Korea. The source of second strain (KACC No: 11052) was *Lespedeza bicolor*, and the location of isolation was same as the first strain. From both strains, a single yellowish colony was further subcultured in YEM agar [18], and liquid medium of YEM was used for testing the good growth of the colony. This colony was maintained as the new isolates of *Rhizobium* sp.

2.6. Bacterial Inoculation Procedure

Rhizobium strains were cultivated for 48 h in 50 mL of YEM at 28 °C and 200 rpm for inoculum preparation. Bacteria were separated through centrifugation and resuspended in a modified 1/10 strength Hoagland mineral solution (without N) of 50 mL [19]. Suspensions of bacteria were diluted to make an optical density of 0.15 (λ = 500 nm), which resembled to a concentration of 3×10^8 bacteria/mL, measured by the Bradford method [20]. After preparation of inoculum suspension, soybean and shrub lespedeza were inoculated after 8 days and 15 days of germination, respectively, by pouring 2 mL of the rhizobia suspension directly onto the base of the root [17]. The first strain (KACC No: 10996) was used for inoculating soybean, and the second strain (KACC No: 11052) was used for inoculating shrub lespedeza in treatment T_2 and T_3.

2.7. Soil Sampling and Analysis

Before starting the experiment, the experimental soil samples (mixture of coal mine soil and piedmont soil) were analyzed for physical and chemical properties (Table 1). Soil samples were dried at 60 °C for 48 h, and thrashed in a 2 mm sieve before analysis. The level of available nitrogen (NH_4^+ and NO_3^-), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), pH, and electrical conductivity (EC) in the sample were determined (Table 1).

Table 1. Physical and chemical characteristics of the experimental soil (mixture of coal mine soil and piedmont soil in 1:1 ratio).

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})**	**EC (dS m^{-1})**
Clay loam	7.4	6	0.4	9	2.4	1.09	4.36	0.0

After 60 days of inoculation, final soil sampling was done for determining the change of available nitrogen and other nutrients in the soil because of fertilization and bacterial inoculation. Soil samples were collected from the base of root of each pot. An equal amount of soil for each replication (six pots per replication) were mixed together to make one sample. Each soil sample was separated into two parts. One part was used for the measurement of available nitrogen (NH_4^+ and NO_3^-). The other part was used for the determination of pH, available phosphorus, potassium, calcium, magnesium, and electrical conductivity (EC). Measurement of available nitrogen (NH_4^+ and NO_3^-) was done in the laboratory of Environmental Resource Analysis Center, Chungbuk National University by using a stream distillation method.

On the other hand, soil samples for measuring soil pH, available phosphorus, potassium, calcium, magnesium, and electrical conductivity (EC) were send to Danyang Agricultural Technology Center, South Korea.

2.8. 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 $p < 0.05$ using GraphPad software (GraphPad Prism version 7.00, GraphPad Software, La Jolla, CA, USA).

3. Results

The mean values of increased NH_4^+-N and NO_3^--N in the soil, at different treatments for soybean and shrub lespedeza, are shown in Tables 2 and 3. In the case of soybean, the highest mean value of increased NH_4^+-N was recorded in treatment T_2, where N-fixing rhizobia bacteria were inoculated on the soil, and highest the NO_3^--N was found in treatment T_3, where both NPK fertilizer and N-fixing rhizobia bacteria were added to the soil. The lowest mean values of both increased NH_4^+-N and NO_3^--N were found in the control treatment (no source of nitrogen was given on the soil). Shrub lespedeza has also shown the highest mean value in treatment T_2 and T_3 for increased NH_4^+-N and NO_3^--N, respectively, and the lowest mean values in the control treatment for both increased concentration of NH_4^+-N and NO_3^--N.

Table 2. The effect of different treatments in increasing NH_4^+-N in coal mining soil by growing soybean and shrub lespedeza (mean ± SD).

Concentration of Increasing NH_4^+-N (mg L^{-1})				
Plants ‡	**T_0**	**T_1**	**T_2**	**T_3**
Soybean	0.67 ± 0.16 ^{b}	1.29 ± 0.11 ^{a}	1.47 ± 0.08 ^{a}	1.34 ± 0.13 ^{a}
Shrub lespedeza	0.12 ± 0.03 ^{c}	0.82 ± 0.23 ^{b}	1.16 ± 0.20 ^{a}	0.89 ± 0.15 ^{ab}

^{‡,a,b,c} indicate significant difference. Mean values (± standard deviation) in the same row followed by the dissimilar letters are significantly different from each other by the Tukey test at the 5% probability level ($p \leq 0.05$). Note: T_0 = Control (no source of nitrogen was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (nitrogen-fixing rhizobia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (both NPK fertilizer and nitrogen-fixing rhizobia bacteria were added to the soil).

Table 3. The effect of different treatments in increasing NO_3^--N in coal mining soil by growing soybean and shrub lespedeza (mean ± SD).

Concentration of Increasing NO_3^--N (mg L^{-1})				
Plants ‡	**T_0**	**T_1**	**T_2**	**T_3**
Soybean	1.86 ± 0.15 ^{c}	3.16 ± 0.22 ^{ab}	3.06 ± 0.23 ^{b}	3.59 ± 0.11 ^{a}
Shrub lespedeza	2.14 ± 0.12 ^{b}	5.05 ± 0.82 ^{a}	5.28 ± 0.25 ^{a}	5.91 ± 0.62 ^{a}

^{‡,a,b,c} indicate significant difference. Mean values (± standard deviation) in the same row followed by the dissimilar letters are significantly different from each other by the Tukey test at the 5% probability level ($p \leq 0.05$). Note: T_0 = Control (no source of nitrogen was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (nitrogen-fixing rhizobia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (both NPK fertilizer and nitrogen-fixing rhizobia bacteria were added on the soil).

Tables 2 and 3 also present the effect of different treatments on the concentration of NH_4^+-N and NO_3^--N in the experimental soil by growing soybean and shrub lespedeza. Comparing to control (T_0), NH_4^+-N and NO_3^--N was significantly increased ($p \leq 0.05$) in the soil at different treatments for both soybean and shrub lespedeza (Tables 2 and 3). The results express that there was no significant difference ($p > 0.05$) in increased concentration of NH_4^+-N between the treatments T_1, T_2, and T_3 when applied to soybean plant, but shrub lespedeza show a low significant difference ($p \leq 0.05$) between fertilizer application (T_1) and bacterial inoculation (T_2) treatments (Table 2). The results in Table 3 show a significant difference ($p \leq 0.05$) in increasing NO_3^--N between bacterial inoculation (T_2) and combined application of fertilizer and bacterial inoculation (T_3) treatments for soybean plant, but shrub lespedeza sown in coal mining soil showed no significant difference ($p > 0.05$) when comparing the treatments with each other, with the exception of the control.

Tables 4 and 5 are showing nodule number and nodule dry weight per plant for soybean and shrub lespedeza, respectively. The highest number of nodules per plant was recorded as 5.73 in soybean and 7.77 in shrub lespedeza. As expected, the highest number of nodules and highest nodule dry weight per plant were found for the bacteria-inoculated treatment in both plants, followed by fertilization with inoculated treatment. On the other hand, the lowest number of nodules and lowest nodule dry weight per plant were found at fertilization treatment in both plants. Nodule number and nodule dry weight per plant for different treatments were comparatively higher in shrub lespedeza than soybean.

The effect of different treatments on soil pH and other available nutrients (P, K, Ca, and Mg) in the coal mining soil for soybean and shrub lespedeza are recorded in Tables 6 and 7, respectively. The highest values of available P and K in the soybean growing soil were found in treatment T_3 and treatment T_1, respectively, whereas maximum mean values of available Ca and Mg were noted in the control. In shrub lespedeza, both P and K have shown the highest value in treatment T_1, but available Ca and K was highest in T_2 and control, respectively.

Table 4. Nodule number and nodule dry weight per plant at different treatments of soybean growing in coal mining soil (mean ± SD).

Soybean		
Treatments ‡	**Number of Nodules/Plant**	**Nodule Dry Weight/Plant (g)**
T_0	0.73 ± 0.06	0.26 ± 0.03
T_1	0.67 ± 0.15	0.19 ± 0.02
T_2	5.73 ± 0.25	1.80 ± 0.07
T_3	2.27 ± 0.25	0.71 ± 0.03

‡ T_0 = Control (no source of nitrogen was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (N-fixing rhizobia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (both NPK fertilizer and N-fixing rhizobia bacteria were added on the soil).

Table 5. Nodule number and nodule dry weight per plant at different treatments of shrub lespedeza growing in coal mining soil (mean ± SD).

Shrub Lespedeza		
Treatments ‡	**Number of Nodules/Plant**	**Nodule Dry Weight/Plant (g)**
T_0	1.40 ± 0.26	0.54 ± 0.06
T_1	0.93 ± 0.12	0.32 ± 0.03
T_2	7.77 ± 0.21	2.76 ± 0.18
T_3	3.53 ± 0.25	0.71 ± 0.07

‡ T_0 = Control (no source of nitrogen was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (N-fixing rhizobia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (both NPK fertilizer and N-fixing rhizobia bacteria were added on the soil).

Table 6. The effect of different treatments on soil pH and different available nutrients in coal mining soil by growing soybean (mean ± SD).

Measured Soil pH and Available Nutrients						
Plant ‡	**Treatments**	**Soil pH**	**P (mg kg^{-1})**	**K (cmol (+) kg^{-1})**	**Ca (cmol (+) kg^{-1})**	**Mg (cmol (+) kg^{-1})**
Soybean	T_0	7.3 ± 0.10 ^{a}	4.7 ± 0.58 ^{b}	0.33 ± 0.06 ^{b}	7.83 ± 0.32 ^{a}	1.83 ± 0.12 ^{a}
	T_1	7.2 ± 0.00 ^{a}	33 ± 2.65 ^{a}	0.53 ± 0.06 ^{a}	7.40 ± 0.30 ^{ab}	1.43 ± 0.15 ^{b}
	T_2	7.1 ± 0.06 ^{a}	4.3 ± 1.15 ^{b}	0.30 ± 0.00 ^{b}	7.53 ± 0.32 ^{ab}	1.53 ± 0.15 ^{ab}
	T_3	7.1 ± 0.12 ^{a}	36.7 ± 2.08 ^{a}	0.47 ± 0.06 ^{a}	6.83 ± 0.15 ^{b}	1.43 ± 0.12 ^{b}

‡,a,b indicate significant difference. Mean values (± standard deviation) in the same column followed by the dissimilar letters are significantly different from each other by the Tukey test at the 5% probability level ($p \leq 0.05$). Note: T_0 = Control (no source of nitrogen was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (nitrogen-fixing rhizobia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (both NPK fertilizer and nitrogen-fixing rhizobia bacteria were added on the soil).

Table 7. The effect of different treatments on soil pH and different available nutrients in coal mining soil by growing shrub lespedeza (mean ± SD).

Measured Soil pH and Available Nutrients						
Plant ‡	**Treatments**	**Soil pH**	**P (mg kg^{-1})**	**K (cmol (+) kg^{-1})**	**Ca (cmol (+) kg^{-1})**	**Mg (cmol (+) kg^{-1})**
Shrub lespedeza	T_0	7.3 ± 0.06 ^{a}	5.3 ± 0.58 ^{b}	0.33 ± 0.06 ^{b}	5.97 ± 0.42 ^{b}	1.87 ± 0.12 ^{a}
	T_1	7.1 ± 0.10 ^{a}	32.3 ± 2.08 ^{a}	0.63 ± 0.06 ^{a}	6.57 ± 0.21 ^{ab}	1.70 ± 0.10 ^{ab}
	T_2	7.2 ± 0.17 ^{a}	5.0 ± 0.00 ^{b}	0.37 ± 0.06 ^{b}	7.17 ± 0.51 ^{a}	1.67 ± 0.06 ^{ab}
	T_3	7.1 ± 0.06 ^{a}	29.7 ± 1.53 ^{a}	0.57 ± 0.06 ^{a}	6.37 ± 0.47 ^{ab}	1.60 ± 0.10 ^{b}

‡,a,b indicate significant difference. Mean values (± standard deviation) in the same column followed by the dissimilar letters are significantly different from each other by the Tukey test at the 5% probability level ($p \leq 0.05$). Note: T_0 = Control (no source of nitrogen was given on the soil), T_1 = Fertilization (NPK fertilizer was added on the soil), T_2 = Bacterial inoculation (nitrogen-fixing rhizobia bacteria was inoculated on the soil), T_3 = Fertilization along with bacterial inoculation (both NPK fertilizer and nitrogen-fixing rhizobia bacteria were added on the soil).

There was a small decrease in the initial soil pH due to the application of fertilizer and inoculation of bacteria for shrub lespedeza and soybean, but there was no significant difference in soil pH between control and other treatments for both plants (Tables 6 and 7). The increase of available P concentration, due to the effect of treatments in both plants, was highly significant ($p \leq 0.05$) in treatment T_1 and T_3 when compared with control and treatment T_2, but there was no significant change between control and treatment T_2 (Tables 6 and 7). The results of available K for both plants shown in Tables 6 and 7 are also similar to the results of available P. There was a significant decrease of available Ca in treatment T_3 for soybean (Table 6), and significant increase of Ca in treatment T_2 for shrub lespedeza (Table 7) when treatments were compared with control. For soybean, there was a significant decrease ($p \leq 0.05$) of available Mg in coal mining soil between control and treatment T_1 and T_3 (Table 6), but in the case of shrub lespedeza, a significant change ($p \leq 0.05$) of Mg from control was found only in T_3 (Table 7).

4. Discussion

4.1. Effects of Fertilization and Bacterial Inoculation on Increasing NH_4^+-N in Coal Mine Soil

For restoring degraded areas, an increase in soil nitrogen content has great significance because they can improve the capability of the system to assist a more complex community [21,22]. N-fixing plants have been used as a source of N in the reclamation of tropical and subtropical systems, including deforested land [23], agroforestry [24], and degraded mining land [21]. In this study, two N-fixing species (*Glycine max* and *Lespedeza bicolor*) were able to increase the concentration of NH_4^+-N in the coal mining soil. The rate of increasing NH_4^+-N for both plants was highest when plants were inoculated with bacteria, followed by fertilization along with bacterial inoculation. This indicates the importance of biological nitrogen fixation (BNF) of legume plants in improving NH_4^+-N in coal mine soil. Nearly all the nitrogen, found in the atmosphere is N_2 gas and most of the nitrogen in soil originated as N_2 gas. Plant cannot use this inert N till it is converted to available ammonium (NH_4^+) or nitrate (NO_3^-) forms. A mutual and beneficial relationship, or symbiosis is formed by N_2-fixing rhizobia bacteria with legume plants. These rhizobia bacteria remove N from air and transform it into other form of N called ammonia (NH_4^+) which can be used by plants [25]. No significant difference was found in increasing NH_4^+-N between fertilization and bacterial inoculation for soybean, and low significant difference between the same treatments for shrub lespedeza, because fertilization also might cause an accumulation of NH_4^+-N (but less than NO_3^--N) in the soil profile [26], but it was significantly lower in the control for both plants than other treatments, due to the absence of additional nitrogen sources. Cakmakci et al. [27] showed a significant increase of NH_4^+-N concentration in the soil of barley plant after 30 days of inoculation and fertilization.

4.2. Effects of Fertilization and Bacterial Inoculation on Increasing NO_3^--N in Coal Mine Soil

In the study, application of fertilizer, along with bacterial inoculation, produced the highest concentration of NO_3^--N in soil, followed by fertilization in soybean and bacterial inoculation in shrub lespedeza. NO_3^- is formed by mineralization of organic N and oxidation of NH_4 from fertilizer, and this form of combined N is mostly available to crop legumes [28]. NH_4^+-N does not stay in the soil for long periods; it is converted to NO_3^--N through a process called nitrification. To increase soil fertility in degraded lands, use of mineral fertilizers such as fertilizer-N is very common practice. Zahran [28] suggested that nitrogen fertilizer could be used to improve fertility in nitrogen-poor soils in order to attain extensive yield of legume plants when it is not possible to afford enough N through symbiotic N_2 fixation for attaining maximum yield. Though the combination of fertilizer application and bacterial inoculation resulted in the greatest increase of NO_3^-N, this treatment was not significantly different from the fertilization treatment and bacterial inoculation treatment for shrub lespedeza, and there was only a low significant difference between bacterial inoculation and fertilized bacterial inoculation treatments for soybean. The reason for this could be the fewer numbers and smaller, less active nodules on the roots of legumes grown in soil because nodulation and, subsequently, the process of N fixation by rhizobia is decreased significantly by the presence of soil nitrates added by fertilization. Hungria et al. [29] reported that nodulation and N fixation are more affected when a large amount of N fertilizer is applied. N_2 fixation rate by a well-nodulated, and active legume is always inhibited by NO_3^- ions [30]. It has been recognized for many years that nodule development [31], root infection [32], and nitrogenase activity [33,34] is inhibited by soil NO_3^- ions. Maximum N_2 fixation requires adequate nodulation in a legume. Therefore, for the fertilized inoculated treatment, NO_3^--N was added to the soil mostly through fertilization, and only a small amount through N fixation, because of poor nodule formation. As developing plants use both N forms to grow, both NH_4^+-N and NO_3^--N would be decreased in non-fertilized non-inoculated treatment (control), but that the concentration of NH_4^+-N and NO_3^--N in this study was increased in the control might be due to mineralization and nitrification of decomposed materials and organic N present in the soil, but this increasing concentration was significantly lower than other treatments applied to soybean and shrub lespedeza. In a previous study, bacterial inoculation and fertilization significantly increased total mineral N and NO_3^--N in soil when applied to barley plant [27].

4.3. Nodule Formation and Nodule Dry Weight

The results of this study about nodule formation and nodule dry weight are similar with Hungria et al. [29], and Janagard and Ebadi-Segherloo [35] who observed that nodule development and nodule dry weight increased in inoculated plants as compared to the uninoculated plants. On the contrary, nodulation and N fixation affected by the presence of NO_3^--N in the soil, resulting in fewer numbers and poor nodules on the roots of legumes [30]. That is why the number of nodules and nodule dry weight of both plants in the fertilized treatment and, even, for fertilization with inoculated treatment, was very low compared to the inoculated treatment of our study.

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

Mineral N also lead to changes in soil pH and other soil properties in addition to increasing the nitrate content in soil [36]. There was a slight decrease in the pH of our experimental coal mining soil at different treatments. Riley and Barber [37] reported that the rhizocylinder (root along with strongly adhering soil) pH decreased by NH_4^+-N and increased by NO_3^--N. The nitrogen source affects the pH of the rhizosphere by three mechanisms [38,39]: (i) dislocation of H^+/OH^- adsorbed on the solid stage; (ii) nitrification and denitrification reactions; and (iii) uptake and release of H^+ by roots in response to the uptake of NH_4/NO_3. Therefore, fertilizer, apart from increasing the nitrate content in soil, also change the soil pH [40].

The maximum available P and K concentration of the soil was found in both soybean and shrub lespedeza when treated with NPK fertilizer, and there was high statistical difference between the fertilized and non-fertilized treatments. Increased concentration of available P and K are expected in the NPK fertilized treatments [36]. By contrast, the concentration of P and K cations decreased in non-fertilized treatments of both soybean and shrub lespedeza. Application of the high rate of N may result in a rather reduced concentration of P, which is possibly induced by declined solubility of phosphorus compounds because of increased soil acidity [41]. Moreover, nitrate enhances the uptake of K cations and ammonium enhances the uptake of available P by plants. Usually, K cations do not compete with NH_4^+ for uptake, rather, they increase NH_4^+ assimilation in plants [42].

The concentration of both Ca and Mg cations decreased in the mining soil at different treatments of the study after growing soybean and shrub lespedeza. Generally, nitrate enhances the uptake of Ca and Mg cations like K because of anion–cation interactions. Apart from fertilization with inoculated treatment in soybean (resulting in the lowest available Ca) and inoculated treatment (resulting in the highest available Ca) in shrub lespedeza, there was no statistical difference of decreasing Ca cations between the control and the other two treatments. The highest concentration of available Mg was found in the control treatment of both plants, which was significantly higher than fertilized and fertilized with inoculation treatments in soybean, and fertilized with inoculation treatment in shrub lespedeza.

In a previous study, Cakmak et al. [36] revealed that reduced concentration of exchangeable Ca, clearly specifies an inequality between its input and output, and the leaching that happened because of soil acidification and nitrification processes, is the main reason for this imbalance. Decreasing tendency in the concentration of Mg in the studied soil also confirm the similar situation when compared to the control. Although Ca requirement is low for plant growth and metabolism, it has a great role in balancing the levels of other nutrients, including N [41].

5. Conclusions

In this study, we evaluated the role of two N-fixing plant species soybean (*Glycine max*) and shrub lespedeza (*Lespedeza bicolor*) in improving the concentration of available N (NH_4^+-N and NO_3^--N) and other soil nutrients in coal mine soil through the application of fertilizer and bacterial inoculation. For this purpose, four treatments, including fertilization, bacterial inoculation (Rhizobia bacteria), and a combination of fertilization and bacterial inoculation and non-fertilized non-inoculation, were used for both plants in the study. Concentration of NH_4^+-N and NO_3^--N increased significantly in the experimental coal mine soil at all fertilized and inoculated treatments applied to soybean and shrub lespedeza as compared to control. Available P and K also increased significantly because of NPK fertilizer application to the plants. There was also a slight change in soil pH and exchangeable Ca and Mg cations in coal mining soil as a result of applied treatments to soybean and shrub lespedeza. Therefore, plantation of soybean and shrub lespedeza for improving the fertility status of coal mining soil could be a good option because of their N-fixing ability and, consequently, for restoring the soil-nutrient cycling in the degraded coal mining soil.

Author Contributions: Conceptualization, C.-S.S. and M.O.S.; Methodology, M.O.S. and H.-Y.L.; Software, M.O.S.; Supervision, C.-S.S.; Investigation, C.-S.S., M.O.S. and H.-Y.L.; Resources, C.-S.S.; Data Curation, C.-S.S. and M.O.S.; Writing—Original Draft, M.O.S.; Writing—Review & Editing, C.-S.S., M.O.S. and H.-Y.L.

Funding: This research received no external funding.

Acknowledgments: We would like to express our special thanks to the Department of Crop Science, Chungbuk National University and National Forest Seed Variety Center, South Korea for providing us the seeds of Soybean and Shrub Lespedeza that required for the experiment. We are also grateful to Batmunkh Munkhgerel for helping us in technical work during the whole experimental period.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ram, L.C.; Masto, R.E. Fly ash for soil amelioration: A review on the influence of ash blending with inorganic and organic amendments. *Earth Sci. Rev.* **2014**, *128*, 52–74. [CrossRef]
2. Moura, G.G.D.; Armas, R.D.; Meyer, E.; Giachini, A.J.; Rossi, M.J.; Soares, C.R.F.S. Rhizobia Isolated from Coal Mining Areas in the Nodulation and Growth of Leguminous Trees. *Rev. Bras. Cienc. Solo* **2016**, *40*, e0150091. [CrossRef]
3. Mukhopadhyay, S.; Maiti, S.K.; Masto, R.E. Development of mine soil quality index (MSQI) for evaluation of reclamation success: A chronosequence study. *Ecol. Eng.* **2014**, *71*, 10–20. [CrossRef]
4. Agrawal, M.; Singh, J.; Jha, A.K.; Singh, J.S. Coal-based environmental problems in a low rainfall tropical region. In *Trace Elements in Coal Combustion Residues*; Keefer, R.F., Sajwan, K.S., Eds.; Lewis Publishers: Boca Raton, FL, USA, 1993; pp. 27–57.
5. Jha, A.K.; Singh, J.S. Spoil characteristics and vegetation development of an age series of mine spoils in a dry tropical environment. *Vegetation* **1991**, *97*, 63–76.
6. Dutta, R.K.; Agrawal, M. Effect of tree plantations on the soil characteristics and microbial activity of coal mine spoil land. *Trop. Ecol.* **2002**, *43*, 315–324.
7. Yang, B.; Shu, W.S.; Ye, Z.H.; Lan, C.Y.; Wong, M.H. Growth and metal accumulation in *vetiver* and two *Sesbania* species on lead/zinc mine tailings. *Chemosphere* **2003**, *52*, 1593–1600. [CrossRef]
8. Song, S.Q.; Zhou, X.; Wu, H.; Zhou, Y.Z. Application of municipal garbage compost on revegetation of tin tailings dams. *Rural Eco-Environ.* **2004**, *20*, 59–61.

9. Zhang, Z.Q.; Shu, W.S.; Lan, C.Y.; Wong, M.H. Soil seed bank as an input of seed sources in vegetation of lead/zinc mine tailings. *Restor. Ecol.* **2001**, *9*, 1–8. [CrossRef]
10. Sheoran, V.; Sheoran, A.S.; Poonia, P. Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. *Int. J. Soil Sediment. Water.* **2010**, *3*, 1–20.
11. Ohyama, T.; Fujikake, H.; Yashima, H.; Tanabata, S.; Ishikawa, S.; Sato, T.; Nishiwaki, T.; Ohtake, N.; Sueyoshi, K.; Ishii, S.; et al. Effect of Nitrate on Nodulation and Nitrogen Fixation of Soybean. In *Soybean Physiology and Biochemistry*; El-Shemy, H.A., Ed.; InTech: Shanghai, China, 2011; pp. 333–364.
12. Peoples, M.B.; Gault, R.R.; Lean, B.; Sykes, J.D.; Brockwell, J. Nitrogen fixation by soybean in commercial irrigated crops of central and southern new south wales. *Soil Biol. Biochem.* **1995**, *27*, 553–561. [CrossRef]
13. Tesky, J.L. Lespedeza bicolor. In *Fire Effects Information System*; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory: West Missoula, MT, USA, 1992. Available online: https://www.fs.fed.us/database/feis/plants/shrub/lesbic/all.html (accessed on 11 October 2018).
14. Geetha, S.J.; Joshi, S.J. Engineering rhizobial bioinoculants: A strategy to improve iron nutrition. *Sci. World J.* **2013**, *13*, 1–15. [CrossRef] [PubMed]
15. Melloni, R.; Moreira, F.M.S.; Nóbrega, R.S.A.; Siqueira, J.O. Efficiency and phenotypic diversity among nitrogen-fixing bacteria that nodulate cowpea [*Vigna unguiculata* (L.) Walp] and common bean (*Phaseolus vulgaris* L.) in bauxite-mined soils under rehabilitation. *Rev. Bras. Cienc. Solo* **2006**, *30*, 235–246. [CrossRef]
16. Lindemann, W.C.; Fresquez, P.R.; Cardenas, M. Nitrogen mineralization in coal mine spoil and topsoil. *Biol. Fertil. Soils* **1989**, *7*, 318–324. [CrossRef]
17. Ferrari, A.E.; Wall, L.G. Nodulation and growth of black locust (*Robinia pseudoacacia*) on a desurfaced soil inoculated with a local Rhizobium isolate. *Biol. Fertil. Soils* **2007**, *43*, 471–477. [CrossRef]
18. Vincent, J.M. Factors controlling the legume-Rhizobium symbiosis. In *Nitrogen Fixation*; Newton, W.E., Orme-Johnson, W.H., Eds.; University Park Press: Baltimore, MD, USA, 1980; Volume II, pp. 103–129.
19. Hoagland, D.R.; Arnon, D.I. The Water-Culture Method for Growing Plants without Soil. In *California Agricultural Experiment Station, Circular-347*; University of California: Berkeley, CA, USA, 1950.
20. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
21. Franco, A.A.; De Faria, S.M. The contribution of N2-fixing tree legumes to land reclamation and sustainability in the tropics. *Soil Biol. Biochem.* **1997**, *29*, 897–903. [CrossRef]
22. Macedo, M.O.; Resende, A.S.; Garcia, P.C.; Boddey, R.M.; Jantalia, C.P.; Urquiaga, S.; Campello, E.F.C.; Franco, A.A. Changes in soil C and N stocks and nutrient dynamics 13 years after recovery of degraded land using leguminous nitrogen-fixing trees. *For. Ecol. Manag.* **2008**, *255*, 1516–1524. [CrossRef]
23. Siddique, I.; Engel, V.L.; Parrotta, J.A.; Lamb, D.; Nardoto, G.B.; Ometto, J.P.H.B.; Martinelli, L.A.; Schmidt, S. Dominance of legume trees alters nutrient relations in mixed species forest restoration plantings within seven years. *Biogeochemistry* **2008**, *88*, 89–101. [CrossRef]
24. Handayanto, E.; Cadisch, G.; Giller, K.E. Manipulation of quality and mineralization of tropical legume tree prunings by varying nitrogen supply. *Plant Soil* **1995**, *176*, 149–160. [CrossRef]
25. Gil-Sotres, F.; Trasr-Cepeda, C.; Leiros, M.C.; Seoane, S. Different approaches to evaluating soil quality using biochemical properties. *Soil Biol. Biochem.* **2005**, *37*, 877–887. [CrossRef]
26. Kolodziejczyk, M. Effect of nitrogen fertilization and application of soil properties improving microbial preparations on the content of mineral nitrogen in soil after spring wheat harvesting. *J. Cent. Eur. Agric.* **2013**, *14*, 306–318. [CrossRef]
27. Cakmakci, R.; Donmez, M.F.; Erdogan, U. The Effect of Plant Growth Promoting Rhizobacteria on Barley Seedling Growth, Nutrient Uptake, Some Soil Properties, and Bacterial Counts. *Turk. J. Agric. For.* **2007**, *31*, 189–199.
28. Zahran, H.H. *Rhizobium*-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 968–989. [PubMed]
29. Hungria, M.; Nogueira, M.A.; Araujo, R.S. Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool improve yield and sustainability. *Am. J. Plant Sci.* **2015**, *6*, 811–817. [CrossRef]
30. Herdina; Silsbury, J.H. Nodulation and early growth of faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) as affected by strain of *Rhizobium*, NO_3^- supply, and growth temperature. *Aust. J. Agric. Res.* **1989**, *40*, 991–1001. [CrossRef]

31. Imsande, J. Inhibition of nodule development in soybean by nitrate or reduced nitrogen. *J. Exp. Bot.* **1986**, *37*, 348–355. [CrossRef]
32. Abdel-Wahab, H.H.; Zahran, H.H.; Abd-Alla, M.H. Root-hair infection and nodulation of four grain legumes as affected by the form and the application time of nitrogen fertilizer. *Folia Microbiol.* **1996**, *41*, 303–308. [CrossRef]
33. Purcell, L.C.; Sinclair, T.R. Nitrogenase activity and nodule gas permeability response to rhizospheric NH_3 in soybean. *Plant Physiol.* **1990**, *92*, 268–272. [CrossRef]
34. Arrese-Igor, C.; Minchin, F.R.; Gordon, A.J.; Nath, A.K. Possible cause of the physiological decline in soybean nitrogen fixation in response to nitrate. *J. Exp. Bot.* **1997**, *48*, 905–913. [CrossRef]
35. Janagard, M.S.; Ebadi-Segherloo, A. Inoculated soybean response to starter nitrogen in conventional cropping system in Moghan. *J. Agron.* **2016**, *15*, 26–32. [CrossRef]
36. Cakmak, D.; Saljnikov, E.; Perovic, V.; Jaramaz, D.; Mrvic, V. Effect of long-term nitrogen fertilization on main soil chemical properties in Cambisol. In Proceedings of the 19th World Congress of Soil Sciences, Brisbane, Australia, 1–6 August 2010.
37. Riley, D.; Barber, S.A. Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. *Soil Sci. Soc. Am. J.* **1971**, *35*, 301–306. [CrossRef]
38. Gahoonia, T.S.; Nielsen, N.E. Control of pH at the soil-root interface. *Plant Soil* **1992**, *140*, 49–51. [CrossRef]
39. Marschner, H.; Romheld, V. Root-induced changes in the availability of micronutrients in the rhizosphere. In *Plant Roots: The Hidden Half*, 2nd ed.; Waisel, Y., Eshel, A., Kafkafi, A., Eds.; Marcel Dekker: New York, NY, USA, 1996; pp. 557–579.
40. Brady, A.C.; Weil, R.R. *The Nature and Properties of Soils*, 13th ed.; Prentice Hall: Upper Saddle River, NJ, USA, 2002.
41. Marsh, K.B.; Tillman, R.W.; Syers, J.K. Charge relationship of sulfate sorption by soil. *Soil Sci. Soc. Am. J.* **1987**, *51*, 318–323. [CrossRef]
42. Aulakh, M.S.; Malhi, S.S. Interactions of nitrogen with other nutrients and water: Effect on crop yield and quality, nutrient use efficiency, carbon sequestration and environmental pollution. *Adv. Agron.* **2005**, *86*, 341–409.