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Article

Pseudomonas fluorescens with Nitrogen-Fixing Function Facilitates Nitrogen Recovery in Reclaimed Soils of Coal Mining Areas

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Abstract: Coal mining has caused a significant loss of nitrogen content of soil in mining areas. This article studies the effects of organic fertilizer, inorganic fertilizer, and combined application of *Pseudomonas fluorescens* with the ability of nitrogen fixation on soil nitrogen accumulation and composition in the reclamation area of Tunlan Coal Mine from 2016 to 2022 under the condition of equal nitrogen application, providing scientific basis for microbial fertilization and rapid increase of nitrogen content in the reclaimed soil of mining areas. The results showed that as the reclamation time increased, the nitrogen content and its composition structure of the soil treated with fertilization rapidly evolved towards normal farmland soil. The soil nitrogen content increased the fastest in the treatment of *Pseudomonas fluorescens* + organic fertilizer (MB), compared with inorganic fertilizer (CF), organic fertilizer (M), and *Pseudomonas fluorescens* + inorganic fertilizer (CFB) treatments, the treatment of *Pseudomonas fluorescens* + organic fertilizer (MB) can bring the total nitrogen (TN) content of the soil to normal farmland soil levels 1-3 years earlier. The comprehensive scores of *Pseudomonas fluorescens* + organic fertilizer (MB) and *Pseudomonas fluorescens* + inorganic fertilizer (CFB) on the two principal components were increased 1.58 and 0.79 than those of organic fertilizer (M) and inorganic fertilizer (CF) treatments, respectively. This further indicates that the combination of *Pseudomonas fluorescens* and organic fertilizer has a better effect on improving soil nitrogen accumulation than the combination of *Pseudomonas fluorescens* and inorganic fertilizer. At the same time, the application of *Pseudomonas fluorescens* will increase the content of unknown nitrogen (UN) in acid-hydrolysable nitrogen (AHN), and decrease the content of amino acid nitrogen (AAN) and ammonia nitrogen (AN), relatively. However, there was no significant effect on the content of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in soil inorganic nitrogen (SMN). When combined with inorganic fertilizer, the contribution of soil mineralized nitrogen (SMN) to total nitrogen (TN) increased by 14.78%, while when combined with organic fertilizer, the contribution of acid-hydrolysable nitrogen (AHN) to total nitrogen (TN) increased by 44.77%. In summary, the use of *Pseudomonas fluorescens* is beneficial for nitrogen recovery in the reclaimed soil of coal mining areas. The optimal fertilization method under the experimental conditions is the combination of *Pseudomonas fluorescens* and organic fertilizer.

Keywords: *Pseudomonas fluorescens*; nitrogen; coal mining area; soil reclamation

1. Introduction

As the largest coal producer and consumer in the world, China's proven the reserves of coal resources by the end of 2020 are 1.73 trillion tons, accounting for 13.3% of the world's reserves. It is estimated that the mining output of coal will remain over 3.7 billion tons by the year of 2030 [1]. At present, land reclamation in coal mining areas generally involves engineering measures such as stripping, backfilling, digging pads, covering soil, and leveling. After the engineering measures are completed, fertilization and planting are carried out to gradually restore soil productivity. However, a series of engineering measures have caused the core soil layer to cover the topsoil layer, leading to severe nutrient loss in the topsoil layer, with nitrogen loss exceeding 70% [1,3]. Nitrogen, as an important material structure indicator, is widely regarded by many scholars as an important basis for the success of soil reclamation in coal mining areas [4–6]. It can be seen that soil nitrogen content can quantify the degree of soil restoration in mining areas. Research has shown that the natural recovery of soil nitrogen is slow, and artificial restoration is currently the main direction of research [7]. Among them, farmland soil fertilization and reclamation have the advantages of shorter reclamation time and higher economic benefits compared to forest land and grassland reclamation, which is currently a research hotspot [8,9].

The supplement of nitrogen in farmland soil mainly comes from external fertilizers such as inorganic and organic fertilizers [10,11]. Adding inorganic and organic nitrogen fertilizers can significantly increase soil nitrogen content, and the growth rate of soil nitrogen also increases with the increase of application years [12]. However, traditional nitrogen fertilizer application methods have drawbacks such as loss of soil microbial diversity and changes in bacterial community composition [13–15], stimulation of soil organic nitrogen mineralization, which may increase nitrogen loss caused by leaching and gas emissions[], and can easily have negative impacts on the environment [16,17]. Therefore, there is an urgent need to find a method that can solve the above hazards while also increasing the nitrogen content of reclaimed soil. Microbial reclamation technology utilizes the advantages of microbial inoculation to increase nitrogen fixation and effective nitrogen content by improving microbial nitrogen fixation activity and accelerating soil organic nitrogen mineralization. It is an effective way to solve a series of problems caused by traditional nitrogen application. As an important plant rhizosphere growth promoting bacterium, *Pseudomonas fluorescens* mainly converts nitrogen in the air into a nitrogen source that can be absorbed and utilized by crops through its own nitrogenase secretion [18,19].In addition, *Pseudomonas fluorescens* can also secrete antibiotics such as *phenazine-1-carboxylic acid* [20], *Pseudomonas aeruginosa* [21], *nitropyrrolidins* [22], *hydrocyanic acid, 2,4-diacetyl phloroglucinol* [23] to inhibit the growth of various pathogenic microorganisms, Synthetic auxin promotes fruit development and induces vascular bundle differentiation in crops [24]. *Pseudomonas fluorescens* has the characteristics of simple nutritional requirements, fast reproduction, and strong competitive colonization ability, and has enormous application potential in agricultural production in coal mining and reclamation areas.

At present, the application of inorganic and organic nitrogen fertilizers is usually adopted to quickly increase the nitrogen content of soil in coal mine reclamation areas and shorten the reclamation period. There have been no reports on the use of *Pseudomonas fluorescens* with nitrogen fixation ability to improve the nitrogen content of reclaimed soil in coal mining areas. This experiment takes the soil of Tunlan coal mine reclamation area in Gujiao City from 2016 to 2022 as the research object, and explores the effect of adding *Pseudomonas fluorescens* with nitrogen fixation ability on different forms of nitrogen in the soil. The aim is to improve the nitrogen utilization rate of the soil in the coal mining reclamation area and accelerate the rate of soil maturation in the reclamation area.

2. Materials and Methods

2.1. Overview of The Experimental Area

The experimental area is located in the coal gangue discharge area of Tunlan Coal Mine, 6 km south of Gujiao City, Shanxi Province (37 ° 53 ′ 12 ″ N, 12 ° 6 ′ 13 ″ E). It belong to Warm temperate semi humid continental monsoon climate. It is in the area of low mountain and hilly areas; and in the Altitude of 1006.80 m; The annual average temperature is 9.50 ℃; The average annual rainfall is 475.00 mm. After the completion of coal mine waste disposal in 2012, soil was taken to cover the coal gangue, with a thickness of about 1.00 meters. The soil type was calcareous brown soil, and its basic physical and chemical properties were: pH value 8.53; Organic matter 3.45 g/kg; Total nitrogen 0.19 g/kg; Effective phosphorus 2.51 mg/kg; Unit weight 1.45 g/cm^3. From 2016 to 2022, a targeted experiment was conducted to improve the nitrogen content of reclaimed soil through fertilization.

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2.2. Test Materials

Test crop: Corn (Dafeng 30).

Test strains: N64-1 and N137-1 were isolated from farmland soil in Shanxi Province. They were identified as *Pseudomonas fluorescens*, with nitrogen fixation amounts of 4.54 and 4.44 mg/L (semi micro Kelvin method), nitrogenase activity of 41.39 and 40.71 nmol/(mg · h) (acetylene reduction method), and showed good growth trends within the range of 0-40 °C and pH 6-9, Multiple carbon sources such as glucose, maltose, lactose, sucrose, and mannitol can be utilized.

Test inorganic fertilizers: urea (N, 46%), superphosphate (P_2O_5, 16%), potassium chloride (K_2O, 60%).

Test organic fertilizer: decomposed chicken manure (N, 1.45%, P_2O_5, 1.14%, K_2O, 0.85%).

2.3. Experimental Design

Following the principle of equal nitrogen application (N approximately 180 kg/hm). Four fertilization treatments were set up in the experiment, **CF**: inorganic fertilizer; **CFB**: *Pseudomonas fluorescens* + Inorganic fertilizer; **M**: Organic fertilizer; **MB**: *Pseudomonas fluorescens* + Organic fertilizer, all four fertilization treatments were planted with corn. Three control treatments, **IS**: no corn planting and no fertilization; **CK:** planting corn without fertilization; **MS**: a normal mature soil for farmland, and the fertilization amount is about 2/3 of the fertilization treatment. Each treatment area is 100m^2 (10m × 10m), each repeated 3 times.

2.4. Test Implementation

Sowing at the end of April in the years of 2016-2022 and harvesting at the end of September of every year, with a growth period of 110 ± 3 days and a planting density of 60000 plants per hectare. N64-1 and N137-1 were inoculated into LB liquid culture medium, and shaken at 28 °C and 150 rpm until logarithmic phase. The effective viable bacterial count reached 10^8 cfu/mL, and they were irrigated and inoculated. Organic and inorganic fertilizers are applied by sowing. *Pseudomonas fluorescens*, organic fertilizer, and inorganic fertilizer were applied once the day before sowing, and weeding was carried out once during the jointing period of corn. No irrigation was conducted during the growth period (Table 1. for fertilizer application amount).

Table 1. Fertilization Plan (Unit: kg/hm).

Treatment	Urea	Superphosphate	potassium chloride	organic fertilizer	*Pseudomonas fluorescens*	LB culture medium	corn
IS							×
CK							√
MS				8000			√
CF	378	855	170			750	√
CFB	378	855	170		750		√
M				12000		750	√
MB				12000	750		√

Note: "×" Indicates planting corn; "√ "indicates not planting corn.

2.5. Sampling and Physicochemical Analysis

During the experiment, soil samples were collected within 24 hours after corn harvest, and a total of 147 soil samples were analyzed. Collect soil samples from 5 sampling points at a depth of 0-20 cm in each plot and mix them into a single sample. After removing visible plant residues and stones, each sample is divided into two parts: one part is stored at -4 °C for the determination of soil mineralized nitrogen (SMN), and the other part is dried and ground for the determination of total nitrogen (TN) and acid-hydrolysable nitrogen (AHN).

The nitrogen composition of soil is shown in Figure 1. The total nitrogen (TN) is determined by Kjeldahl method; Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in soil mineralized

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nitrogen (SMN) were extracted using 0.5mol/L K_2SO_4 and measured using a flow analyzer; The soil organic nitrogen components were determined using the Bremner organic nitrogen classification method, with acid-hydrolysable nitrogen (AHN) determined using the Kjeldahl method, non-hydrolysable nitrogen (THN) obtained by subtracting total nitrogen (TN) from acid-hydrolysable nitrogen (AHN), and soil mineralized nitrogen (SMN). Ammonia nitrogen (AN) was determined using the MgO distillation method, and ammonia nitrogen (AN)+amino sugar nitrogen (ASN) was determined using the phosphate borax salt buffer distillation method. The amino acid nitrogen (AAN) was determined by ninhydrin oxidation and phosphate borate buffer distillation method, while the amino acid nitrogen (ASN) and unknown nitrogen (UN) were obtained by subtraction method.

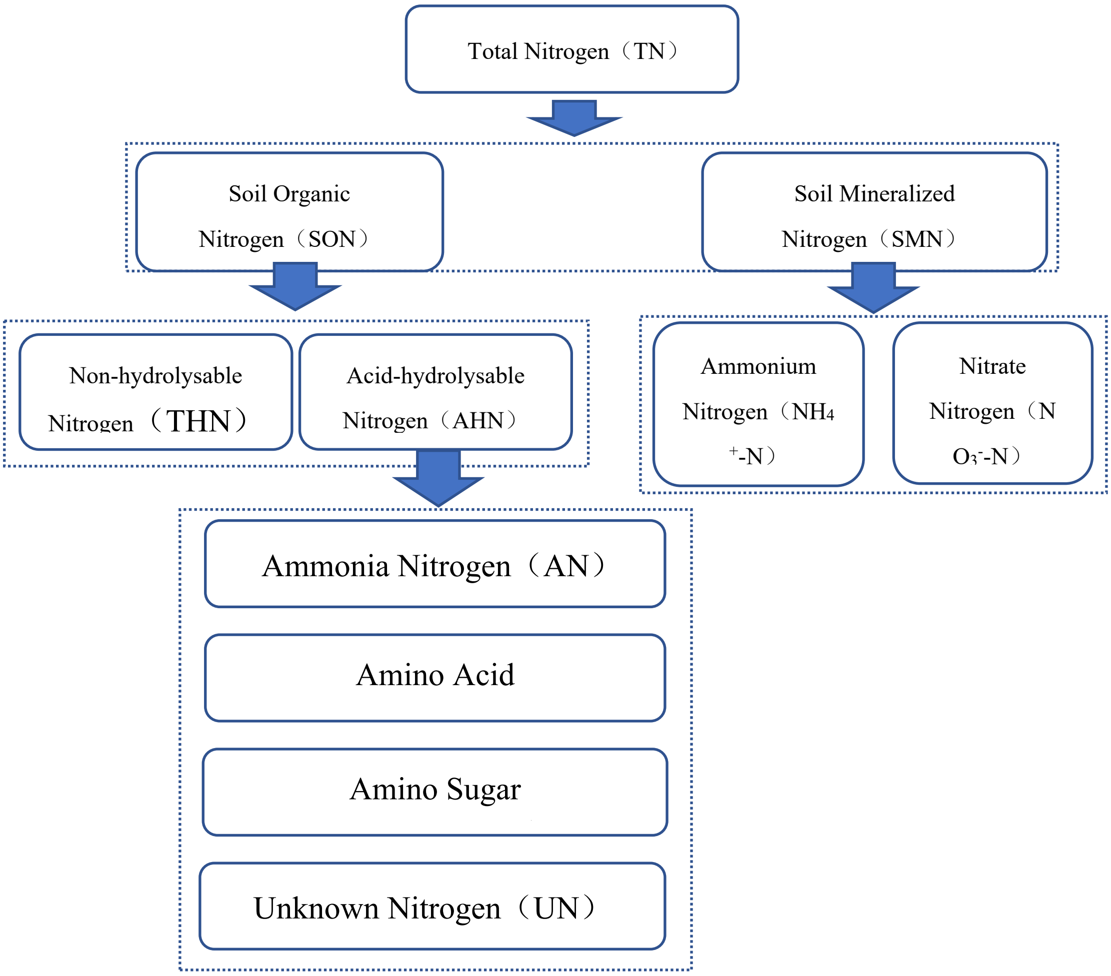

Figure 1. Soil nitrogen composition.

2.6. Statistical Analysis

Using Excel 2007 for data processing, SPSS 22 was used for analysis of variance, data fitting, multiple linear regression analysis and principal component analysis.

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3. Results

3.1. Changes in TN Content

As shown in Figure 2, with the increase of reclamation years, the TN content of MS and IS treatments varied between 1313.72~1387.87 mg/kg and 178.61~194.81 mg/kg, with no significant increase or decrease trend. The fitting results showed that due to the lack of external nitrogen input, the TN content of CK treatment decreased year by year at a rate of 4.88 mg/kg/y. The TN content of CF, CFB, M, and MB treatments showed an exponential increase trend, and the difference reached a significant level between 2019 and 2022. MB treatment is expected to reach the normal farmland soil (MS) level after 10 years, which is 1-3 years earlier than CF, CFB, and M treatments. There was no significant difference in TN content between CFB and CF treatments. Compared with M treatment, the TN content of MB increased by 1.47% to 18.38%, and the difference reached a significant level from 2020 to 2022.

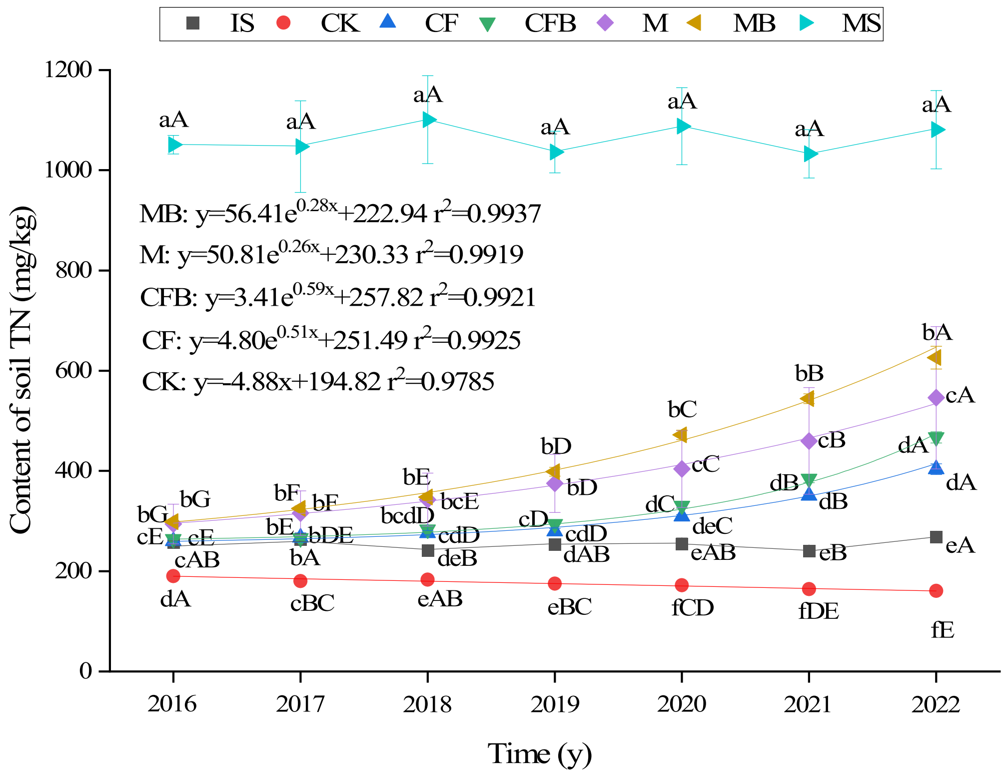

Figure 2. TN content of soil under different fertilization treatments. Note: Lowercase letters indicate significant differences in treatment for the same age group, capital letters indicate significant differences between different years of treatment for the same treatment (P<0.05), same below.

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3.2. Changes in SMN Content

As shown in Figure 3, with the increase of reclamation time, the NO_3^--N and NH_4^+-N content of MS, IS, and CK treatments showed no significant increase or decrease trend. The fitting results showed that the NH_4^+-N and NO_3^--N content showed an exponential increase trend in MB and M treatments, while the NH_4^+-N content showed a linear increase trend in CFB and CF treatments, while the NO_3^--N content showed an exponential increase trend. The order in which different fertilization treatments improve the NH_4^+-N and NO_3^--N content in reclaimed soil is MB>M>CFB>CF. In 2022, the NH_4^+-N and NO_3^--N content in MB treatment is about 75.59% and 47.03% of that in MS treatment. It is expected that MB treatment can reach normal farmland soil (MS) levels after 8-10 years. Compared with CF treatment, the NH_4^+-N content of CFB increased by 3.77%~22.13%, and the difference reached a significant level in 2016, 2019~2022. The NO_3^--N content significantly increased by 13.94% and 14.23% in 2021 and 2022. Compared with M treatment, the NH_4^+-N content significantly increased by 16.60% to 33.94% from 2016 to 2022, and the NO_3^--N content significantly increased by 12.79% to 24.63% from 2019 to 2022.

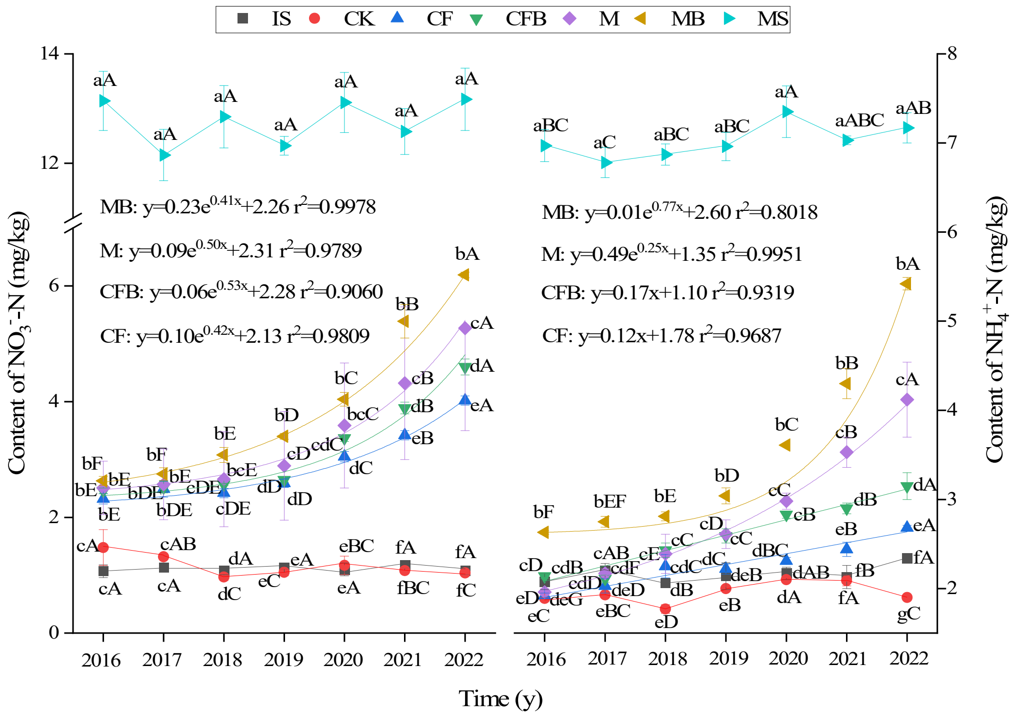

Figure 3. NO_3^--N and NH_4^+-N content under different fertilization treatments.

As shown in Figure 4, the NH_4^+-N dominates in the SMN treated with IS and CK treatments, while the NO_3^--N dominates in the SMN treated with MS, CF, CFB, M, and MB treatments, indicating that fertilization significantly affects the relative content of NH_4^+-N and NO_3^--N in SMN. The regression equation indicates that, the increased SMN content in CFB and CF treatments includes 68.51% and 71.89% NO_3^--N, 31.49% and 42.20% NH_4^+-N, the increased SMN content in MB and M treatments includes 57.34% and 57.80% NO_3^--N, 42.66% and 42.20% NH_4^+-N, it can be seen that *Pseudomonas fluorescens* can reduce the relative content of NO_3^--N in SMN, but the effect is not significant, especially when combined with organic fertilizer, the NO_3^--N content is only reduced by 0.46%.

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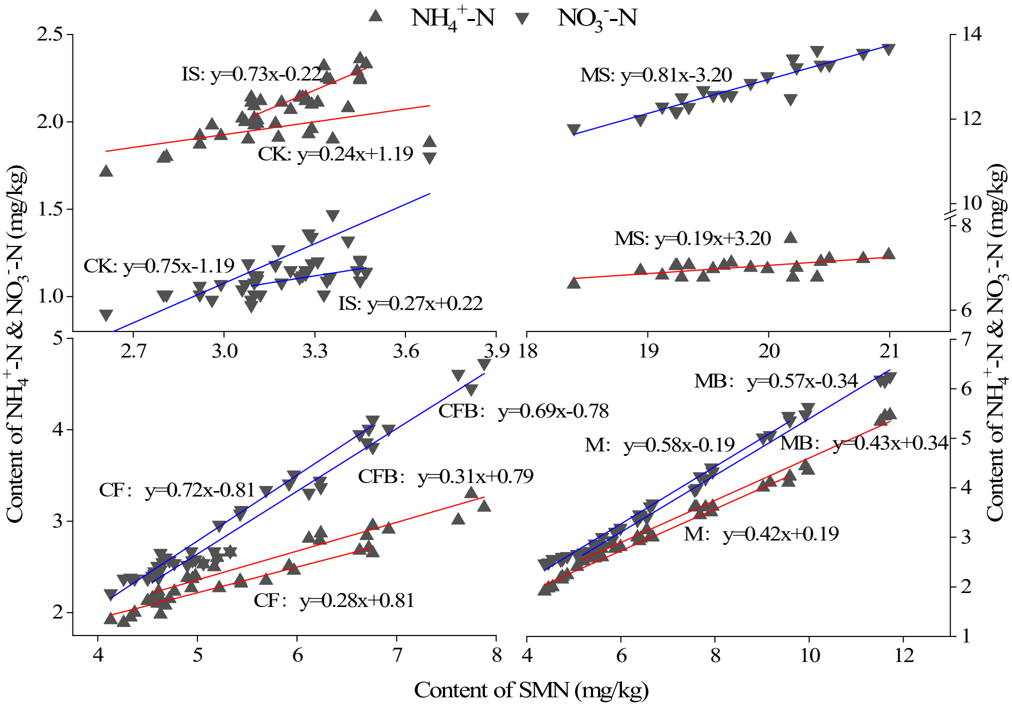

Figure 4. Relative contents of NH_4^+-N and NO_3^--N in SMN under different fertilization treatments.

3.3. Changes in AHN Content

As shown in Figure 5, The AAN and AN content of MS, IS, and CK treatments fluctuated within a small range. MB treatment has the best effect on improving the AAN and AN content of reclaimed soil. The fitting results showed that the AAN content of MB treatment showed an exponential increase trend, while M, CFB, and CF treatments showed a linear increase trend. MB treatment is expected to reach normal farmland soil (MS) levels after 9 years, which is more than 10 years earlier than CF, CFB, and M treatments. The AN content of CF, CFB, M, and MB treatments showed a linear increasing trend. In 2022, the AN content of MB treatment increased by 7.61% to 24.39% compared to CF, CFB, and M treatments. The UN content was the same as the ASN results, and the MS treatment was significantly higher than the fertilization treatment and IS, CK treatments. The ASN and UN contents of fertilization treatment did not show an increasing or decreasing trend with the increase of reclamation time. Compared with CF treatment, the AAN content of CFB treatment significantly increased by 9.10% to 17.38%, while the AN and UN content significantly increased by 11.99% and 14.46% only in 2020. Other time differences did not reach a significant level, while there was no significant difference in ASN content. Compared with M treatment, the content of AAN and AN in MB significantly increased by 6.13% -16.77%, 9.48% -17.82%, and the content of UN significantly increased by 97.10% -117.66% from 2019 to 2022. The content of ASN only increased significantly by 6.20% and 8.82% in 2020 and 2021. This indicates that the effect of *Pseudomonas fluorescens* on AAN content is higher than that of AN, UN is higher than that of ASN, and the effect of the combination of *Pseudomonas fluorescens* and organic fertilizer on the AHN content of reclaimed soil in coal mining areas is higher than that of the combination of *Pseudomonas fluorescens* and inorganic fertilizer.

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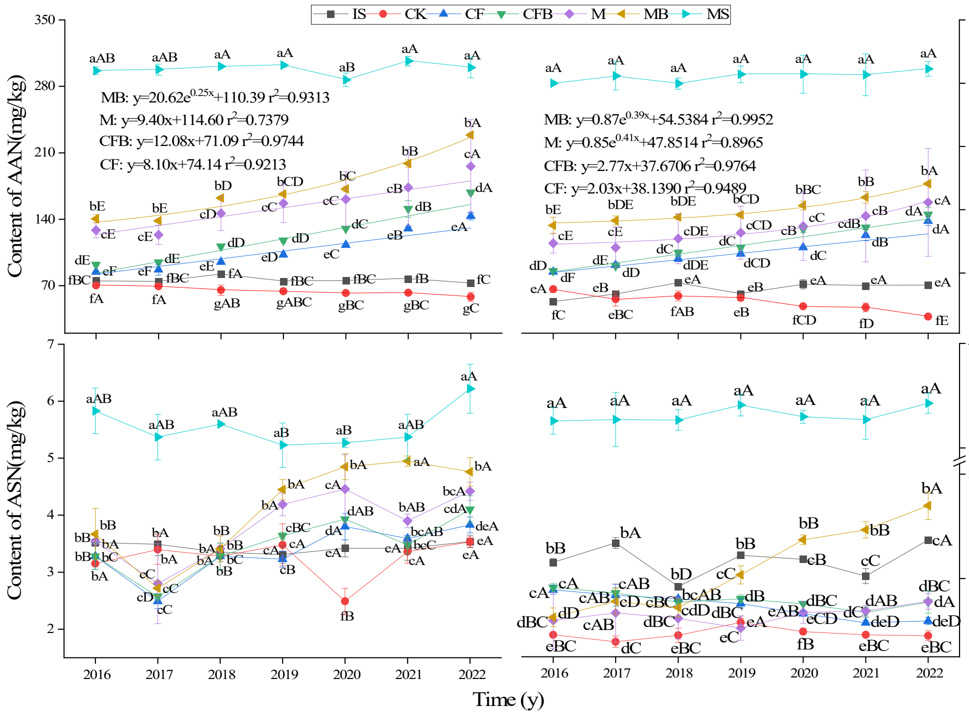

Figure 5. Content of AAN, AN, ASN, and UN under different fertilization treatments.

As shown in Figure 6, the order of soil organic nitrogen components in CK, CF, CFB, M, and MB treatments is AAN>UN>AN>ASN, while in IS and MS treatments, the order is UN>AAN>AN>ASN. It can be seen that fertilization, crop growth, and their interactions are all factors that affect the relative content of organic nitrogen components. As the reclamation time increased, the relative content of AN and AAN in AHN increased with CF and CFB treatments, while the relative content of UN decreased. There was no significant change in the relative content of ASN. Compared with CF, the relative content of AAN and AN in CFB treatment decreased by 33.77% and 10.67%, while the relative content of UN increased by 45.01%. Among the AHN increased by MB treatment, 39.90% is AAN, 53.34% is UN, 5.87% is AN, and 0.59% is ASN, compared with M treatment, the relative content of UN increased by 35.43%, while the relative content of AAN and AN decreased by 27.26% and 7.86%. The combination of *Pseudomonas fluorescens* with inorganic and organic fertilizers can reduce the relative content of AAN and AN, increase the relative content of UN, and have no significant effect on the relative content of ASN.

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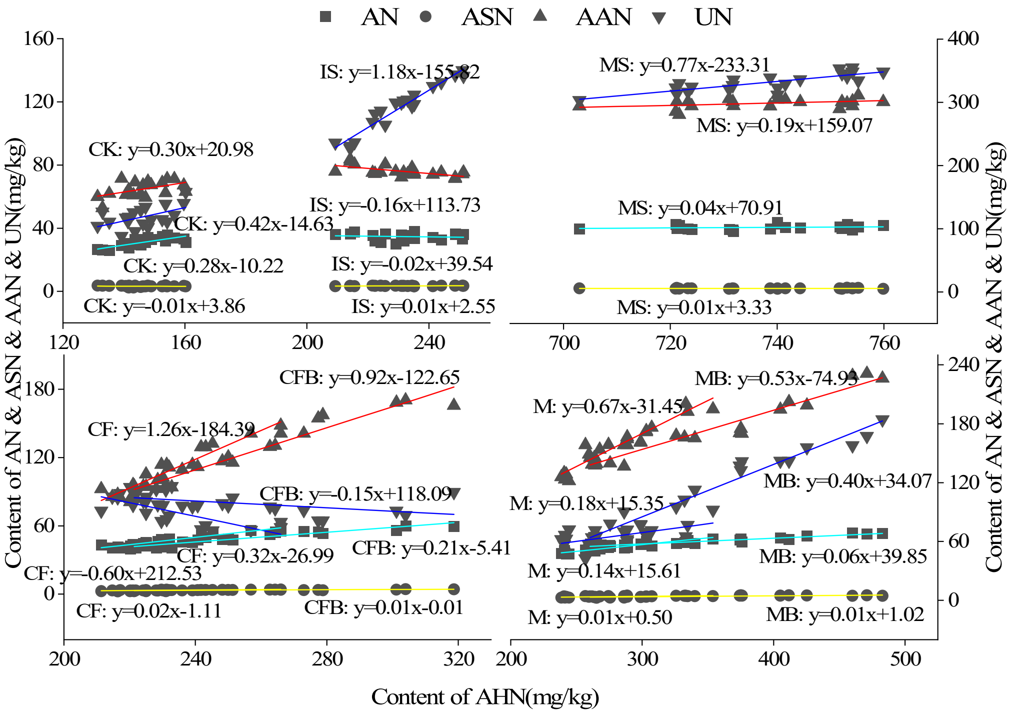

Figure 6. Relative content of AAN, AN, ASN, UN in AHN under different fertilization treatments.

3.4. Principal Component Analysis and Regression Analysis of Nitrogen in Reclaimed Soil

To further explore the effect of the application of *Pseudomonas fluorescens* on nitrogen accumulation and composition in reclaimed soil, principal component analysis and multiple linear regression analysis were conducted on the fertilization treatment.

As shown in Figure 7, based on the eigenvalues >1, two principal components were extracted, and the variance contribution rates of PC1 and PC2 were 95.20% and 3.00%, respectively, explaining a total of 98.20% variation in the variance variable. The seven treatments were copolymerized into 4 clusters. In PC1 axis direction, the clusters were CK, IS treatments, fertilization treatment and MS treatment from left to right. The same fertilization treatment is arranged from left to right along the PC1 axis direction in chronological order, indicating that soil nitrogen forms gradually succession towards normal farmland soil along the time dimension during the reclamation process. The spatial positions of fertilization treatment and MS treatment in the same year, from far to near, are CF, CFB, M, and MB treatments, indicating that *Pseudomonas fluorescens* is beneficial for the restoration of nitrogen content and composition in the reclaimed soil of coal mining areas. The combination of *Pseudomonas fluorescens* and organic fertilizer have the best effect.

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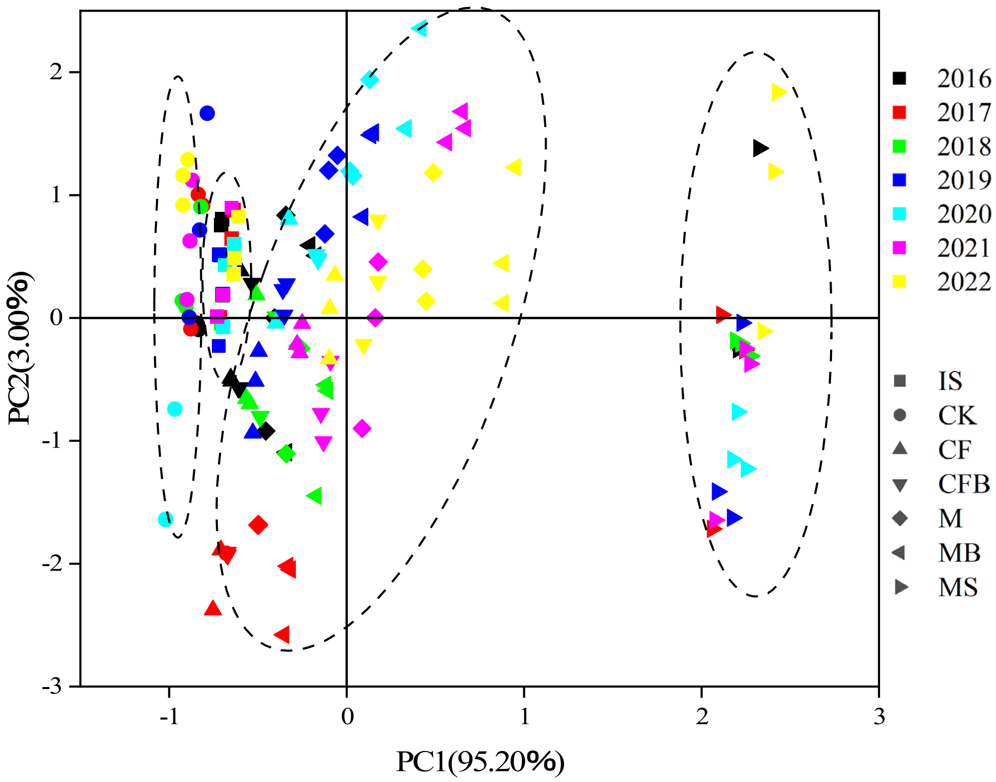

Figure 7. Principal component analysis of reclaimed soil nitrogen.

As shown in Figure 8, there is a significant difference in the comprehensive score results of fertilization treatment on the two principal components, manifested as MB>M>CFB>CF. The comprehensive scores of MB and CFB treatments increased by 1.58 and 0.79 respectively compared to M and CF treatments, indicating that the application of *Pseudomonas fluorescens* can increase the nitrogen content of reclaimed soil. The combination of *Pseudomonas fluorescens* and organic fertilizer has a better effect than the combination of inorganic fertilizer.

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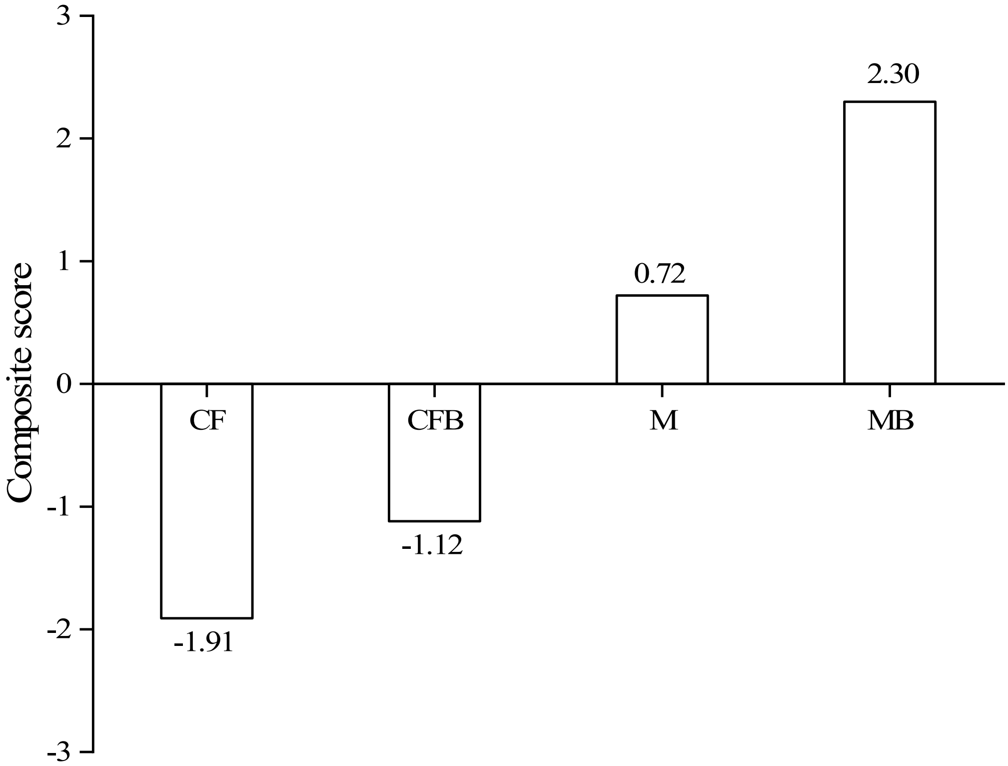

Figure 8. Comprehensive scores of fertilization treatment on two principal components.

$$Y(CF)=0.0081X_1+0.0034X_2+0.0713X_3+0.0061X_4+0.2831X_5+0.1661X_6+0.4620X_7 \quad (1)$$
$$Y(CFB)=0.0091X_1+0.0041X_2+0.0744X_3+0.0058X_4+0.3124X_5+0.0983X_6+0.4959X_7 \quad (2)$$
$$Y(M)=0.0112X_1+0.0084X_2+0.0552X_3+0.0065X_4+0.2685X_5+0.1085X_6+0.5416X_7 \quad (3)$$
$$Y(MB)=0.0106X_1+0.0077X_2+0.0378X_3+0.0068X_4+0.2546X_5+0.3359X_6+0.3466X_7 \quad (4)$$

Note: Y:TN; X_1:NO_3^--N; X_2:NH_4^+-N; X_3:AN; X_4:ASN; X_5:AAN; X_6:UN; X_7:THN.

Through the regression equation, it can be seen that the contribution of NO_3^--N, NH_4^+-N, and ASN to TN is significantly lower than that of AN, AAN, AUN, and UN. Compared with CF treatment, the contribution of SMN (NO_3^--N+NH_4^+-N) in CFB treatment increased by 14.78%, while the contribution of AHN (AN+ASN+AAN+UN) decreased by 7.19%. Compared with M treatment, the contribution of SMN (NO_3^--N+NH_4^+-N) in MB treatment decreased by 7.10%, while the contribution of AHN (AN+ASN+AAN+UN) increased by 44.77%. This indicates that the combination of *Pseudomonas fluorescens* and inorganic fertilizer will increase the contribution of SMN to TN, and the combination of organic fertilizer will increase the contribution of AHN to TN.

4. Discussion

4.1. Effect of Pseudomonas fluorescens on Soil Nitrogen Accumulation

Similar to most research results [25], with the increase of reclamation time, the soil TN, SMN, and AHN contents of CF, CFB, M, and MB treatments showed an increasing trend. The combination of *Pseudomonas fluorescens* with inorganic and organic fertilizers can increase the nitrogen content of reclaimed soil in coal mining areas, this is because *Pseudomonas fluorescens* N137-1 and N64-1 are autotrophic nitrogen fixing bacteria that can convert N_2 from the atmosphere into ammonia and store it in the soil [26], although the nitrogen fixation ability of *Pseudomonas fluorescens* is not as good as that of symbiotic and combined nitrogen fixing bacteria such as rhizobia and nitrogen fixing spirochetes, it has advantages such as low host specificity, wide distribution, and strong adaptability, compared to symbiotic nitrogen fixing bacteria and combined nitrogen fixing bacteria, they are more conducive to colonization and function in coal mining and reclamation areas with harsh environments. Moreover, *Pseudomonas fluorescens* can directly reduce the pH value of surrounding soil by synthesizing organic acids such as indole-3-acetic acid [27], reducing the loss of NH_4^+-N caused by acid-base reactions. In the preliminary experiment, we found that *Pseudomonas fluorescens* is similar to biofertilizers and has a disadvantage of poor stability. Therefore, we used a combination of *Pseudomonas fluorescens* and inorganic and organic fertilizer carriers to increase the stability of *Pseudomonas fluorescens* in the soil of coal mining and reclamation areas [28,29].

The research results showed that the growth rate and accumulation of soil nitrogen in the combination of *Pseudomonas fluorescens* and organic fertilizer were significantly higher than those in the combination of inorganic fertilizer. This is because after inorganic fertilizer (urea) enters the soil, the amide nitrogen is hydrolyzed to NH_4^+-N under the action of soil urease. Part of NH_4^+-N is directly absorbed by crops and converted into crop tissue nitrogen, while the remaining NH_4^+-N is converted into NO_3^--N through nitrification, which is absorbed or leached by crops, or directly volatilized. Especially in calcaric soil, the ammonia volatilization loss can be as high as 30% of the nitrogen applied. Finally, only a small part of amide nitrogen is dissolved in soil solution, absorbed by soil through hydrogen bonding, or transformed into soil microbial biomass and organic nitrogen by biological assimilation [30], which remain in the soil.

Therefore, we believe that under appropriate nitrogen application rates, the increase in soil nitrogen content under CF and CFB treatments mainly comes from the increase in biomass such as roots and leaves caused by absorption and assimilation of microorganisms and crops [31], rather than the residue of inorganic fertilizers. As an important plant rhizosphere growth promoting bacterium, *Pseudomonas fluorescens* can simulate the synthesis of plant hormones to directly regulate plant growth and development. It promotes plant growth by increasing the nitrogen and minerals in the soil that can be utilized by plants. Therefore, the comprehensive score of CFB treatment on two principal components is 0.79 higher than that of CF treatment. Organic fertilizer (chicken manure), on the one hand, controls the mineralization of SON and the fixation of SMN by regulating soil C/N, promoting soil nitrogen to meet crop needs, maximizing soil nitrogen utilization efficiency, avoiding soil nitrogen leaching and gaseous loss [32,33]. On the other hand, applying organic fertilizer to reclaimed soil with insufficient organic matter content can also have a negative excitation effect, increasing soil nitrogen accumulation. Longer time of research have also shown that under equal fertilization conditions, the soil nitrogen accumulation content of organic fertilizer treatment is much higher than that of inorganic fertilizer treatment [34]. At the same time, organic fertilizer can also provide a more suitable culture medium for soil microorganisms, including *Pseudomonas fluorescens*, improving soil microbial activity and quantity. This is reflected in the comprehensive score of MB treatment on two principal components being 1.58 higher than M treatment. This research result also confirms the view that organic cultivation is more conducive to microbial nitrogen fixation [35].

4.1. Effect of Pseudomonas fluorescens on Soil Nitrogen Accumulation

Soil nitrogen can be divided into SMN and SON according to its occurrence form. In terms of soil fertility, NH_4^+-N and NO_3^--N are the two most important types of SMN [36], so this study assumes that SMN=NH_4^+-N+NO_3^--N. The results showed that the relative content of NH_4^+-N and NO_3^--N in MB treatment increased and decreased by 0.46% compared to M treatment, while the relative content of NH_4^+-N and NO_3^--N in CFB treatment decreased and increased by 3.38% compared to CF treatment. This indicates that *Pseudomonas fluorescens* has no significant effect on the relative content of NH_4^+-N and NO_3^--N, this is mainly due to the long-term nitrification and nitrate dissimilation reduction of NH_4^+-N and NO_3^--N in the soil, which to some extent achieve dynamic equilibrium[37], *Pseudomonas fluorescens* is unable to effectively alter factors such as soil clay mineral types, acidity, and alkalinity that affect the fixation process of NH_4^+-N minerals, and increase or decrease the leaching of NO_3^--N. However, we also found that the contribution of SMN to TN in CF treatment is 0.0115, which is lower than 0.0132 in CFB treatment, this is due to the use of *Pseudomonas fluorescens* in the absence of external organic matter addition, which resulted in a significant portion of soil carbon being consumed as energy, leading to a rapid decrease in soil C/N. After its mineralization exceeded assimilation [38], the supply of NH_4^+-N and NO_3^--N significantly increased, thus increasing the contribution of SMN to TN. The contribution of SMN to TN in MB treatment is 0.0183, which is lower than 0.0196 in M treatment, this is due to competition between crops and microorganisms in the process of absorbing and assimilating NH_4^+-N and NO_3^--N in the soil. The addition of *Pseudomonas fluorescens* is beneficial for microbial competition. From the perspective of soil nitrogen cycling, microorganisms absorb and assimilate NH_4^+-N and NO_3^--N into organic components, thus reducing the contribution of SMN to TN.

At present, people's understanding of SON is still limited, and there is no method to separate different chemical forms of nitrogen from soil without damaging the components of soil organic nitrogen. This study chose to use acid hydrolysis method to divide organic nitrogen into THN and AHN for research. The results showed that after 7 years of continuous fertilization, the order of soil organic nitrogen components in CF, CFB, M, and MB treatments was AAN>UN>AN>ASN. Although *Pseudomonas fluorescens* did not change the order of soil organic nitrogen component size, it could increase the relative content of UN and reduce the relative content of AAN and AN. This is because UN is composed of non α-amino acid nitrogen, fatty amines and aromatic amines, nitrogen-containing heterocyclic compounds, and amino acids directly connected to aromatic rings through C-N bonds, its content is not related to the mineralization rate of SMN [39]. As a temporary nitrogen reservoir containing a large amount of easily mineralized organic nitrogen, AN is the main available nitrogen in the soil that can be directly absorbed and utilized by crops in the current season, mainly derived from fixed ammonium, especially newly formed fixed nitrogen [40]. AAN is the main source of available nitrogen absorbed by soil microorganisms and plants, especially small molecule amino acids that can be directly assimilated and absorbed by microorganisms [41]. AAN and AN are the two most important organic nitrogen components that determine the potential for nitrogen mineralization and are the main sources of mineralization. *Pseudomonas fluorescens* accelerates the mineralization of AN and AAN, resulting in an increase in the relative content of UN. ASN mainly comes from the cell wall of soil microorganisms, which accounts for about 5% of soil organic nitrogen. Although *Pseudomonas fluorescens* can increase the number of soil microorganisms, its colonization time in reclaimed soil is about 80 days. Its remains participate in the internal circulation of soil nitrogen in the subsequent time. After 30 days of sampling and measurement, only a portion continues to remain in the form of ASN in the soil, indicating no significant change in the relative content of ASN. Wang's research results also indicate that there is no correlation between ASN and other nitrogen forms in the soil [42]. CF and CFB treatments due to the absence of exogenous AHN addition, require microbial mineralization to convert the original AHN in the soil into SMN for crop use after the consumption of SMN. *Pseudomonas fluorescens* promotes the mineralization of AHN, manifested as the contribution of ANH to TN in CFB treatment being 0.4909, lower than that of CF treatment being 0.5266. Research has shown that the contribution of organic nitrogen components is closely related to the difficulty of mineralization [43,44]. When exogenous AHN is added to M and MB, *Pseudomonas fluorescens* causes a decrease in the contribution of organic nitrogen components that are easily mineralized by AN and AAN, and an increase in the contribution of organic nitrogen components that are not easily mineralized by UN. Specifically, the contribution of AN+AAN decreases by 0.0313 and the contribution of UN increases by 0.2274.

5. Conclusions

Comprehensive analysis shows that continuous 7 years of fertilization and cultivation have led to a rapid evolution of nitrogen content and composition structure in reclaimed soil towards normal mature farmland soil. *Pseudomonas fluorescens* can increase the relative content of unknown nitrogen (UN) in soil nitrogen, and reduce the relative content of amino acid nitrogen (AAN) and ammonia nitrogen (AN). The combination of *Pseudomonas fluorescens* and inorganic fertilizer can increase the contribution of inorganic nitrogen (SMN) in soil total nitrogen (TN), while the combination of *Pseudomonas fluorescens* and organic fertilizer can increase the contribution of acid-hydrolysable nitrogen (AHN) in soil total nitrogen (TN). *Pseudomonas fluorescens* is beneficial for nitrogen recovery in coal mining areas, and the optimal fertilization method under this experimental condition is the combination of *Pseudomonas fluorescens* and organic fertilizer. This study can provide scientific basis for the restoration of soil nitrogen by *Pseudomonas fluorescens* coal mining areas.

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