

Effect of Combined Fertilization on Plant Biomass of Waste Land and Comprehensive Benefit Evaluation in Grassland Mining Area

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Abstract: The dump site formed by the large-scale open-pit coal mining waste piles has poor soil environment and difficult vegetation restoration, and is in the grassland area with relatively weak ecological environment. A completely randomized block design was used to conduct separate application of microbial fertilizer and the combined application of microbial fertilizer and organic fertilizer in this study. The results showed that "Sino Green Agri-Biotech" soil conditioner combined with organic fertilizer had a higher plant biomass by comparing different fertilization methods, the plant biomass increased with the increase of microbial fertilizer application. The RSR (Rank Sum Ratio) method was used to analyze the comprehensive benefits of different fertilization methods from the aspects of microbial community diversity, soil enzyme activity, soil fertility, vegetation growth status and economic benefits. Evaluation results showed that application of microbial fertilizer had an important role in increasing soil fertility and accelerating vegetation construction the combined benefit of E-2001 microbial fertilizer application rate of 0.60 ml/m² and organic fertilizer was the highest, priority can be given to the process of ecological restoration on coal mine dump in grassland. It is preferred to use a combination of E-2001 microbial fertilizer application rate of 0.60 ml/m² and organic fertilizer in the soil improvement process of vegetation restoration and reconstruction in the grassland mining area.

1. Introduction

Inner Mongolia has become one of the most important energy bases in China because of its abundance of coal. Most coal resources are concentrated in grassland areas with relatively fragile ecological environment in Inner Mongolia. The large-scale development of coal has brought negative impacts on the grassland ecosystem and the production and livelihood of local inhabitants, the most obvious impact is the environmental damage caused by open-pit coal mining[1]. The waste land produced by open-pit mining is mainly coal mine dump on grassland[2]. A large number of waste soil and residue are piled up in the coal mine dump, and the original mechanical composition and structure of the soil are changed. These characteristics of the coal mine dump severely restrict the ecological restoration and vegetation reconstruction of the grassland mining area, such as poor soil, low water content, and low biological activity[3-4]. Soil microorganisms are an important part of the ecosystem and the most active part of the soil ecosystem. They are also extremely important decomposers in the soil system. Soil microorganisms play a leading role in soil nutrient transformation, being resistance to external disturbances, maintaining system stability, and sustainable soil development[5]. The use of modern microbial technologies such as autogenous nitrogen-fixing bacteria, phosphorus bacteria, potassium

bacteria, compound bacteria, and mycorrhizal roots to comprehensively treat and improve soil waste in coal mines can use the advantages of microbial inoculation to re-establish soil that has lost microbial activity and restore soil microorganism System to promote the maturation of raw soil and shorten the ecological restoration cycle[6].

At present, there are many results in the field of using microbial technology to restore damaged land in coal mines, and the repair mechanism of soil by microorganisms is relatively clear[7-10]. However, the practical application of microbial technology is not ideal due to the limitations of microorganisms' own characteristics and changes in environmental factors[11-12]. The application of microbial technology in the ecological restoration of grassland mining areas is relatively small in the arid and semi-arid grassland areas of northern China; it is affected by factors such as the harsh natural climate and the relatively low attention of the grassland coal industry. Based on the fact that the lower climatic temperature in the northern grassland of China could not fully activate the bacterial flora in the microbial fertilizer, this study analyzed the change of plant biomass under the combined application of microbial fertilizer and organic fertilizer and evaluated the comprehensive benefits. The combined application of microbial fertilizer and organic fertilizer can improve soil infertility, poor microbial activity and low water-holding capacity in coal mine dumps on grassland mining areas.

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2. Materials and methods

2.1. Study area

The study area was located at the coal mine dump of Shengli East No.2 Open-pit Coal Mine of Datang International in Xilinhot, Xilinguole League, Inner Mongolia. The coal mine dump was located in the southeast of the mining area and covered an area of 13.66 km². It is a stepped landform with platforms and slopes distributed and the relative height is 100m. The height of each step is about 20m. The coal gangue was mixed with soil and then discharged to the dump and then covered with soil 50 cm ~ 80 cm [13]. A test area was set up on the sundeck platform of the dump. The soil quality is poor and the nutrient content is low according to the test of soil physical and chemical properties. The test area was located in the middle of the Inner Mongolia Plateau and it belonged to the arid and semi-arid climate in the mid-temperate zone. The average annual rainfall is 289 mm, which is concentrated in June to August, accounting for more than 70% of the annual precipitation. The local multi-year average evaporation is 1830.8 mm. The native soil in the test area is typical chestnut soil, and the original vegetation belongs to typical steppe vegetation. *Stipa krylovii* and *Leymus chinensis* are the main group species, and the vegetation coverage is 35% -50%.

2.2. Experimental design

There were two kinds of microbial fertilizer used in the test, namely "E-2001 microbial fertilizer" (E) and "Sino Green Agri-Biotech" soil conditioner (L). For organic fertilizer, fermented cow dung granules (F) were selected, the raw materials were mainly fungal bran and cow dung. E-2001 microbial fertilizer is a microbial liquid bacterial fertilizer, produced by Agricultural Research Technologies, LTD, American, it was approved by the Fertilizer Registration Review Committee of the Ministry of Agriculture and obtained the fertilizer registration certificate of the People's Republic of China in 2001. "Sino Green Agri-Biotech" soil conditioner is a high-tech product successfully developed by Sino Green Agri-Biotech (Beijing) Biotechnology Co., Ltd. and China Agricultural University. The test area was set on an open-pit mine dump platform that had undergone natural restoration for three years. The random block design was adopted, with no fertilization as the control treatment (CK), E-2001 microbial fertilizer alone, "Sino Green Agri-Biotech" soil conditioner alone, organic fertilizer alone, comparative experiment on different application rates of microbial fertilizer and organic fertilizer. The area of each test plot was 6 m² (2 m×3 m), and the test plot spacing was 0.5 m. Three application rates were set for two microbial fertilizers and organic fertilizer, and three test levels were set for E-2001 microbial fertilizer, which was E1-0.36 ml/m², E2-0.48 ml/m², E3-0.60 ml/m²; "Sino Green Agri-Biotech" soil conditioner set three test dosages as L1-2 g/m², L2-4 g/m², L3-6 g/m²; organic fertilizer set three test dosages as F1-10 g/m²,

F2-20 g/m², F3-30 g/m².

2.3. Plant biomass

At the end of each growing season, around the beginning of September, the aboveground biomass of plant communities was determined by the method of cutting. Randomly select a 1 m×1 m sample plot, cut the plants in the plot uniformly, and stubble the height of about 1cm. After cutting, the plants are placed in the drying box without distinguishing the species. The drying box facility is 80 °C. The plants are dried to constant weight in the drying box for about 24-48 hours. The dry weight is weighed with a 0.01g balance after cooling.

3. Results and analysis

3.1. Change of plant biomass

The perennial forage is generally green in April and yellow in October. The plant biomass in the whole growing season shows obvious seasonal dynamics. During the test, at the end of the growing season (early September), the aboveground biomass measurement of plant communities with different fertilization methods showed that the growth conditions of the plants in the test area in 2018 were better, showing higher biomass values. The average biomass reached 161.53 g/m², the plant biomass treated by E3F3 reached 220.45 g/m², and the aboveground biomass of the non-fertilized control plot also had 84.64 g/m². The average biomass in the test plot was significantly higher than in 2017, the average value of plant biomass was 47.80 g/m² in 2017.

Comparing the changes of plant biomass with different fertilization measures (Table 1), the plant biomass of each fertilization treatment at the end of the 2017 growing season was 34.36 g/m² to 66.31 g/m². After analysis of variance, the difference between the treatments did not reach a significant level, indicating that in the first year of the experiment. The effect of combined fertilization on plant biomass was small. Plants biomass with different fertilization methods varied greatly at the end of the growing season in 2018, and the biomass changed from 80.75 g/m² to 220.45 g/m². The above-ground biomass of the vegetation was ranked as L3 + F> E3 + F> L2 + F> L> E2 + F> F> E1 + F> L1 + F> E> CK. The plant biomass of each test plot was significantly higher than that of the control plot (P <0.05), indicating that the effect of combined fertilization on plant growth was more obvious. In each fertilization method, "Sino Green Agri-Biotech" soil conditioner combined with organic fertilizer had higher plant biomass, and the biomass increased with the increase of fertilization. The combined application of "Sino Green Agri-Biotech" soil conditioner and organic fertilizer can promote the growth of vegetation more than other fertilization measures in the process of vegetation restoration in abandoned grassland mining areas, according to the change of plant biomass at the end of the growing season.

Table 1. Effects of combined fertilization on plant biomass in 2017 and 2018 (g/m²)

treatment	2017				
	Fertilizer application level			Average value	Standard error
	1	2	3		
E	40.85	43.16	51.16	45.06a	4.418
L	46.70	56.87	40.65	48.07a	6.693
F	55.01	50.74	62.35	56.03a	4.795
E1+F	35.25	37.53	44.45	39.08a	3.912
E2+F	51.32	58.63	53.72	54.56a	3.042
E3+F	49.51	54.65	66.31	56.82a	7.029
L1+F	40.01	34.49	49.20	41.23a	6.067
L2+F	34.36	42.76	53.18	43.43a	7.698
L3+F	40.18	50.31	56.16	48.88a	6.601
CK				38.82a	
treatment	2018				
	Fertilizer application level			Average value	Standard error
	1	2	3		
E	95.89	111.65	165.32	124.29bc	29.720
L	179.10	176.45	173.40	176.32a	2.329
F	162.30	147.39	185.80	165.16ab	15.811
E1+F	100.20	167.34	177.26	148.27ab	34.229
E2+F	191.68	1.00	167.13	119.94ab	84.695
E3+F	181.05	193.64	176.53	183.74a	7.239
L1+F	96.09	156.82	180.64	144.52ab	35.597
L2+F	174.58	172.68	203.34	183.53a	14.027
L3+F	165.58	173.59	220.45	186.54a	24.200
CK				84.64c	

Note: Significant level $P < 0.05$, lower case letters indicate comparison between treatments.

3.2. Comprehensive benefit analysis

3.2.1. Investment cost of different fertilizing materials. Through experiments and investigations, E-2001 microbial fertilizer is a liquid fertilizer, which is diluted and applied after the bacteria are activated, and the economic cost is 960 yuan/hm². "Sino Green

Agri-Biotech" soil conditioner is a solid granular fertilizer, which is easy to transport and apply, and the economic cost is 720 yuan/hm². Organic fertilizer cow dung granules have low nutrient content, large application amount, economic cost is about 1200 yuan/hm², and economic input is at a high level.

Table 2. Investment cost survey of test fertilizers used in the test

Test fertilizer		Advantages	Disadvantages	Economic cost
Microbial fertilizer	E-2001 microbial fertilizer	It is a pure natural microbial inoculant. The decomposition of beneficial living microorganisms can promote nutrient circulation and produce a variety of vegetative bacteria and enzymes that rejuvenate the soil.	It is a kind of living fertilizer. Temperature and moisture have certain limiting effects on the activity of bacterial fertilizer. Short validity period, 18 months.	960 yuan/hm ²
	"Sino Green Agri-Biotech" soil conditioner	The beneficial microorganism flora regulates the community structure of soil microorganisms, rapidly decomposes soil organic matter, and promotes the formation of soil aggregates.	The effectiveness of microbial fertilizers may be affected by the soil microbial flora and quantity.	720 yuan/hm ²
Organic Fertilizer	Cow dung pellets	Contains functional bacteria and organic matter, can improve the soil and promote the release of nutrients fixed by the soil.	The nutritional content is low and the application amount is large.	1200 yuan/hm ²

3.2.2. *Comprehensive evaluation of RSR method.* RSR method (Rank Sum Ratio method) is a statistical analysis method that combines classical parameter statistics with modern non-parametric statistics and combines their respective advantages. RSR method can also be applied to the comprehensive evaluation of classification and measurement data. This method is mainly used for comprehensive evaluation and analysis of multiple indicators, statistical forecast and prediction, and statistical quality control[14-15]. The average of the ranks of multiple evaluation indexes is a continuous variable of 0 to 1 in multi-index comprehensive evaluation, and it is a non-parametric statistic. In comprehensive evaluation RSR method, the value of rank sum ratio can contain information of all evaluation indicators, showing the comprehensive level of these evaluation indicators. A larger RSR value indicates a better overall evaluation.

① *Principle of RSR method.* Suppose a matrix has n rows (n evaluation objects) and m columns (m evaluation indexes). The formula for calculating the RSR value is as follows: $RSR_i = \sum_{j=1}^m \frac{R_{ij}}{m \times n}$

Where, $i=1,2, \dots, n$; $j=1,2, \dots, m$; R_{ij} is the rank of the element in the i -th row and the j -th column. The RSR value is dimensionless, with a minimum of $1/n$ and a maximum of 1.

The basic idea of the RSR method is to rank each index value in a matrix with n rows and m columns, and then calculate the RSR value, which is a dimensionless statistic, the parameter statistical method is applied to study the distribution of RSR values, and finally sorts according to the size of the RSR values and archived for

classification. The theoretical significance of the RSR method is to expand the function of nonparametric statistics, effectively combine classical parametric statistics and modern nonparametric statistics, and their advantages and characteristics have complemented each other.

② *Index value calculation.* Five indicators were selected for comprehensive evaluation of the RSR method, including indicators of microbial community diversity, indicators of soil enzyme activity, indicators of soil fertility, indicators of vegetation growth and economic benefits. The microbial community diversity index selected the *simpson* diversity index. Catalase was selected as the index of soil enzyme activity. Organic matter was selected as an indicator of soil fertility. Above-ground biomass was selected as an indicator reflecting the growth status of vegetation. The economic benefit index selected the investment cost.

When the RSR value is larger, the comprehensive benefit of the evaluated model is higher, and when the RSR value is smaller, the comprehensive benefit of the evaluated model is lower according to the evaluation principle of the RSR method. The comprehensive benefits of different fertilization measures were ranked based on the calculation results of RSR values, that was $E3+F > L3+F > E2+F > L1+F > L2+F > E1+F > L > E > F$ (Table 3). The results showed that the combined benefit of E-2001 microbial fertilizer application rate of 0.60 ml/m² and organic fertilizer was the highest, followed by the combination of "Sino Green Agri-Biotech" soil conditioner application rate of 6 g/m² and organic fertilizer, and the comprehensive benefit of organic fertilizer alone lowest.

Table 3. Comprehensive benefit indicators of different fertilization methods

Treatment	Comprehensive Benefit Index										RSR value
	<i>simpson</i> diversity index	rank order	catalase	rank order	Organic matter	rank order	aboveground biomass	rank order	investment cost	rank order	
E	0.949	4.5	34.447	5.0	7.13	2.0	124.29	3.5	0.096	4.5	0.433

L	0.946	3.0	25.595	2.5	7.70	3.0	176.32	6.5	0.072	8.0	0.511
F	0.921	2.0	21.135	1.0	7.83	3.5	165.16	5.5	0.120	1.0	0.289
E1+F	0.968	7.0	35.666	6.0	8.43	5.0	148.27	3.5	0.068	8.5	0.667
E2+F	0.980	9.0	41.219	8.8	8.57	5.5	119.64	1.5	0.076	7.0	0.707
E3+F	0.971	7.5	41.260	9.0	8.37	4.5	183.74	8.0	0.084	6.5	0.789
L1+F	0.960	6.0	26.444	4.5	10.10	8.5	144.52	3.8	0.069	8.5	0.696
L2+F	0.961	6.0	26.173	3.5	8.40	5.0	183.53	8.0	0.072	8.0	0.678
L3+F	0.978	8.5	25.302	2.0	9.57	7.5	186.54	9.0	0.075	7.0	0.756

4. Discussion

The grassland coal mine dump site matrix is mainly mining stripping, mining waste and ash. The native vegetation on the coal mine dump is extremely scarce due to insufficient topsoil, low soil fertility, and water shortage. The vegetation in the test area was naturally restored for five years, and the plant species composition was single, mainly perennial grasses and one or two year weeds. The grass layer was low and the differentiation was not obvious. Plant biomass is a comprehensive reflection of the structure and function of natural ecosystems, and is the product of the combined effects of plant biocological characteristics and external environmental conditions. The result showed that the average plant biomass in the test plots in 2018 was significantly higher than in 2017. The temperature in the test area since the beginning of spring (March to May) was abnormally high in 2018, with an average temperature of 8.0°C, 3.4°C higher than the same period of the year, and precipitation of 41.7 mm, 21% more than the same period of the year 21%, Especially in July and August 2018, the accumulated precipitation was 132.8mm, which was an increase of 51.3mm over the same period in 2017. A positive correlation between grassland productivity and annual precipitation has been recognized. Most studies believe that there is a simple linear relationship between the two[16-18], and some studies believe that the two are exponential[19-21]. Regardless of the relationship, rainfall can promote plant growth, and grassland productivity can be determined through higher annual precipitation. On the other hand, a higher climatic temperature is beneficial to the rapid propagation of beneficial living microorganisms in microbial fertilizers, enabling the advantages of microbial fertilizer inoculation to be exerted, and accelerating the conversion of fixed nutrients to effective nutrients in the soil, thereby promoting plant roots and aboveground Growth, so higher precipitation and temperature in 2018 increased plant biomass significantly.

There are large differences in the economic costs of microbial fertilizer and organic fertilizer through comparison. In the process of vegetation restoration and reconstruction in the abandoned land of steppe mining, the comprehensive benefit evaluation of different combined fertilization methods can reflect which fertilization method is more conducive to artificial remodeling and soil improvement. The comprehensive benefit evaluation results of the RSR method showed that

the combined application of microbial fertilizer and organic fertilizer was better than the single application of any fertilizer in improving soil and promoting vegetation growth. The combination of E-2001 microbial fertilizer with an application rate of 0.60 ml/m² and organic fertilizer has obvious effects in improving soil microbial composition, increasing soil fertility, and increasing aboveground biomass, and the investment cost is at a medium level.

5. Conclusion

5.1 "Sino Green Agri-Biotech" soil conditioner combined with organic fertilizer had a higher plant biomass, and the plant biomass increased with the increase of fertilizer application. The combined application of "Sino Green Agri-Biotech" soil conditioner and organic fertilizer could promote the growth of vegetation more than other fertilization methods.

5.2 The maximum RSR value was the combination of E-2001 microbial fertilizer application rate of 0.60 ml/m² and organic fertilizer based on the comprehensive benefit analysis, which indicates that it can be preferentially used in the soil improvement process of vegetation restoration and reconstruction in the abandoned land of steppe mining.

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References

1. Bai Z K, Zuo X, Guo Q X, et al. Study on land reclamation planning in large-scale opencast coal mine. *Journal of Soil and Water Conservation*, 2001, 15(4): 118-121.
2. Huang D Y. Investigation and practice on mining land rehabilitation. *Hunan Nonferrous Metals*, 2011, 27(6): 45-49.
3. Bai Z K, Wang Z G, Zhao J K, et al. characteristics of soil erosion and its control in antaibao open-pit mine. *Journal of China Coal Society*, 2002, 14(3): 27-30.
4. Ministry of Water Resources, Chinese Academy of Sciences, Chinese Academy of Engineering.

- Prevention and control of soil erosion and ecological security in China, Development and construction activity volume. Beijing: Science Press, 2010.
5. Zhang W, Wei H L, Gao H W, et al. Advance of studies on soil microbial diversity and environmental impact factors. Chinese Journal of Ecology, 2005, 24(1):48-52.
 6. Wang H X, Li F P, Zhang J. Review of microbic reclamation technology for mining areas. METAL MINE, 2004, 8: 96-99.
 7. Auernik K S, Maezato Y, Blum P H, et al. The genome sequence of the metal-mobilizing, extremely thermoacidophilic archaeon *Metallosphaera sedula* provides insights into bioleaching-associated metabolism. Appl Environ Microbiol, 2008, 74(3): 682-692.
 8. Mera-Nappa A, Picioreanu C, Asenjo J A. Nonhomogeneous biofilm modeling applied to bioleaching processes. Biotechnol Bioeng, 2010, 6(4): 660-762.
 9. Jin L, Wang H F, Hong J P. Effects of bacterial manure on soil enzymatic activity in core-mining subsidence area. Journal of Shanxi Agricultural Sciences, 2010, 38(2): 53-54.
 10. Du S Z, Bi Y L, Wu W Y, et al. Ecological effects of arbuscular mycorrhizal fungi on environmental phytoremediation in coal mine areas. Transactions of the CSAE, 2008, 24(4): 113-116.
 11. Xv J M. Effect of biological bacterial fertilizer on the phosphorus, organic matter and microorganism in the reclamation soil in mining area. Journal of Shanxi Agricultural Sciences, 2011, 39(3): 250-252.
 12. Cui S J, Gu L K, Lian Y X, et al. Research of microbiology technology in ecological remediation of the abandoned coal mining land. METAL MINE, 2010, 406(4):76-79.
 13. Liu W M, Xing W S. Study on the mining technology of the two open pit mine. Opencast Mining Technology, 2009, 4: 14-16.
 14. Yu C, Su B. A non-parametric sequential rank-sum probability ratio test method for binary hypothesis testing. Signal Processing, 2004, 84(7):1267-1272.
 15. Hong L G. Study on the present situation of grassland utilization and protection countermeasures in Xilin River Basin. Inner Mongolia University, 2009.
 16. Cai X C, Li Z Q, Chen Z Z. The relationship between aboveground biomass and precipitation on *Stipa grandis* steppe in Inner Mongolia. Acta Ecologica Sinica, 2005, 25(7): 1657-1662.
 17. Bai Y, Wu J, Xing Q, et al. Primary production and rain use efficiency across a precipitation gradient on the mongolia plateau. Ecology, 2008, 89(8):2140-2153.
 18. T G O'Connor, L M Haines, H A Snyman. Influence of precipitation and species composition on phytomass of a semi-arid African grassland. Journal of Ecology, 2001, 89:850-860.
 19. Hu Z M, Fan J W, Zhong H P, et al. Spatiotemporal dynamics of aboveground primary productivity along a precipitation gradient in Chinese temperate grassland. Science in China, 2007, 50(5):754-764.
 20. Hu Z, Yu G, Fan J, et al. Precipitation-use efficiency along a 4500-km grassland transect. Global Ecology & Biogeography, 2010, 19(6):842-851.
 21. Ma W H, Yang Y H, He J S, et al. Above-and belowground biomass in relation to environmental factors in temperate grasslands, Inner Mongolia. Science in China Series C: Life Sciences, 2008, 51:263-270.