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Full Length Article

Preliminary Investigation of the Relationship between Vegetation Types and Soil Microbial Flora and Biomass in Northern Shanxi Province

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Abstract

Five vegetation types in the Shuozhou ecological reclamation area of Shanxi Province (*Populus simonii* forest, *Robinia pseudoacacia* plantation, *Pinus tabulaeformis* plantation, *Caragana seabuckthorn* mixed forest and natural grassland) were studied, and the relationship between soil microbial biomass carbon, nitrogen content, abundance of bacteria and fungi, and soil basic chemical properties was analyzed. The results showed that the soil quality of the four types of vegetation improved to a different degree than that of natural grassland, which showed the trend of *P. simonii* forest and natural grassland. The biomass carbon and nitrogen of the *P. simonii* forest soil were highest; those of the plantation were second, and those of the natural grassland lowest, and the soil organic carbon, total nitrogen. There was a significant positive correlation between alkali and nitrogen, the lowest bacterial and fungal abundance was observed in natural grassland soil, and the content of bacteria and fungi in plantation soil was significantly lower than that of the *P. simonii* forest. Overall, the results indicated that vegetation restoration has a positive effect on soil quality in the study area, and different vegetation types have different effects on soil quality and microbial flora.

Keywords: Vegetation type; Soil microorganism; Microbial organic matter; Vegetation

Introduction

Soil microorganisms, which comprise the most active fraction of soil ecosystems, participate in the energy conversion and material metabolism of ecosystems (Hatris, 2003). Soil microbes influence the global biochemical cycle, while also playing an important role in improving the physicochemical properties of soil (Taylor *et al*., 2002). In addition, soil microorganisms can indirectly promote the growth of vegetation. Soil microbial biomass is a repository of soil nutrients that can be used as an index of soil material cycling. Soil microbial biomass is closely related to soil nutrient and health status (Singh *et al*., 1989; Smolander *et al*., 1994; Li *et al*., 2004), with a higher content reflecting a higher activity of the microbial community. Accordingly, soil microbial biomass can be used to measure the material cycling ability of the ecosystem and its ability to promote the growth and development of vegetation to some degree (Rogers and Tate, 2002). Soil microorganisms are very sensitive to environmental changes, and different vegetation types can cause differences in microbial biomass in soil, even under the same site conditions (Waid, 1999), thus changing its ecosystem function.

The Shanxi area is one of the most serious soil and water loss areas in the Loess Plateau because of the destruction of natural vegetation in the area as a result of coal mining and other anthropogenic activities. Accordingly, a large restoration plantation has been implemented to improve the local environment. However, there is controversy regarding the current income and expenditure status of forest ecosystems, with some studies considering these ecosystems to be carbon sources and others considering them carbon sink (Ma and Wang, 2015). This divergence mainly stems from the output of carbon from forest ecosystems. Abnormal soil respiration is the only way for carbon to leave the soil carbon pool; therefore, it is essential to understand the soil microbial diversity and characteristics of soil microorganism in different vegetation types to better understand the income and expenditure of soil ecosystem carbon pools in this region. Many studies have shown that the characteristics of soil microorganisms are affected by numerous factors (Fisk *et al*., 2003). For example, vegetation cover can increase soil microbial biomass carbon and nitrogen content, and vegetation type can lead to selection within the microbial population (Bever, 1994; Zhu *et al*., 2007). Moreover, microbial responses to

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environmental factors vary among ecosystems, and significant differences in soil microbes under different vegetation types occur in areas under the same climatic conditions and soil types (Powlson *et al*., 1987). The ecological system in the Shanxi area is diverse, its topography is complex, and the soil microbial characteristics of different vegetation types in this area are not well understood.

To discuss the relationship between vegetation types and soil microbial biomass, in this study, plantations in the Shuozhou ecological reclamation area in Shanxi Province (*Populus simonii* forest (SP1), *Robinia pseudoacacia* plantation (SP2), *Pinus tabulaeformis* plantation (SP3), *Caragana Seabuckthorn* mixed forest (SP4), and natural grassland (CK)) were investigated. Surface soil (0–20 cm) from each of the five vegetation types was collected and the bacterial and fungal populations they contain were investigated. The number and diversity of soil microbes, as well as the characteristics of soil microbial biomass carbon and nitrogen are useful to the prediction and control of the direction, velocity and global carbon cycle of carbon flow in future ecosystems, as well as to providing a theoretical basis for understanding the income and expenditure of forest ecosystems in this area. These factors also can also provide a reference for different vegetation types and structural adjustment in the ecological restoration area investigated herein.

The objectives of this study were to understand the laws of soil microbial biomass and related ecological processes in the northern Shanxi region, the role and status of different vegetation types in regional and global carbon cycles, and to clarify the response of different vegetation types to regional and global climate change in the northern Shanxi region, as well as to predict the future global in the future. It is of great theoretical and practical significance to provide scientific basis for climate change trend.

Materials and Methods

Overview of Research Areas

Ping-Shuo Mining area is located in the Loess Plateau, Shanxi-Shaanxi-Mongolia bordering the Black Triangle and the northern Shuozhou territory at Longitude E 112°11′ to 113°30′, Latitude N 39°23′ to 39°37′. Winter and spring drought less rain, cold, windy, summer and autumn precipitation concentration, warm and cool less wind. The annual precipitation in the region is about 428.2 mm. The soil in this area is in the transition zone between chestnut and chestnut brown soil; the plant coverage is low and the plants are more tolerant to drought tolerance. After more than 20 years of reclamation, the ecological system of the mining area has been effectively restored, and it has formed a Joe irrigation grass structure with a multi-level plant layout composed primarily of *R. pseudoacacia*, *P. tabulaeformis*, *seabuckthorn* and Elm trees (Yuan *et al*., 2016).

Sample Ground Setting and Soil Sample Collection

Three standard strains were selected from each sample to collect soil samples based on the average morphological characteristics of the different vegetation types. At the lower wind direction of each standard plant, three sampling points were set at a distance of 60 cm from the center of the standard plant. The samples collected from the three points for each plant were uniformly mixed into one composite sample and then returned to the laboratory in a self-made bag. The relative indexes of soil nutrient content and biological properties were then determined (Table 1).

Determination of Soil Physical and Chemical Properties

The soil alkalinity was determined using the potential method, the soil organic matter was evaluated by the volumetric method, the total nitrogen was determined using the Kjeldahl method, the alkali nitrogen was evaluated by the alkali solution diffusion method, and the total phosphorus and available phosphorus were evaluated using molybdenum antimony to resist the colorimetric method (Kalembasa and Jenkinson, 1973; Lao, 1988). The soil organic carbon was measured by extracting 0.5 g soil samples with 5 mL of 1 N $K_2Cr_2O_7$ and 10 mL of concentrated H_2SO_4 at 150°C for 30 min, followed by titration of the extracts with standardized $FeSO_4$. Total N of soil was measured with the Kjeldahl method. Soil-determination of total phosphorus by alkali fusion-Mo-Sb Anti spectrophotometric method. Determination of available nitrogen in soil by alkali solution diffusion method and determination of available phosphorus in soil by molybdenum antimony colorimetric method.

Determination of Microbial Biomass in Soil

After adjusting the moisture content of fresh soil to 60%, it was placed in a jar and cultured for 7 D with fresh-keeping membrane at 25°C, after which the microbial biomass carbon (MBC) and nitrogen (MBN) were determined. Microbial biomass carbon nitrogen using chloroform fumigation-leaching reference (Lin *et al*., 1999), microbial biomass carbon (MBC) and nitrogen (MBN) were extracted by potassium sulfate, the organic carbon in the extraction solution was determined by the automatic organic carbon analyzer, the conversion coefficients were 0.38, the total nitrogen in the leaching solution was eliminated by Kjeldahl method, and the automatic nitrogen determination device was measured (Vance *et al*., 1987; Ocio and Brookes, 1990; Inubushi *et al.*, 1991).

Number of Soil Bacteria, Fungi and DGGE Analysis

The taxa of soil microorganisms were enumerated by the dilution coating plate method as previously described (Kucharski and Wyszkowska, 2004). Bacteria were cultured on beef paste peptone medium. Soil micro-biological diversity was evaluated by denaturing gradient gel electrophoresis (DGGE) as previously described (Muyzer *et al*., 1993; May *et al*., 2001) using the 16S rDNA V3 region and the 18S rDNA amplification products for bacteria and fungi, respectively. Samples were run in 8% polyacrylamide gel using 1×TAE electrophoresis buffer according to the manufacturer's instructions. The Quantity One 4.6.2 software was then used to analyze the obtained images and the Shannon-Weiner index was employed to indicate the diversity of soil microbial communities.

Data Processing

The measured data were statistically analyzed using Microsoft Excel 2003 and SPSS 19.0, while the Quantity One 4.6.2 software was used to analyze the obtained images, and the diversity of soil microbial communities was indicated by the Shannon-Wiener index.

Results

Soil Properties under Different Vegetation Types

In this study, all soils were slightly alkaline, with pH values ranging from 8.21 (*P. simonii* forest) to 8.47 (natural grassland). The pH among sample locations did not differ significantly. In the present study, the nutrient accumulation of the *P. simonii* forest was better than that of the *R. pseudoacacia* forest, oil pine and mixed shrub forest. When compared with the natural grassland, the soil nutrient content of the artificial forest was obviously higher, which shows the change trend of natural forest > artificial forest > natural grassland (Table 2). Thus, different vegetation types have different effects on soil quality, and natural forests lead to greater improvements in pH and soil nutrients than plantations and grasslands.

Soil Microbial Biomass Carbon and Nitrogen in Different Vegetation Types

In this study, the content of MBC was not the same as that of 20.60–58.84 mg/kg and the content of MBN was 3.43–9.80 mg/kg. Analysis of variance revealed that the MBC content of different vegetation types differed significantly, with that of the *P. simonii* forest being higher than that of the *R. pseudoacacia* plantation, *P. tabulaeformis* plantation and mixed shrub forest. There was no significant difference in the MBC of the *R. pseudoacacia* plantation and the *P. tabulaeformis* plantation, but both were significantly higher than those of the shrub forest, and that of the woodlands was higher than that of the grasslands. The MBN content of areas of different vegetation type differed significantly, with the grassland content being the lowest and that of the *P. simonii* forest being significantly higher than that of the *R. pseudoacacia* plantation, *P. tabulaeformis* plantation and mixed shrub forest. Additionally, the MBN content of the latter three areas mentioned above did not differ significantly (Table 3). Overall, the soil MBC and MBN showed that the soil microbial biomass of the natural forest was highest, followed by that of plantations, and then grasslands.

Number and Diversity of Soil Bacteria and Fungi In Different Vegetation Types

Under different vegetation types, the number of soil bacteria in the *P. simonii* forest was significantly higher than in the *R. pseudoacacia* plantation, *P. tabulaeformis* plantation, and shrub and grasslands; however, there was no significant difference between the Chinese pine forest and shrub forest. The number of fungi was also highest in the *P. simonii* forest, while there was no significant difference between the *P. tabulaeformis* plantation and the *R. pseudoacacia* plantation. Overall, the bacterial and fungal diversity occurred in the following order: *P. simonii*forest > *R. pseudoacacia* plantation >*P. tabulaeformis* plantation > shrub mixed forest > grassland.

The MBC and MBN were positively correlated with organic carbon, total nitrogen and available phosphorus in this study, which is significantly positively correlated with total phosphorus. Additionally, both the MBC and MBN were significantly positively correlated with alkali nitrogen. When compared with natural grassland, vegetation cover can significantly increase the number of soil bacteria and community diversity (Table 4), visible bacterial abundance and soil organic carbon, total nitrogen, total phosphorus, available phosphorus significant positive correlation (Table 5). The abundance of soil fungi in areas of different vegetation types also differed, was positively correlated with the total phosphorus, and was significantly positively correlated with organic carbon, total nitrogen and available phosphorus.

Discussion

All soils in this study were slightly alkaline, and the pH of grassland soils was significantly higher than that of areas of other vegetation type, indicating that the natural forest improved soil pH to a greater degree than the plantations. Soil organic carbon reserves and their activities are influenced by many natural factors, among which vegetation type is an important driving factor of soil organic carbon turnover (Tian *et al*., 2017). Different types of vegetation input varying types and amounts of soil organic matter; thus, affecting microbial activity. Moreover, vegetation input has the potential to change the soil function and properties of forest soil to become carbon sources or sinks. The litter input of different woodlands during the growth process provides the source material for nutrient replenishment and improvement of the soil system.

Table 1: A survey of the sample plots in research area

Vegetation pattern	Planting time	Geographical position
Natural grassland (CK)	-	N:39°29.474′E:112°18.367′
*Populussimonii*forest (SP1)	-	N: 39°30.842′E: 112°20.040′
*Robiniapseudoacacia*plantation (SP2)	1992	N: 39°27.709′E: 112°20.044′
Pinustabulaeformis plantation (SP3)	1992	N:39°27.367′E:112°19.400′
CaraganaSeabuckthorn mixed forest (SP4)	1992	N: 39°27.844′E: 112°19.940′

Table 2: Chemical properties of soil under different vegetation types

Sample	pH	SOC	TN	AN	TP	AP
CK	8.47±0.04 a	8.36±2.68 a	0.19±0.01 a	5.60±1.62 a	0.33±0.02 a	1.07±0.02 a
SP1	8.21±0.03 a	31.24±10.81 a	0.61±0.02 c	76.69±3.88 c	0.47±0.01 c	9.02±0.18 d
SP2	8.32±0.02 a	25.06±0.70 a	0.40±0.01 b	41.30±8.77 b	0.45±0.03 c	7.54±0.07 c
SP3	8.35±0.03 a	23.32±5.35 a	0.26±0.01 a	35.23±8.99 b	0.41±0.03 b	7.05±0.33 c
SP4	8.46±0.02 a	20.24±9.41 a	0.26±0.01 a	27.30±5.98 b	0.36±0.01 a	6.12±0.07 b

Note: different lowercase letters in the same column indicate a significant difference ($P<0.05$)

Table 3: Soil microbial biomass carbon and nitrogen under different vegetation types

Sample	MBC	MBN
CK	20.60±1.164 a	3.43±0.009 a
SP1	58.84±6.915 c	9.80±0.055 c
SP2	34.68±3.836 b	5.47±0.024 b
SP3	31.36±6.113 b	5.33±0.012 b
SP4	29.54±3.173 a	5.00±0.012 b

Note: different lowercase letters in the same column indicate a significant difference (P<0.05)

Table 4: The number and diversity of soil bacteria and fungi under different vegetation types

Sample	Number of bacteria	Number of fungi	Bacterial diversity	Fungal diversity
CK	4.75±0.66 a	9.90±0.39 a	0.61	0.51
SP1	16.33±1.56 d	21.25±1.75 c	1.46	1.55
SP2	10.80±0.15 c	16.29±0.24 b	1.35	1.10
SP3	7.90±0.06 b	15.68±0.43 b	0.92	0.96
SP4	7.77±0.12 b	12.33±1.48 a	0.79	0.72

Note: different lowercase letters in the same column indicate a significant difference (P<0.05)

Table 5: Correlation of soil microbial biomass and biomass carbon, nitrogen and soil chemical properties in areas of different vegetation type

Sample	pH	SOC	TN	AN	TP	AP
MBC	-0.258	0.721**	0.646**	0.782**	0.540*	0.756**
MBN	-0.314	0.893**	0.711**	0.443*	0.509*	0.835**
NB	-0.440	0.909**	0.585**	0.243	0.662**	0.720**
NF	-0.139	0.493*	0.536*	0.239	0.549**	0.504*

Note: * indicates a significant correlation (P< 0.05), ** indicates a very significant correlation (P < 0.01), MBC: microbial biomass C, MBN: microbial biomass nitrogen, NB: bacteria number, NF: fungi number

Specifically, leaf litter is transformed into humus by microorganisms, resulting in the accumulation of soil nutrients, and improved soil carbon and nitrogen reserves (Gil-Sotres *et al*., 2005). In this study, a general change in soil nutrient status of natural forest > artificial forest > grassland was observed. These findings indicate that the soil quality in this area is being improved at different rates, which indirectly affects soil microorganisms.

Soil microbial biomass is an important biological index that reflects soil quality, which can sensitively reflect changes in the soil properties (Waid, 1999). Soil microbial biomass is affected by many ecological factors (Fisk *et al*., 2003), including type of vegetation (Bever, 1994; Zhu *et al*., 2007). The soil microbial biomass and biomass of natural forests were higher than those of the plantations, while those of natural grasslands were smallest, similar to the results reported by Zhang (Zhang *et al*., 2003), Wang (Wang *et al*., 2004). These findings indicated that vegetation cover could increase the biomass carbon and nitrogen content of soil microorganisms. Vegetation growth increases vegetation coverage, reduces direct light and provides roots, which help maintain soil and water, thereby improving the microclimate environment (Bever, 1994; Zhu *et al*., 2007) and facilitating soil microbial growth and development.

Soil loss and erosion of shrub forestlands are relatively heavy in the study area, which is unfavorable to the regeneration and maintenance of soil (Tateno *et al*., 2007). Grassland soil in the face of direct sunlight, soil and water conservation capacity is relatively poor, and the loss of nutrients is serious; therefore, these systems are not conducive to microbial growth. From the results of soil microbial biomass, carbon and nitrogen, the vegetation coverage is larger than that of grassland; broadleaf species are larger than coniferous trees; natural forests are larger than plantations, which are similar to many scholars (Wang and Wang, 2006). In addition, the changes in MBC and MBN with organic carbon were basically consistent with those of total nitrogen and available phosphorus, indicating that the content of organic carbon, total nitrogen and available phosphorus may regulate soil microbial biomass. The vegetation types and quantity of underground biomass differed among land types, and the content of organic carbon, total nitrogen and available phosphorus in forestland improved remarkably, resulting in increased microbial growth and reproduction. Different vegetation types have different root densities and ranges, and the changes in soil water condition and nutrient supply status differed; therefore, the effects of organic carbon, total nitrogen and total phosphorus content differed (Bever, 1994; Zhu *et al*., 2007). The content of organic carbon, total nitrogen and available phosphorus all have important effects on soil microorganisms (Shen *et al*., 1989).

When compared with the grassland, the abundance and diversity of soil bacteria in woodland and shrub forests increased significantly, indicating that the vegetation mulch can improve the soil quality. This is because bacteria can produce extracellular metabolites such as polysaccharides, lipids and proteins, which help stabilize soil aggregates (Kirk *et al*., 2004) and promote soil quality. Fungi decompose cellulose, lignin, pectin and other refractory substances (Kabri and Koide, 2000). Moreover, the accumulation of fungal hyphae can improve the physical structure of soil (Kabri and koide, 2000), so it can also reflect the state of soil to some extent. The soil MBC/MBN ratio can be used to reflect the species composition of soil microorganisms. In the present study, the MBC/MBN was about 6, indicating that actinomycetes were relatively abundant, which may be related to the conditions of actinomyces growth and reproduction (Rogers and Tate, 2002). The most suitable pH for soil actinomycetes is slightly alkaline; which is likely to favor their growth in the region (Griffiths *et al*., 2011). Actinomycetes can decompose many organic compounds, including aromatic compounds, cellulose, lignin and other complex chemicals (Tang *et al*., 2012). Overall, actinomycetes, bacteria and fungi have a positive effect on soil that occurs through different biochemical effects.

Conclusion

Different vegetation types have different effects on soil microbial biomass. The soil nutrient status and microbial biomass and quantity showed a similar trend, with the highest levels being in the forest, followed by the plantation, and then the grassland. Soil MBC and MBN showed significant positive correlations with organic carbon, total nitrogen and available phosphorus, indicating that soil fertility in the area had an obvious effect on soil MBC and MBN, and that these could be used as biological indexes to judge soil fertility. Soil bacterial and fungal abundance were positively correlated with soil nutrient status in different vegetation types, and the effects of the *R. pseudoacacia* plantation on improving soil quality were better than those of *P. tabulaeformis*. Therefore, it is recommended that *R.pseudoacacia* be planted to improve the soil environment and microbial resources.

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