Relationships among Vegetation Type, Soil Microbial Community, and Biomass in the Loess Hilly Region (Postprint)

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Abstract

Taking five different vegetation types (artificial Robinia pseudoacacia forest, natural Platycladus orientalis forest, natural Quercus wutaishansea forest, shrubland, and bare land) in the Loess Hilly Region of Yan' an, Shaanxi as research objects, we analyzed the variation patterns of soil microbial biomass carbon and nitrogen contents, bacterial and fungal abundances, and their relationships with basic soil chemical properties. The results showed that: (1) Soil quality under the four vegetation types exhibited varying degrees of improvement compared with bare land, with the general trend being natural forests > artificial forests > bare land; (2) Soil microbial biomass carbon and nitrogen followed the general trend of being highest in natural forests, followed by artificial forests, and lowest in bare land, and were extremely significantly positively correlated with soil organic carbon (SOC), total nitrogen (TN), and available phosphorus (AP) ($P < 0.01$); (3) Bare land exhibited the lowest bacterial abundance, and fungal content in artificial Robinia pseudoacacia forest was significantly lower than that in natural Quercus wutaishansea forest. Bacterial abundance was significantly positively correlated with soil nutrient status ($P < 0.05$), whereas fungi showed no significant correlation with soil nutrients, being only negatively correlated with soil pH. These results indicate that in this study area, vegetation type and soil quality exert varying degrees of influence on microbial resources.

Full Text

Preamble

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Relationship among vegetation types and soil microbial biomass in the Loess Hilly region of China

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Abstract

To remedy adverse impacts on ecological environments, vegetation restoration has been completed on the Loess Hilly region of China, thus yielding changes in vegetation types, and secondary forests and exotic vegetation have reclaimed most of this area. Vegetation type plays an important role in soil quality, and similar land-use types usually possess similar soil properties and, most likely, similar microbial communities. To assess the effects of vegetation type on soil development, we measured the basic physicochemical properties, microbial biomass carbon (MBC) and nitrogen (MBN), and the number of bacteria and fungi of soils from areas with five different vegetation types, including a secondary natural oak forest (*Quercus liaotungensis*), a non-typical natural forest of oriental arborvitae (*Platycladus orientalis*), a natural shrub land, a planted forest of black locust (*Robinia pseudoacacia*), and a plot of unforested bare land.

We found that (1) soil properties improved with the presence of vegetation, compared with the abandoned bare land, and that there were differences in the physicochemical properties of soil from the different land-use types. Soils from the bare land and black locust forest yielded higher pH values than the natural forests, and the overall trend of soil quality was: natural forest > plantation > bare land. This indicated that vegetation coverage, especially natural types, has a beneficial effect on soil nutrient conditions and soil pH. (2) Similar to the trend of the other soil parameters, both MBC and MBN were highest in the oak forest and shrub land and lowest in the bare land and black locust forest. These two indices (MBC and MBN) were also strongly correlated with specific soil properties (soil organic carbon, total nitrogen, and phosphorus, $r = 0.736$, $r = 0.725$, and $r = 0.775$; $P < 0.001$), suggesting that vegetation type and soil properties influence microbial biomass. Thus, microbial biomass can

be used to assess soil trophic status and which lower in oligotrophic soils on the Loess Plateau of China. In our study area, the soil was alkaline (pH 8.18–8.48), and the MBC/MBN ratio was ~6, indicating that actinomycetes were dominant, possibly facilitated by the alkaline soil conditions. (3) Bare land harbored the lowest abundance of bacteria, and the abundance of fungi in the black locust was lower than that of the oak forest. The abundance of bacteria exhibited a strong relationship with specific soil properties and was positively correlated with C/N ratio ($r = 0.754$, $P < 0.001$), soil organic carbon ($r = 0.636$, $P < 0.05$), total nitrogen ($P < 0.05$), and phosphorus ($r = 0.775$, $P < 0.05$), and negatively correlated with pH ($r = 0.761$, $P < 0.001$). In contrast, the abundance of fungi was only correlated with soil pH, thus confirming previous reports that bacteria and fungi respond to different environmental factors. This discrepancy may result from the different functions of bacteria and fungi. Fungi are primarily responsible for the decomposition of recalcitrant soil organic matter in forest soils. Thus, the overall trend of soil characteristic was: natural forest > plantation > bare land, indicating that the recovery of soil quality differed among plots with different vegetation types, and both soil microorganisms and soil microbial biomass, to a certain extent, can be used as an important index of soil fertility.

Keywords: vegetation type; soil bacteria; soil fungi; soil properties; the Loess Hilly region

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1. Study Area Overview

The study area is located in the Gonglu Mountain forestland of Liulin Town, Baota District, Yan'an City, Shaanxi Province (36°25.40 N, 109°31.53 E, 1353 m a.s.l.), situated in the transitional zone between forest and forest-steppe regions at the northern edge of the forest distribution area. The landform belongs to the loess plateau hilly-gully region. According to meteorological data from Yan' an (1971–2010), the area has a temperate semi-arid climate with an average annual precipitation of 504.7 mm, with 60–70% concentrated in July–September, and an average annual temperature of 10.1°C. The soil type is primarily loess soil (pH 7.8–8.5). Main vegetation types include natural forests of *Quercus liaotungensis*, *Platycladus orientalis*, *Pinus tabuliformis*, and *Larix principis-rupprechtii*, as well as natural shrublands such as *Caragana microphylla*, *Cotoneaster multiflorus*, *Artemisia gmelinii*, *Syringa oblata*, *Bothriochloa ischaemum*, and *Betula platyphylla*, and planted forests of *Robinia pseudoacacia* and *Populus davidiana*.

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2. Plot Setup and Soil Sampling

In August 2015, five different vegetation types were selected as experimental plots in the Gonglu Mountain forest area: (1) planted black locust forest (PL), (2) natural oriental arborvitae forest (NA), (3) natural oak forest (NO), (4) natural shrub land (NS), and (5) abandoned bare land (BL) as the control plot. All selected forestlands were mature stands with tree heights of 8–12 m and canopy density of 30–40%. The plots were spatially adjacent within a few hundred meters and shared similar climatic conditions. Soils were collected using a diagonal five-point sampling method from the 0–20 cm surface layer. One portion was stored at 4°C for microbial analysis, while another was air-dried and sealed for preservation.

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3. Determination of Soil Microbial Biomass

Fresh soil samples were adjusted to 50% of saturated water-holding capacity, placed in wide-mouth bottles, sealed with plastic wrap, and incubated at 25°C for 7 days in the dark before microbial biomass carbon and nitrogen determination. Microbial biomass carbon (MBC) and nitrogen (MBN) were measured using the chloroform fumigation-extraction method. Fumigated and non-fumigated soils were extracted with 0.5 M K SO . Organic carbon in the extracts was analyzed using a Tekmar-Dohrmann Apollo 9000 TOC Combustion Analyzer. Total nitrogen in the extracts was determined using a Kjeldahl automatic nitrogen analyzer. The conversion factor (K_EC) was 0.45.

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4. Determination of Soil Chemical Properties

Air-dried and sieved soil samples were analyzed using conventional methods for soil organic carbon (SOC), total nitrogen (TN), pH, available phosphorus (AP), and available potassium (AK).

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5. Quantification of Bacteria and Fungi by qRT-PCR

Soil microbial DNA was extracted using the PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) and detected by 1% agarose gel electrophoresis. The DNA was recovered using a gel extraction kit (TianGen Biotech Co., Ltd.). Quantitative PCR primers for bacteria and fungi were Eub338/Eub518 and nu-SSU-1196F/nu-SSU-1536R, respectively. The PCR reaction system contained 12 L of SYBR Green, 1.0 L each of forward and reverse primers, 2.0 L of DNA template, and 4 L of ddH O in a total volume of 20 L. The PCR amplification program was: 95°C for 2 min, followed by 45 cycles of 95°C for 10 s, 55°C for 10 s, and 72°C for 10 s, with a final extension at 72°C for 1 min.

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6. Data Processing

Data were organized and subjected to statistical analysis. Differences among vegetation types were analyzed using One-Way ANOVA, and correlations among factors were evaluated using Pearson correlation coefficients. All data are presented as means of three replicates, with least significant difference (LSD) used for multiple comparisons.

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1. Soil Chemical Properties Under Different Vegetation Types

All soils in this study were slightly alkaline. The pH values of bare land and black locust forest were significantly higher than those of natural forests (8.55 and 8.12 vs. 8.18–8.27, $P < 0.05$), indicating that natural forests have a certain ameliorating effect on soil pH. Numerous studies have shown that similar vegetation types usually possess similar soil physicochemical properties. In this study, natural forests were superior to planted black locust in soil nutrient accumulation. Compared with forest plots, bare land had relatively poor soil nutrition, with significantly lower SOC, TN, AP, AK, and C/N ratio ($P < 0.05$). The SOC and TN contents of black locust forest were significantly lower than those of other natural forests ($P < 0.05$). The overall trend of soil nutrition was: natural forest and shrub land > black locust forest > bare land, demonstrating that different vegetation types have varying effects on soil quality, with natural forests and shrublands being more effective than black locust plantation and bare land in improving soil structure and nutrients.

Table 1 Soil chemical properties of different land use types (mean±SE)

Vegetation type	Organic C (%)	Total N (%)	Available K (mg/kg)	Available P (mg/kg)	pH	Soil C/N ratio
Planted Black Locust (PL)	1.08(0.09) c	0.11(0.00) c	190.13(10.67) a	2.12(0.07) d	8.45(0.01) a	9.54(0.50) b
Natural Oriental Arborvitae (NA)	1.88(0.24) b	0.17(0.02) b	139.73(33.94) b	2.10(0.17) d	8.18(0.06) b	11.08(0.15) a
Natural Oak forest (NO)	2.86(0.42) a	0.26(0.02) a	142.88(7.76) bc	3.31(0.35) a	8.23(0.10) b	11.07(0.93) a

Vegetation type	Organic C (%)	Total N (%)	Available K (mg/kg)	Available P (mg/kg)	pH	Soil C/N ratio
Natural Shrub Land (NS)	2.26(0.27) ab	0.23(0.02) a	174.17(14.45) ac	2.46(0.12) b	8.27([illegible]) b	[illegible](0.23) b
Bare Land (BL)	0.70(0.09) c	0.08(0.01) c	76.22(7.11) d	1.65(0.05) c	8.48([illegible]) a	[illegible](0.59) b

Note: Different lowercase letters in the same column indicate significant differences (P < 0.05). PL: Planted Black Locust, NA: Natural Oriental Arborvitae, NO: Natural Oak forest, NS: Natural Shrub Land, BL: Bare Land; SOC: Soil organic carbon, TN: Total nitrogen, AK: Available potassium, AP: Available phosphorus.

2. Soil Microbial Biomass Carbon, Nitrogen and Their Ratios Under Different Vegetation Types

Microbial biomass carbon (MBC) and nitrogen (MBN) contents varied significantly among vegetation types, ranging from 139–98 mg/kg and 21–13 mg/kg, respectively. MBC and MBN contents in natural forests (NA, NO) and shrub land (NS) were significantly higher than in bare land (BL) and black locust forest (PL) ($P < 0.05$), being 2.0–2.9 times and 2.1–2.9 times higher than bare land, respectively. The contents in natural forests and shrub land were also significantly higher than in black locust forest ($P < 0.05$), while no significant differences were observed between natural forests and shrub land. The overall trend of soil microbial biomass carbon and nitrogen was: natural forest and shrub land > plantation > bare land, consistent with previous studies.

Variance analysis showed that MBC and MBN were extremely significantly positively correlated with SOC, TN, and AP ($P < 0.01$), significantly positively correlated with AK and C/N ratio ($P < 0.05$), but not significantly correlated with pH. This indicates that soil microbial biomass is closely related to soil nutrients and vegetation type. The MBC/SOC ratio ranged from 4.05%–6.93% across different vegetation types, while the MBN/TN ratio showed no significant differences among vegetation types.

[Figure 1: see original paper] **Fig. 1** The microbial biomass carbon and nitrogen under different vegetation types

Table 2 Correlation among soil microbial biomass carbon, nitrogen and soil chemical properties

Microbial biomass	SOC	TN	AP	AK	pH	C/N ratio
MBC	0.725**	0.661**	0.736**	0.679*	-0.304	0.393
MBN	0.775**	0.689**	0.432	-0.371	0.189	0.432

Note: indicates significant correlation (P < 0.05), ** indicates extremely significant correlation (P < 0.01). MBC: microbial biomass carbon, MBN: microbial biomass nitrogen.*

Table 3 Soil microbial biomass ratio of different land use types (mean ± SE)

Vegetation type	MBC/MBN	MBC/SOC (%)	MBN/TN (%)
Planted Black Locust (PL)	5.91±2.35	6.84±2.86 b	11.69±5.13
Natural Oriental Arborvitae (NA)	6.62±1.16	5.17±0.54 a	8.8±1.35
Natural Oak forest (NO)	6.34±1.75	4.05±1.08 a	7.17±1.83
Natural Shrub Land (NS)	6.46±0.80	6.24±1.47 ab	9.42±1.16
Bare Land (BL)	6.79±2.24	6.93±1.53 b	9.39±0.35

Note: Different letters in the same column indicate significant differences (P < 0.05).

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3. Quantities of Soil Bacteria and Fungi Under Different Vegetation Types

Compared with bare land, vegetation cover significantly increased soil bacterial genomes ($P < 0.05$). Forest soils contained (7.05–9.46) × 10 copies/g, while bare land contained only 2.9 × 10 copies/g. Bacterial abundance was significantly lower in bare land than in forest and shrub land ($P < 0.05$), but no significant differences were observed among different forest types. Bacterial abundance was significantly positively correlated with SOC, TN, and AP ($P < 0.05$), extremely significantly positively correlated with C/N ratio ($P < 0.01$), and extremely significantly negatively correlated with pH ($P < 0.01$).

Fungal abundance in black locust forest was significantly lower than in natural oak forest ($P < 0.05$), but showed no significant differences compared with bare land, natural oriental arborvitae forest, and natural shrub land. Fungal abundance was only significantly correlated with soil pH ($P < 0.05$), showing a significant negative correlation, but did not reach significant correlation with soil organic carbon, available potassium, or C/N ratio.

[Figure 2: see original paper] **Fig. 2** qRT-PCR analysis of bacteria and fungi in different forestry soil

Table 4 Correlation among bacterial number, fungal number and soil properties

Microbial type	SOC	TN	AP	AK	pH	C/N ratio
Bacteria (NB)	0.636*	0.611*	0.518*	0.246	-0.761**	0.754**
Fungi (NF)	0.404	0.354	0.429	-0.304	-0.546*	0.429

Note: indicates significant correlation (P < 0.05), ** indicates extremely significant correlation (P < 0.01). NB: number of bacteria, NF: number of fungi.*

3. Discussion

All soils in this study were slightly alkaline. The pH values of bare land and black locust forest were significantly higher than those of natural forests ($P < 0.05$), indicating that natural forests have an ameliorating effect on soil pH. Vegetation type is an important driver of soil organic carbon turnover, while black locust forest shows weaker improvement capacity. Soil organic carbon storage and its activity are influenced by many natural factors. Different vegetation types input different types and quantities of organic matter into soil, thereby affecting soil microbial activity and altering soil functions and properties that determine whether forest soils become carbon sources or sinks.

In this study, natural forests had significantly higher litter input during growth than black locust plantation and bare land. Moreover, natural forests had well-developed root systems, and natural shrub lands had diverse vegetation species, all providing abundant material sources for soil nutrient replenishment and improvement. These materials formed humus after microbial decomposition, which was more conducive to soil nutrient accumulation, consistent with previous research findings. The overall trend of soil nutrition in this study was natural forest and shrub land > plantation > bare land, indicating that soil quality in this region is improving at different rates.

Soil microbial biomass is an important biological indicator that reflects soil quality and can sensitively indicate changes in soil properties. Microbial biomass is influenced by many ecological factors, among which different vegetation types mainly affect soil microbes through environmental improvement and energy material input. The smallest soil microbial biomass in black locust plantation and bare land indicates that vegetation cover can increase soil microbial biomass carbon and nitrogen content. Natural forest soil microbial quantities and contents were higher than those of plantations, especially natural oak forest, which accumulated soil microbial biomass carbon and nitrogen more intensively than black locust plantation and bare land, similar to findings by Zhang et al. Forest vegetation growth increases vegetation coverage, reduces direct sunlight, and the extensive root systems effectively maintain soil moisture, thereby improving the soil microclimate environment and favoring microbial growth and development. However, black locust plantation has problems such as excessive water consumption and susceptibility to soil erosion, which are unfavorable for soil regeneration and maintenance. Bare land soils are exposed to sunlight with harsh environmental conditions, severe nutrient loss, and poor soil quality.

The trend of MBC and MBN was consistent with changes in SOC and TN, showing significant positive correlations ($P < 0.05$), indicating that soil resources may be regulatory factors for soil microbial biomass. Since different vegetation types input different types and quantities of underground biomass, organic carbon content, and litter quality, natural forests and shrub lands had higher SOC and TN contents with fewer growth limitations for microbes, which was conducive to their growth and reproduction. Different vegetation root densities also alter soil moisture conditions and nutrient supply status, affecting microbial growth. Some reports suggest soil microbial biomass is more easily limited by carbon and nitrogen contents in soil, and this study also showed significant correlations with SOC and TN, similar to Wang and Bing' s research results, indicating that both contents play important roles for soil microbes in this region.

Compared with bare land, forest and shrub land soils showed significantly increased bacterial genomic content ($P < 0.05$), possibly because bare land has poor soil nutrition while forest soils have high nutrient content. Bacterial abundance was significantly positively correlated with SOC, TN, and AP, and extremely significantly negatively correlated with pH ($P < 0.01$), indicating that soil quality has improved after returning farmland to forest. Soil pH has a decisive role in structuring soil bacterial communities, and lower pH is conducive to microbial growth. Bacteria can produce extracellular metabolites such as lipids and proteins that cement soil particles, stabilizing aggregates and resulting in higher soil quality in forest land than bare land.

However, soil fungi did not show significant correlation with soil nutrient content, which may be related to their functions and characteristics, such as decomposing recalcitrant substances like cellulose, lignin, and pectin. Fungal hyphal accumulation can improve soil physical structure and reflect soil fertility, but fungal abundance was only significantly correlated with pH. The harsh environment of excessive water consumption in black locust plantation and bare land makes soils prone to drought, resulting in shorter optimal metabolic times for microbes, thus requiring higher MBC/SOC to maintain populations.

The MBC/SOC ratio can reflect microbial community composition. The bacterial ratio is generally 2%–4%, while actinomycetes require 4%–6%. The MBC/MBN ratio in this study was ~6, indicating relatively abundant actinomycetes, possibly because actinomycetes thrive in microalkaline conditions, and the study area' s alkaline soil (pH 8.18–8.48) provides a suitable environment.

Actinomycetes can decompose many organic compounds, including aromatic compounds and lignin, playing a positive role in natural material cycling and promoting soil aggregate formation.

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4. Conclusion

Different vegetation types create different soil nutrient conditions that substantially influence soil microbial biomass. In this study area, soil nutrients and microbial biomass showed consistent trends: natural forest and shrub land were highest, plantation intermediate, and bare land lowest. MBC and MBN exhibited significant positive correlations with SOC and TN, indicating that soil fertility has a primary limiting effect on soil microbes in this region. Therefore, MBC and MBN can serve as biological indicators of soil fertility status.

Bacterial abundance was significantly positively correlated with soil nutrient status across different vegetation types, while fungal abundance showed no significant correlation, suggesting that the alkaline soil environment in this region is unfavorable for fungal survival. Native species showed advantages in improving soil quality, thus we recommend selecting native tree species to improve soil environment and microbial resources.

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Note: Figure translations are in progress. See original paper for figures.