Effects of slope aspect on soil nitrogen and microbial properties in the Chinese Loess region

Yi-Mei Huang, a,b,*, Dong Liu, b Shao-Shan An, a

a State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, China
b Key Laboratory of Plant Nutrition and the Agri-environment in Northwest China, Ministry of Agriculture, College of Resources and Environment, Northwest A&F University, Yangling 712100, China

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 13 September 2014
Accepted 22 September 2014
Available online 5 November 2014

Key words:
Caragana korshinskii
Slope aspect
Soil microbial activity
Nitrogen
PLFA

ABSTRACT

Revegetation is a very important way to improve soil quality and fertility in the hilly–gully region of the Loess Plateau, where serious soil nutrient loss and degradation due to water loss and soil erosion were experienced. The creation of an artificial forest is an important measure to restore the local vegetation. Caragana korshinskii (CK) is an ideal tree species in the recovery of vegetation in the Loess hilly regions because it has a strong compatibility and reproduction ability and the ability to artificially regenerate vegetatively. The slope aspects of the Loess Plateau influenced its community and asexual reproduction characteristics. Determining the relationship between the soil physicochemical properties and the soil microbial characteristics under different slope aspects can provide useful information for vegetation restoration in the Loess Plateau. This study aimed to investigate the effects of different slope aspects on soil nitrogen, soil microbial activity and community structures in a Loess hilly–gully region. According to the levels of sun exposure, four sample sites (about 35-year-old CK plantation) were selected from four different slope aspects, including sunny, shady, half-shady and hilltop slopes in the Zhifanggou watershed of Ansai (Shaanxi Province). The soil samples were collected at three different depths (0–10 cm, 10–30 cm and 30–60 cm) at each site and analyzed to determine the nitrogen (N) content, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), basal respiration (BR) and level of microbial phospholipid fatty acids (PLFAs). The slope aspect affected (p < 0.05) the six nitrogen forms (nitrate-N, organic N, mineralizable N, MBN, soluble organic nitrogen (SON) and ammonium-N) and the proportion of each form of nitrogen. Five of the nitrogen forms (with the exception of SON), the basal respiration (BR), the microbial quotient (MBC/total organic C) and the relative abundance of anaerobe on the shady/half-shady slopes were significantly (p < 0.05) greater than those on the sunny or hilltop slopes. The relative abundance of Gram-negative (G−) bacteria and aerobes was the greatest on the shady slope. On the hilltop, the content of available P and MBP, as well as the proportion of SON and ammonium-N, was the greatest, whereas the total PLFA was the lowest. The canonical variation mainly reflected the relationship between the mineralizable N and the MBC, which were the most sensitive indicators that were related to slope changes.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The Loess hilly–gully area is one of the most fragile zones in the Chinese Loess Plateau region, but it is also a key area of vegetation construction in the Loess Plateau. Due to unreasonable slope cultivation and low natural vegetation cover, the Loess Plateau is still considered to be one of the most severely eroded areas in the world (Kimura et al., 2004; Wang et al., 2006). Wind and water erosion has occurred in as much as 80% of the region. Accelerated erosion has been a constant threat to the livelihoods of rural families and a major problem for the ecosystem and environment (van den Elsen et al., 2003). In an attempt to control the soil and water losses and to improve the ecological environment of the area, the Chinese Central Government has enacted a policy entitled “Shift from Cropland to Forest or Grassland” for the restoration of vegetation since the late 1990s. Vegetation has become a tool in restoring the slope’s physical condition and stability throughout the succession process (Osman and Barakbah, 2011). Caragana korshinskii (CK), a legume shrub, has strong reproductive and self-renewal ability and is a valuable shrub species in the hilly–gully zone of northern China (Niu et al., 2003). CK not only effectively improves the soil structure and nutrition but also significantly conserves the soil and water resources (An and Huang, 2009; Zhang et al., 2010). The environmental heterogeneity arising from slope changes in soil 

http://dx.doi.org/10.1016/j.catena.2014.09.010
0341-8162/© 2014 Elsevier B.V. All rights reserved.
differences greatly affects the growth, population density and water use efficiency of CK (Wang and Wang, 2010).

The soil microclimate conditions (e.g., soil temperature and moisture) on different slope types can influence the soil development and processing (Egli et al., 2006) and can affect the stability of the soil aggregate (Schoorl et al., 2004). The properties of soil are closely linked to the region from which the soil is derived (Tsui et al., 2004). In addition, slope differences can directly affect the environmental conditions of the soil, including its temperature, light exposure and moisture levels (Bennie et al., 2008; Sidari et al., 2008). The slope angle and aspect, as well as plant growth, have significant effects on the bulk density, porosity, the availability of nutrients and hydraulic conductivity of the associated soil (Wang et al., 2008). The slope aspect also has significant effects on the spatial variability of some soil physicochemical properties (Bennie et al., 2008; Sidari et al., 2008; Salehi et al., 2011) and on the productivity and species composition of hilly grasslands (Gong et al., 2008). The slope aspect and vegetation also affect the organic carbon and total nitrogen stocks in the soil (Yimer et al., 2006).

Nitrogen is one of the most important soil nutrients, and it affects the plant growth and viability, which can affect the plant water use efficiency (Sardans et al., 2008). Therefore, research into the influence of the slope aspect on the soil nitrogen is of great importance to understanding the growth and population succession of CK. Previous studies have indicated that the soil nutrient cycling is driven by microbial activity (Burke et al., 2011). The soil microbial biomass, microbial quotient and microbial physiological indices (e.g., F/B, G+/G−) are all sensitive bio-indicators that can be used to estimate the soil quality (Harris, 2003) and health (Zhou and Ding, 2007).

As an index of the total soil microbial activity (Insam, 1990), soil respiration quotient (qCO2) has a great potential for improving our understanding of the development of living microbial communities in the ecosystem (Anderson and Domsch, 1990, 2010; Bastida et al., 2008). Fungi/bacteria ratio can be used as an important index that reflects physiological state of the microbial communities especially associated with organic matter transformation and storage (Bailey et al., 2002), as well as ecosystem's self-control and buffering capacity (Bardgett and McAlister, 1999; Bossio et al., 1998). To date, most studies have focused on the changes in the soil microorganisms during ecological restoration (Zhang et al., 2012; Xue et al., 2008) or the transfer of soil inorganic N on the Loess slope (Xue et al., 2013; Zhang et al., 2007). However, little is known about the different forms of N or the relationship among the soil N, the microbial biomass and the microbial diversity on different slopes that are populated by CK. The objectives of this study were, therefore, 1) to investigate the different forms of soil N and their relative abundance, as well as the microbial activity and community structures on different slopes; 2) to explore the relationship between N and microorganisms; and 3) combined with canonical correlation analysis, to identify the most significant soil N and microbial parameters associating with slope aspect change. The results from this study may offer theoretical guidance for CK reproduction and for the ecological restoration of the loess plateau.

2. Materials and methods

2.1. Study area description

The study area is located in the Zhifanggou watershed, which belongs to the Ansai Research Station of Soil and Water Conservation of Chinese Academy of Science. It is located in the northern Shaanxi Province, China (108°5′–109°26′E, 36°30′–37°39′N), with an elevation of 1010–1400 m. The mean annual temperature of the area is 8.8 °C, the mean annual precipitation is 513 mm, the aridity index is 1.48, the annual sunshine hours are 2300–2400 h, the total solar radiation is 492.95 kJ cm−2 and the frost-free period is approximately 160 days. The climate is a typical temperate continental semi-arid monsoon climate. The region’s soil is classified as a typical loess soil and is susceptible to erosion. The vegetation type is of the forest-grassland belt variety, which represents a transitional environment between the warm, temperate deciduous broadleaf forest and the dry grassland belt (Xu et al., 2009).

The Zhifanggou Watershed is located in the second sub-region of the gullied rolling Loess area. The valley is a secondary forest with an area of 8.27 km². In 1938, the entire basin contained 24 households, 94 people, 13.4% of the index of cultivation, a forest coverage rate of 76.5%, and a per unit area yield of grain of 1449 kg/km². Due to the great increase in the population demand for food and wood, the vegetation was greatly destroyed, and the capacity of agricultural production continued to decline until 1958, when the index of cultivation was 51.5%, only fruit trees and shrubs were left in a 3.5 hm² area and the per unit area yield of grain was 415.5 kg/km². In the early 1970s, the comprehensive experiment of small watershed management was performed in this area. Vegetation restoration measures began, and artificial forests and artificial grasslands were created according to the natural environment in the watershed. In 1986, this valley was listed as the comprehensive restoration management area of the Loess Plateau experimental demonstration zone, and it went into a sustained restoration and reconstruction period. In 2008, this valley contained 124 households, 562 people, a vegetation coverage rate of 56.53% and a per unit area yield of grain of 4289 kg/km² (Wang et al., 2009). These measures gradually restored the damaged vegetation. Among the construction plantations of the region, C. korshinskii is the main afforestation tree species. This forest in this study was 35 years old, and the soil preparation was consistent with other CK plantations within the watershed.

2.2. Soil sampling design and sampling

In July of 2011, four different slope research sites with an area of 100 m × 100 m were selected based on their variations in sun exposure and were used as four experimental treatments termed sunny, shady, half-shady and hilltop. The geographical and undergrowth characteristics of the investigated sites are given in Table 1.

In each site, two subplots with a “S” shape random-sampling strategy, with an area of 10 m × 10 m of approximately 35 years of artificial C. korshinskii (CK) shrub land (with Artemisia sacrorum and Lespedeza dahurica as major vegetative species) were selected for the soil sampling and analysis. Two plots in each site were used as two replicates for each treatment. Six soil sampling points were selected in a zigzag sampling layout from each plot, and in each point, three samples were taken from depths of 0–10 cm, 10–30 cm and 30–60 cm separately by soil auger or shovel and then hammering the corer to the desired soil depth, the corer was removed, and the drying soil was weighed. The fresh soil samples were then mixed to form a pooled sample of approximately 1 kg from each depth of each plot. At the same time, six cutting ring soil samples were collected in each plot to determine the soil bulk density. The fresh soil samples were then sealed in plastic bags and transported to the laboratory on ice boxes. Parts of each sample were sieved (<2 mm) to remove large rocks, stones and macro-fauna and were air dried to measure the soil physical and chemical properties. Parts of the samples were stored at −20 °C for the phospholipid fatty acid analysis or at 4 °C for the microbial biomass and basal respiration analyses.

2.3. Soil sample analysis method

2.3.1. Physical and chemical properties of the soil

The soil water content was measured gravimetrically by weighing the soil sample, drying it in an oven at 105 °C for 24 h and then re-weighing the sample. The bulk density was determined using the cutting ring method. After removing the top mineral soil with an auger or shovel and then hammering the corer to the desired soil depth, the corer was removed, and the drying soil was weighed. The volume of the corer and the bulk density were calculated by dividing the drying soil weight by the volume of the soil. The soil pH and electric conductivity were determined in a 1:2.5 soil:water slurry using a Delta
Table 1
The general geographical and vegetative features of the investigated sites.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Altitude (m)</th>
<th>Degree</th>
<th>Latitude (N)</th>
<th>Longitude (E)</th>
<th>Light illumination of community (lx)</th>
<th>Undergrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hilltop</td>
<td>1188</td>
<td>12</td>
<td>36°45.588′</td>
<td>109°15.495′</td>
<td>163,125</td>
<td>Artemisia sacrorum</td>
</tr>
<tr>
<td>Sunny slope</td>
<td>1276</td>
<td>11</td>
<td>36°45.783′</td>
<td>109°15.953′</td>
<td>96,423</td>
<td>Lespedeza dahurica</td>
</tr>
<tr>
<td>Shady slope</td>
<td>1378</td>
<td>14</td>
<td>36°43.628′</td>
<td>109°14.407′</td>
<td>47,543</td>
<td>Arctium capillare</td>
</tr>
<tr>
<td>Half-shady slope</td>
<td>1232</td>
<td>13</td>
<td>36°45.116′</td>
<td>109°14.998′</td>
<td>58,924</td>
<td>Lespedeza dahurica</td>
</tr>
</tbody>
</table>

* The data come from Bao et al. (2010).

320 pH meter (Mettler-Toledo Instruments (Shanghai, China) Co., Ltd.). The soil organic carbon was determined via wet oxidation using dichromate in an acid medium followed by the FeSO4 titration method (Bao, 2007). The soil-available P was measured by NaHCO3 extraction and colorimetry. The total N was measured by Kjeldahl digestion and distillation azotometry. The nitrate-N and ammonium-N were measured by flow injection analysis. The mineralizable nitrogen was determined by the aerobic culture method (Nu, 1999).

2.3.2. **Soil basal respiration (BR) and microbial biomass**

The soil basal respiration (BR) was determined by measuring the CO2 production from 50 g of fresh field soils. The homogenized soil samples were first placed in a 500-mL glass jar in which the soil water content was conditioned to 50% of the field capacity. Then, an absorption bottle that was filled with 4 mL of 0.1 mol/L NaOH solution was carefully hung upon the soil in the glass jar, and the glass jar was sealed with a plastic film. The jar was then incubated at 25 ± 1 °C for 24 h. The evolved CO2 was trapped by NaOH and determined by the titration of 0.05 mol/L standard HCl.

The microbial metabolic quotient (qCO2) was calculated by dividing the basal respiration (mg CO2 d−1) per kg by the microbial biomass C (Anderson and Domsch, 1990).

The soil microbial biomass carbon (MBC) and the microbial biomass nitrogen (MBN) were measured using the chloroform fumigation–extraction method (Ross, 1990). The soil samples that were subjected to the fumigation and non-fumigation treatments were extracted in 0.5 M K2SO4 at a ratio of 1:4. The amount of K2SO4-extracted C was determined using a total organic carbon analyzer (Phoenix 8000). A KEC factor of 0.45 (Wu and Brookes, 2005) was used for converting the extractable-C to the microbial biomass C. The amount of K2SO4-extracted total N was analyzed using a modified method of potassium peroxydisulfate digestion-ultraviolet spectrophotometry analysis (Zhou and Li, 2005). A KEP factor of 0.45 (Wu and Brookes, 2005) was used for converting the extractable-N to the microbial biomass N. The soil microbial biomass phosphorous (MBP) was determined using the chloroform fumigation–extraction inorganic phosphorous analysis (FE-Pi) method (Brookes et al., 1982). The soil samples that were subjected to the fumigation and non-fumigation treatments were extracted using 0.5 M NaHCO3 extract at a ratio of 1:20. The amount of extracted phosphate was analyzed at a ratio of 1:2:0.8. The lipids were separated into neutral lipids, glycolipids and phospholipids on a silica gel column. The phospholipids were subjected to a mild alkaline methanolysis. The samples were analyzed using gas chromatography coupled with mass spectrometry (GC–MS) by Trace GC Ultra/DSQ II (Thermo Fisher Scientific, Waltham, USA) with a BP-5MS column of 30 m × 0.25 mm × 0.25 μm. Splitless injection was employed. The temperature specifications were set as follows: injector temperature 280 °C; detector temperature 260 °C; and temperature program 70 °C for 1 min, then 3 °C/min to 280 °C maintained for 15 min. The carrier gas used for all of the analyses was helium. Methyl non-adcanoeato fatty acid (19:0) was added as an internal standard to quantify the peak areas. The individual PLFAs were identified using fatty acid methyl ester standard compounds (Bacterial Acid Methyl Esters Mix; Supelco, nr. 47080-U) as qualitative standards. The sum of the fatty acids that were indicative of Gram-positive bacteria (G+), Gram-negative bacteria (G−) and actinomyces, plus the four additional fatty acids that were listed, was used to measure the total bacterial biomass. The ratio of the fungal/bacterial (F/B) PLFAs was used as a biomass index to indicate changes in the fungal-to-bacterial biomass ratio (Bååth, 2003). The physiological state of the microbial communities was determined using the ratio of saturated fatty acids to monosaturated fatty acids (S/M) and the ratio of cyclopropyl PLFAs to monoenoic precursors ([cy17:0 + cy19:0]/(16:1ω7 + 18:1ω7)) (Bossio et al., 1998; Kieft et al., 1997). The total PLFA, expressed in nmol g−1 fresh soil, was used as a measure of the microbial biomass. To characterize the community structure, the PLFAs were subdivided into various functional groups. The classification of fatty acids is shown in Table 2.

Table 2
The characterization of the microbial phospholipid fatty acids.

<table>
<thead>
<tr>
<th>Microbial group</th>
<th>Specific PLFA markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonspecific bacteria</td>
<td>14:0, a14:0, i14:0, 15:0, i15:0, a15:0, 16:0, i16:0, a16:0, 17:0, a17:0, 17:0, 16:1ω7t, 16:1ω7c, 17:0, 19:0</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>a14:0, a14:0, i14:0, 15:0, a15:0, 16:0, i16:0, a16:0, 17:0, a17:0</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>16:1ω7c, 16:1ω7t, 17:0, 18:1ω7t, 19:0</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>16:1ω7t, 16:1ω7t, 16:1ω7t, 16:1ω7t</td>
</tr>
<tr>
<td>Methane-oxidizing bacteria</td>
<td>16:1ω5c, 18:1ω8c</td>
</tr>
<tr>
<td>Fungi</td>
<td>18:2ω6t, 18:1ω9c, 18:1ω9c</td>
</tr>
<tr>
<td>cy/pre ratio</td>
<td>(cy17:0 + cy19:0)/(16:1ω7 + 18:1ω7)</td>
</tr>
<tr>
<td>S/M ratio</td>
<td>(14:0, 15:0, 16:0, 17:0, 18:0, 20:0)/(16:1ω5, 16:1ω5c, 18:1ω7t, 16:1ω7t, 16:1ω7t, 17:0, 18:1ω8c, 18:1ω9c, 18:1ω9c)</td>
</tr>
<tr>
<td>G−/G+ ratio</td>
<td>F/B ratio</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>16:1ω7t, 16:1ω7t, 16:1ω7t, 16:1ω7t</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>16:1ω7t, 16:1ω7t, 16:1ω7t, 16:1ω7t</td>
</tr>
<tr>
<td>Fungi</td>
<td>18:2ω6t, 18:1ω9c, 18:1ω9c</td>
</tr>
</tbody>
</table>
2.4. Data analysis

All figures were made with Sigma Plot 10.0 software for soil physical and chemical properties, nitrogen content and microbial activity. For ANOVA, Student–Newman–Keuls (S–N–K) least significant difference was adopted for testing significant differences of the three soil layers (0–10 cm, 10–30 cm and 30–60 cm) and across different slopes (sunny, shady, half-shady and hilltop) within same soil layer at the 5% level. SPSS 17.0 was used for canonical correlation coefficients (CCA) between soil nutrient and microbial activity.

A canonical correlation analysis (CCA) is a method that is used to assess the relationship between two datasets. This method is designed to identify linear combinations of variables in one dataset that account for the greatest variation in a linear combination of variables for the other dataset (Lattin et al., 2003). In this study, the CCA was used to evaluate the relationship between the soil N content and the microbial activity index. Six indices of the soil N canonical variate (N-CV) were used: organic N (X1), SON (X2), MBN (X3), nitrate-N (X4), ammonium-N (X5) and mineralizable N (X6). Five microorganism activity indices in the microbial canonical variate (M-CV) were used: basic respiration (Y1), respiratory quotient (Y2), microorganism quotient (Y3), MBC (Y4) and MBP (Y5).

3. Results

3.1. The soil physicochemical properties on the different slopes

The organic C and total N contents on the shady and half-shady slopes were significantly (p < 0.05) higher than those on the other slopes (Fig. 1). The organic C content on the sunny slope was the lowest. On the hilltop, the total N content was the lowest, whereas the amount of available P was significantly (p < 0.05) higher than that on the other slopes.

![Fig. 1. The basic physical and chemical properties of the three soil layers on the different CK slopes. Note: Different letters indicate significant differences at the 5% level; capital letters indicate differences among the three soil layers; lower-case letters indicate differences within the same soil layer across different slopes.](image-url)
The soil density increased with the increasing soil depth, and this effect was more obvious on the hilltop and the sunny slope. The soil pH (>8) also increased with the increasing soil depth; the pH at 30–60 cm was significantly (p < 0.05) higher than that at 10–30 cm. The electric conductivity of the soil also increased with the increasing soil depth. The electric conductivity at 0–10 cm was significantly (p < 0.05) higher on the hilltop than on the other slopes, and the shady slope had the greatest conductivity at depths of 10–30 and 30–60 cm.

3.2. The soil nitrogen content on the different slopes

As shown in Fig. 2-A, -C and -D, the levels of organic N, nitrate N and mineralizable N contents were significantly (p < 0.05) higher at 0–10 cm than at the other depths, and the concentration of these three forms of N on the sunny, shady and half-shady slopes was higher than that at the hilltop. Specifically, the concentration of organic N at depths of 0–10 and 10–30 cm on these slopes was almost twice as high (2.5 times) as that on the hilltop. Similarly, the nitrate N level at each depth and mineralizable N level at 0–10 cm were also significantly (p < 0.05) higher on these slopes than on the hilltop (2.6 and 4.2 times, respectively).

As shown in Fig. 2-B, the ammonium N content significantly differed among the three soil depths. At 0–10 cm, the ammonium N content on the shady and half-shady slopes was 138–162% higher than that on the hilltop and sunny slopes. At 10–30 cm, the ammonium N content on the shady and sunny slopes was 1.6 and 2.1 times higher than that on the hilltop and half-shady slopes, respectively.

A comparison of Fig. 2-E and -F reveals that on the shady and half-shady slopes, the microbial biomass N (MBN) content was significantly higher (p < 0.05) than that on the sunny slope and hilltop; whereas, the soluble organic N (SON) content was significantly lower (p < 0.05) than that on the sunny slope and hilltop. However, the MBN content was significantly lower (p < 0.05) on the hilltop and sunny slopes than on the shady and half-shady slopes. The SON content on the hilltop and sunny slopes was almost twice as high as that on the shady and half-shady slopes. The levels of nitrate N, organic N, mineralizable N, MBN and SON decreased significantly with the increasing soil depth.

Most of the nitrogen that was found on the different slopes was in the organic N form, with the content ranging from 98.21% to 99.05%. The proportion of nitrate-N and ammonium-N (<2%) was the lowest (<10 mg kg⁻¹). The other nitrogen forms were present in the following proportions:

Fig. 2. The soil nitrogen content at three soil layers on the different CK slopes. Note: Different letters indicate significant differences at the 5% level; capital letters indicate differences among the three soil layers; lower-case letters indicate differences within the same soil layer across different slopes.
order: MBN > SON > mineralizable N. On the hilltop, the content of nitrate-N was the lowest, while the contents of ammonium-N and SON were the highest. The content of mineralizable N was the highest at a depth of 0–30 cm on the half-shady slope, whereas the MBN content was the highest at a depth of 10–60 cm on the shady slope.

3.3. The soil microbial activity and biomass on the different slopes

As shown in Fig. 3-A, -D and -E, the MBC, soil basal respiration (BR) and metabolic quotient (qCO₂) were the greatest in the half-shady slope, followed by the shady, sunny and hilltop slopes. The BR within the soil differed significantly (p < 0.05) among the different slopes; the BR on the half-shady slope was approximately twice that on the shady slope and approximately four times higher than that on the hilltop and sunny slopes. The BR was also significantly (p < 0.05) different among the three soil layers under the hilltop and sunny slopes. The MBC content was significantly (p < 0.05) higher on the shady and half-shady slopes than on the hilltop and sunny slopes. The MBC content decreased significantly (p < 0.05) with increasing depth, and the average magnitude of this decline was approximately 80% for each slope. The qCO₂ at 30–60 cm was significantly (p < 0.05) higher on the shady and sunny slopes than on the other slopes. The qCO₂ increased significantly (p < 0.05) with the increasing soil depth. The qCO₂ on the hilltop, sunny, shady and half-shady slopes at 10–30 cm was 4.2, 6.5, 5.1 and 1.3 times higher than that at 0–10 cm, respectively, and it was 14.2, 25.6, 20.1 and 4.7 times higher at 30–60 cm than at 0–10 cm.

As shown in Fig. 3-B, -C and -F, the microbial quotient (MBC/total organic C) on the hilltop was significantly (p < 0.05) lower than that on the other slopes, whereas the MBP on the hilltop was approximately 10 times higher than that on the other slopes. The MBN content was significantly (p < 0.05) higher on the shady and half-shady slopes than on the hilltop and sunny slopes. The MBN on the hilltop and sunny slopes decreased significantly (p < 0.05) with the increasing soil depth; the magnitude of this decline was 12.5- and 14.1-fold at depths of 10–30 cm and 30–60 cm, respectively.

3.4. The microbial phospholipid fatty acids (PLFAs) on the different slopes

The total PLFA encompasses the total biomass of living cells. Here, we found that the total PLFA content on the sunny slope was significantly (p < 0.05) higher than that on the other slopes (Fig. 4-A), whereas the

![Fig. 3. The soil microbial activity at three different soil layers on the different CK slopes. Note: Different letters indicate significant differences at the 5% level; capital letters indicate differences among the three soil layers; lower-case letters indicate differences within the same soil layer across different slopes.](image-url)
The total PLFA content was the lowest on the hilltop. The total PLFA content of the shady and half-shady slopes did not differ significantly.

To characterize community structure, the PLFAs were subdivided into various microbial functional groups. The relative abundance of the individual groups was expressed as mol% (Fig. 4-B). The highest fraction of the total PLFA was accounted for by bacteria, followed by fungi, actinomycetes and unknown bacteria (protozoa were not detected). The relative abundance of bacteria and actinomycetes on the shady slope was significantly ($p < 0.05$) higher than that on the other slopes, and the relative abundance of fungi on the hilltop was significantly ($p < 0.05$) higher than that on the other slopes. There were no significant differences in the levels of unknown bacteria among the different slopes. Overall, bacteria and fungi were the two main microbial groups that were identified.

Regarding the bacterial distribution (Fig. 4-C), Gram-positive bacteria ($G^+$) and aerobes accounted for the largest groups in the total PLFA. The proportion of anaerobes and Gram-negative bacteria ($G^-$) was less than 5%. The $G^+$ levels on the sunny and shady slopes were significantly ($p < 0.05$) higher than those on the hilltop and half-shady slopes, and the aerobe and $G^-$ levels on the shady slope were significantly ($p < 0.05$) higher than that on the other slopes. Overall, the number of fungi and the total PLFA were highest on the sunny slope, while the amount of bacteria, $G^+$ and aerobes was the greatest on the shady slope.

The F:B ratio on the shady slope was significantly ($p < 0.05$) lower than that on the other slopes (Table 3), which indirectly reflects the low self-buffering and C storage capacity of the shady slope. The increase in the ratio of $G^+$ PLFA to $G^-$ PLFA suggests that $G^-$ are replaced with $G^+$, which indicates that chemolithoautotrophic communities are converted to heterotrophic communities (Dagmar et al., 2004; Bartelt-Ryser et al., 2005). The $G^+:G^-$ ratio on the hilltop and shady slopes was significantly ($p < 0.05$) higher than that on the sunny slope. The $G^+:G^-$ ratio on the half-shady slope was significantly ($p < 0.05$) higher than that on the other slopes, which indirectly indicates that more chemolithoautotrophic communities are converted into heterotrophic communities on the half-shady slope.

The ratio of saturated fatty acids to monounsaturated fatty acids (S/M) is an indicator of the quality of microbial nutrition. An increase

![Fig. 4](image-url) The soil microbial phospholipid fatty acid levels in the different CK slopes. Note: Different letters indicate significant differences at the 5% level; capital letters indicate differences among the three soil layers; lower-case letters indicate differences within the same soil layer across different slopes.

### Table 3
The physiological indices of the PLFAs on the different CK slopes.

<table>
<thead>
<tr>
<th>Sites</th>
<th>F:B</th>
<th>$G^+:/G^-$</th>
<th>S/M</th>
<th>cyc/prec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hilltop</td>
<td>0.72a</td>
<td>24.33b</td>
<td>0.79b</td>
<td>0.27b</td>
</tr>
<tr>
<td>Sunny</td>
<td>0.60a</td>
<td>9.50c</td>
<td>0.80b</td>
<td>0.45a</td>
</tr>
<tr>
<td>Shady</td>
<td>0.37b</td>
<td>20.20b</td>
<td>1.47a</td>
<td>0.36b</td>
</tr>
<tr>
<td>Half-shady</td>
<td>0.73a</td>
<td>40.02a</td>
<td>0.72b</td>
<td>0.32b</td>
</tr>
</tbody>
</table>

Note: $G^+:/G^-$: Gram-positive bacteria/Gram-negative bacteria; F:B: fungi/bacteria; cyc/prec: cyclopropyl (cy17:0 + cy19:0)/precursors (16:1ω7+ 18:1ω7); S/M: saturated fatty acids/monounsaturated fatty acids. Different lowercase letters indicate significant differences at $P < 0.05$ among different slopes.
in the S/M ratio indicates a microorganismal response to soil nutrient deprivation. A high S/M ratio suggests an increased organic C input, whereas a low S/M ratio suggests a lower availability of substrate (Lucía et al., 2010; Zelles et al., 1995). The S/M ratio on the shady slope was significantly (p < 0.05) higher than that on the other slopes, which indicates that the shady slope had the highest quality of soil microbial nutrition.

The cy/pre ratio can be used as an indicator of the physiological status of the G-communities. A smaller cy/pre ratio reflects less community stress (e.g., in terms of carbon or oxygen limitation). If the cy/pre ratio exceeds 0.5 may indicate serious environmental stress (Allison et al., 2005). The cy/pre ratio for the sunny slope was significantly (p < 0.05) higher than that for the other slopes (Table 3). Overall, the cy/pre ratio was lower than 0.5 for all of the slope types that were analyzed, which suggests that all of the slopes presented relatively low-stress environments for bacterial growth.

3.5. A correlation analysis between the soil microbial PLFAs and other indices

As shown in Table 4, the contents of anaerobes and actinomycetes were positively correlated with the bulk density as well as the content of total N, organic N and nitrate-N (p < 0.05) in the soil, but were negatively correlated with the amount of available P and MBP (p < 0.05). The G− and fungi levels were positively correlated with the levels of organic N (p < 0.05).

3.6. A canonical correlation analysis of the indicators of the soil nitrogen and microbial activity

The CCA was performed using the soil N and microbial principal indicators, and five pairs of canonical variates (CVs) were extracted. The canonical correlation between the first soil N canonical variate (N-CV1) and the first microbial canonical variate (M-CV1) was significant (R = 0.964) with a good fit (p = 0.0001). The first canonical variate mainly reflected the relationship between the mineralizable N and the MBC. Approximately 50% of the variance in the M-CV1 was explained by the N-CV1 (Table 5). The contributions (evaluated by the absolute value of canonical coefficient) from the different forms of N as evaluated by the canonical coefficient of CV were on the order of mineralizable N > organic N > MBN > nitrate-N > ammonium-N. In contrast, the microbial index contributions (evaluated by the absolute value of canonical coefficient) were on the order of MBC > MBP > microorganism quotient > respiration quotient > basic respiration.

### Table 4

The correlation coefficients between the soil PLFAs and the physical and chemical properties, soil nitrogen content and microbial activity.

<table>
<thead>
<tr>
<th></th>
<th>Total PLFA</th>
<th>Bacteria</th>
<th>G+</th>
<th>G−</th>
<th>Aerobes</th>
<th>Anaerobes</th>
<th>Fungi</th>
<th>Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic C</td>
<td>0.40</td>
<td>−0.48</td>
<td>0.43</td>
<td>−0.55</td>
<td>−0.47</td>
<td>−0.25</td>
<td>−0.22</td>
<td>−0.30</td>
</tr>
<tr>
<td>Total N</td>
<td>0.45</td>
<td>0.50</td>
<td>0.48</td>
<td>0.21</td>
<td>0.52</td>
<td>0.76*</td>
<td>0.20</td>
<td>0.71*</td>
</tr>
<tr>
<td>Available P</td>
<td>0.57</td>
<td>−0.61</td>
<td>−0.59</td>
<td>−0.37</td>
<td>−0.65</td>
<td>−0.82*</td>
<td>−0.31</td>
<td>−0.81*</td>
</tr>
<tr>
<td>Bulk density</td>
<td>0.42</td>
<td>0.53</td>
<td>0.46</td>
<td>0.26</td>
<td>0.58</td>
<td>0.73*</td>
<td>0.06</td>
<td>0.70*</td>
</tr>
<tr>
<td>Soil pH</td>
<td>0.08</td>
<td>−0.06</td>
<td>−0.07</td>
<td>−0.31</td>
<td>0.02</td>
<td>0.27</td>
<td>−0.18</td>
<td>0.24</td>
</tr>
<tr>
<td>Electric conductivity</td>
<td>0.48</td>
<td>−0.62</td>
<td>−0.54</td>
<td>−0.35</td>
<td>−0.62</td>
<td>−0.77</td>
<td>−0.07</td>
<td>−0.78</td>
</tr>
<tr>
<td>Organic N</td>
<td>0.45</td>
<td>0.50</td>
<td>0.48</td>
<td>0.21</td>
<td>0.52</td>
<td>0.76*</td>
<td>0.20</td>
<td>0.71*</td>
</tr>
<tr>
<td>Soluble organic N</td>
<td>0.64</td>
<td>0.55</td>
<td>0.61</td>
<td>0.72*</td>
<td>0.47</td>
<td>0.31</td>
<td>0.77*</td>
<td>0.32</td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>0.49</td>
<td>0.52</td>
<td>0.50</td>
<td>0.24</td>
<td>0.57</td>
<td>0.78*</td>
<td>0.27</td>
<td>0.71*</td>
</tr>
<tr>
<td>Ammonium-N</td>
<td>−0.17</td>
<td>−0.12</td>
<td>−0.16</td>
<td>−0.36</td>
<td>−0.04</td>
<td>0.14</td>
<td>−0.28</td>
<td>0.05</td>
</tr>
<tr>
<td>Mineralizable N</td>
<td>0.28</td>
<td>0.22</td>
<td>0.26</td>
<td>0.00</td>
<td>0.30</td>
<td>0.56</td>
<td>0.27</td>
<td>0.48</td>
</tr>
<tr>
<td>Basic respiration</td>
<td>−0.18</td>
<td>−0.29</td>
<td>−0.24</td>
<td>−0.45</td>
<td>−0.19</td>
<td>12.08</td>
<td>−0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Respiration quotient</td>
<td>−0.13</td>
<td>−0.27</td>
<td>−0.20</td>
<td>−0.38</td>
<td>−0.19</td>
<td>0.06</td>
<td>0.10</td>
<td>−0.05</td>
</tr>
<tr>
<td>Microorganism quotient</td>
<td>−0.06</td>
<td>0.07</td>
<td>−0.01</td>
<td>−0.21</td>
<td>0.11</td>
<td>0.30</td>
<td>−0.39</td>
<td>0.30</td>
</tr>
<tr>
<td>Microbial biomass C</td>
<td>−0.21</td>
<td>−0.22</td>
<td>−0.22</td>
<td>−0.47</td>
<td>−0.13</td>
<td>0.17</td>
<td>−0.25</td>
<td>0.08</td>
</tr>
<tr>
<td>Microbial biomass N</td>
<td>0.22</td>
<td>0.29</td>
<td>0.25</td>
<td>0.00</td>
<td>0.35</td>
<td>0.60</td>
<td>−0.05</td>
<td>0.56</td>
</tr>
<tr>
<td>Microbial biomass P</td>
<td>−0.60</td>
<td>−0.64</td>
<td>−0.62</td>
<td>−0.41</td>
<td>−0.68</td>
<td>−0.84*</td>
<td>−0.35</td>
<td>−0.84*</td>
</tr>
</tbody>
</table>

*Indicates significant correlation at p = 0.05.

4. Discussion

Environmental factors (e.g., light and moisture) directly impact the growth processes of CK, such as photosynthesis and root growth, and the spatial variability in these features may therefore affect the soil nutrient cycles (Yimer et al., 2006). The shady and half-shady slopes are likely to have more preferable environmental conditions (e.g., moisture and temperature) for CK growth than do hilltop and sunny slopes, as suggested by previous studies (Sun et al., 2010; Zhang et al., 2007; Zhang et al., 2010). Although the available P content was higher on the hilltop than on the other slopes, there was generally no difference in the bulk soil content, pH and electric conductivity among the four slope types that were studied here.

Plants can only take up phosphorus in the orthophosphate form (available P). Therefore, the soluble soil P pool is of pivotal importance for plant growth, as it provides the only measurable source of mobile P. The concentration of P is often higher in the surface soil than in the subsoil. Therefore, when it rains, P losses will occur more readily on the slopes than on the hilltop. Indeed, Liu et al. (2009) found a negative correlation between the P content and altitude. This finding agrees with
our results, as the hilltop was at the lowest altitude and also had the highest P levels.

The total N in the nitrogen pool is often used as a measure of the soil fertility, although the level of available N does not typically limit plant growth (van Wijnen and Bakker, 2000). Heterotrophic microbes drive the mineralization of organic matter, providing a basic mechanism for producing plant-available N. During plant growth, organic N compounds in the soil are strongly affected by microorganisms, which produce various forms of N that are available for plant absorption. Thus, mineralizable N is thought to represent the total N within the soil that can be used for plant nutrition (Parkin et al., 2002).

The MBN is used to predict the soil N mineralization rate (Oils, 1993). In the present study, the amount of mineralizable N on the hilltop, which was strongly related to the amount of N that was available for plant use, was low (Fig. 2). It is likely that soil nutrients limit the accumulation of N to a certain degree (Guo and Li, 2012; Sammul et al., 2012). A possible explanation for the high ammonium-N content and soil EC on the shady slope is that ammonium-N remains stable at high moisture levels (Davenport et al., 2012; Fangueiro et al., 2012) because it is easily formed and can persist in the soil in a solid phase. Although soil moisture is important for limiting the movement of ammonia, it does not ultimately determine whether the NH₄⁺ will remain in the soil. If the soil oxygen availability, moisture levels and temperature are all favorable, the ammonium-N will be converted into nitrate-N to provide a more balanced nitrogen proportion (Smith et al., 2009). To some extent, this conversion might explain the lack of significant difference in the soil nitrate-N content between the sunny and shady slopes. Because a reduced nitrate-N content was found at increasing soil depths, the loss of N through leaching effects was likely also weaker, given the arid weather in this region. The combination of the high MBN and low SON on the shady and self-shady slopes was previously reported by Bardgett et al. (2003) and was used to suggest that the soil SON may be directly absorbed by the plants or microorganisms. In this way, the SON could promote microbial activity within the soil.

In general, the soil N content on the shady and half-shady slopes was higher than that on the hilltop and sunny slopes. It is likely that the good moisture, soil nutrient content and organic matter content on the shady and half-shady slopes produced suitable conditions for both the N mineralization and the accumulation of organic and inorganic N (Qin et al., 2008; Zhang et al., 2010). In agreement with previous studies, a canonical correlation analysis showed that the soil mineralizable N, organic N and MBP were all sensitive indicators that reflected changes in soil N (Bremer and Kuikman, 1997; Murphy et al., 1998).

4.2. The influence of the slope aspect on microbial activity and structures

The MBC content is an important index that reflects the quality and health of the soil (Gil-Sotres et al., 2005) and also serves as an effective index for evaluating changes in the soil properties and the degree of soil degradation (Smith and Paul, 2006). The MBC content reflected the impact of the processes on different slopes, as it had the largest canonical coefficient. This result is consistent with previous results suggesting that the MBC content is closely related to the growth of vegetation and to changes in the microorganism content of the soil (Bi et al., 2008). Moreover, in the present study, the total N, organic N and nitrate-N levels were positively related to the MBC content and the amount of anaerobic bacteria and actinomycetes. The level of carbon that is available to the soil microorganisms is possibly increased by CK through root exudates (Berg and Smalla, 2009; Dennis et al., 2010).

An increase in the microbial activity could increase the MBC and organic N contents of the soil. At high levels of microbial activity, the nutrient turnover rate, mineralization levels and nutrient levels that are required for plant growth through the C and N cycles will also be high. High levels of basic respiration (Gamboa and Galicia, 2011), as observed on the shady and half-shady slopes, reflect microbial soil conditions that are favorable for CK growth. Our results demonstrate that the cyc/prec ratio was lower than 0.5 for all of the sites that were studied, which indicates that the carbon and oxygen contents of the soil were sufficient for microbial growth at a relatively low level of environmental stress (Allison et al., 2005).

The observed decrease in the soil respiration quotient (qCO₂) may reflect an increase in the ecosystem stability and carbon use efficiency (CUE) (Wardle and Ghani, 1995). In our study, the qCO₂ was found to be the lowest on the shady slope, which indicates a high CUE. Furthermore, the CUE increased with the increasing soil depth and with a corresponding decrease in the qCO₂.

Microbial mineralization is an important mechanism for the conversion of organic P into plant-available P (Dai et al., 2008). Moreover, the available soil nutrients change with the specific microbial biomass and plant species (Wu et al., 2007). The amounts of P and MBP that were available for plant use on the hilltop were significantly higher than those on the other slopes, as previously observed by Joergensen et al. (1995). In addition, the correlation analysis revealed that the levels of available P and MBP were negatively correlated with the levels of anaerobes and actinomycetes. The change in the microbial quotient could reflect changes in the content and effectiveness of the organic C within the soil microbial communities of an ecosystem (Yan et al., 2003). In general, the microbial quotient is higher on shady and half-shady slopes than on the hilltop and sunny slopes, which may be related to the higher MBN levels relative to the total N levels on these two slopes. The microorganism population is, therefore, promoted by favorable environmental conditions (Zhang et al., 2009).

5. Conclusions

1) Slope aspect differences can significantly alter the proportion of the six soil nitrogen forms (nitrate-N, organic N, mineralizable N, microbial biomass nitrogen (MBN), soluble organic N and ammonium-N).
2) The content of soil nitrogen, soil basal respiration and microbial entropy were significantly higher on the shady and half-shady slopes than on the sunny slope and the hilltop, whereas the microbial biomass phosphorous MBP on the hilltop was significantly higher than that on the other slopes.
3) The CCA reflected the relationship between the mineralizable N and microbial biomass carbon (MBC). The relative contributions (evaluated by the absolute value of canonical coefficient) of the different forms of N were of the order mineralizable N > organic N > MBN > nitrate-N > ammonium-N, whereas the relative contributions (evaluated by the absolute value of canonical coefficient) of the microbial index were of the order MBC > MBP > microorganism quotient > respiration quotient > basic respiration.
4) Anaerobes and actinomycetes were positively correlated with the soil bulk density and total N, organic N and nitrate-N contents (p < 0.05) and were negatively correlated with the available P and MBP (p < 0.05). The G− bacteria and fungi levels were positively correlated with the level of organic N (p < 0.05)

Acknowledgments

This research was funded by the National Natural Science Foundation of China (41101254, 41171226), the Foundation of State Key Laboratory of Soil Erosion and Dry Land Farming on the Loess Plateau, the Institute of Soil and Water Conservation, the Ministry of Water Resources, the Chinese Academy of Sciences (K318009902–1321) and the Foundation of Basic Research Youth Project of Northwest A&F University (QN2011020). We also thank Dr. Jeff Gale for his help in editing the grammar and syntax.

References


