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Dynamic of soil microbial-community: Terminal restriction fragment length polymorphism analysis on natural secondary succession of *Pinus tabulaeformis* Carr. in the forest region of Loess Plateau, China

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In order to assess the dynamics of microbial-diversity through vegetation successions, sample sites for soil investigation at the 10a, 25a, 40a, 75a natural secondary *Pinus tabulaeformis* Carr. forests were established in the forest region of Loess Plateau, China. With the succession extension, soil microbial biomass carbon (MBC), moisture content, soil organic carbon (SOC) as well as total N also exhibited an increase before decreasing trend with succession gradient. Soil indexes of the soil shannon diversity index (H), which incorporates both richness and evenness of all soil microbial-species observed in the plots according to T-RFs peaks composition determined by terminal restriction fragment length polymorphism (T-RFLP), also exhibited a similar trend with soil physicochemical properties during the succession. In the late-succession of 75a, its soil fertility and microbial-community structure is distant from other three stages, suggesting that it may be a specific stage to the recession succession. The result of a dendrogram of hierarchical cluster of the microbial-community structure of four succession stages showed that 75a was distant to the other three sample sites, and 25a and 40a represented the most similar microbial-community structure of all stages. It was concluded that the underground succession of natural secondary P. tabulaeformis Carr. forests in the forest region of Loess Plateau, China, is significantly linked to its aboveground. We infer that natural secondary succession of *P. tabulaeformis* Carr forest will be replaced by the *Qercu sliaotungensis* in the coming centuries in the forest region of Loess Plateau, China.

Key words: Soil microbial-community; natural secondary succession, T-RFLP, Loess Plateau.

INTRODUCTION

Succession generally refers to the biological changes that occur in an ecosystem after the clearing or exposure of an area, often resulting in predictable sequences of species composition shifts. Plants and sessile animals have long been the focus of succession studies, primarily due to the perceived physical and ecological dominance of the irrespective landscapes (Redford and Fierer, 2009; Wehenkel et al., 2011). Despite their ubiquity, abundance, and diversity, surprisingly few studies have examined succession patterns in microbial communities. This is partly due to methodological reasons, as it is difficult to describe the full extent of microbial diversity in a given sample using traditional, culture-based methods (Li et al., 2004).

Current methods for assessing soil quality give only an incomplete picture of the status of the soil system. Several indicators for assessing soil quality, namely organic matter, topsoil depth, infiltration, aggregation, pH,
electrical conductivity, suspected pollutants, and soil respiration have been investigated (Arshad and Martin, 2002) but they lack any indication of the dynamics of soil microbial community structure and function. Therefore, a comprehensive determination of soil microbial community characteristics is needed. Understanding soil biology and ecology is increasingly recognized as important for the restoration and sustainability of ecosystems. In all ecosystems, soil microbes play important roles in decomposition of organic matter, nutrient cycling, and plant nutrient availability (Steenwerth et al., 2002).

Terminal restriction fragment length polymorphism (T-RFLP) is a fingerprinting technique following the same principle as restriction fragment length polymorphism (RFLP) and amplified ribosome DNA restriction analysis (ARDRA), except one PCR primer is labeled with a fluorescent dye, such as 4,7,2′,7′-tetrachloro-6-carboxyfluorescein (TET) or phosphoramidite fluorochrome 5-carboxyfluorescein (6-FAM) (Liu et al., 1997). It has been proven to be a sensitive and effective method to study complex bacterial community in water, soil and feces (Osborne and Rees, 2006).

China is one of the most severely desertified countries in the world with up to 3.3 million km² desertified lands. In recent years, desertification has become a major environmental problem, attracting wide spread attention in China, especially in the Loess Plateau (Zhong and Qu, 2003).

*Pinus tabulaeformis* Carr. of the subgenus Diploxylon (Pinaceae) is a major and widespread component of coniferous forests in northern China, which extend from northeast China to northwest China (Chen et al., 2008). It has a strong adaptability to dry and barren habitats. It grows quickly and possesses a strong ability to develop roots. Meanwhile, it also has the function of conserving soil and water, enriching headwaters and improving soil fertility so that it has been considered a dominant species in the project of transforming farming land into forests in the Loess Plateau (Zhang et al., 2006). The Chinese government has launched a series of nation-wide conservation projects focused on the ecological restoration, mainly by building population; the Loess Plateau is one of the key areas for this project. Therefore, understanding the dynamics of soil properties and natural vegetation succession characteristics of Chinese pine forest with different ages is no doubt helpful to the recovering pine population in the Loess Plateau, China. Also, it was the hope that this study might contribute new information to Chinese pine forest succession.

**MATERIALS AND METHODS**

**Study site**

The study was conducted in Zhongwan forest region in Ziwuling Mountain of the Loess Plateau, Zhengning County, Gansu Province, China (108°27′E, 35°17′N) as shown in Figure 1. The site has the topography characterized by hills and gullies resulting from continuous alternative effects of loess deposition and river erosion, and an elevation ranging from 1246 to 1756 m above sea level. It has a semi-humid climate, showing obvious continental climatic characteristics. The mean annual temperature is 8.3°C and the total annual precipitation is 623.5 mm; 63% of which concentrates from July to September. The annual evaporation is 1500.8 mm, the aridity index is 0.72, and the atmospheric relative humidity is between 60 and 70%. The annual solar radiation is 2200 to 2400 h, and the frost-free period is about 163 days. In general, sienna soil is mainly distributed on sunny and desert slope, and castaneous-calcareous soil is mainly found in the sheltered and semi-sheltered slope (Zhang et al., 2006).

**Soil sampling**

We established sample sites for soil investigation in the 10a, 25a, 40a, 75a natural secondary *P. tabulaeformis* Carr. forests. Soil samples were collected at layer of 0 to 20 cm in late July (summer) 2011, with three replicates of each succession stage. In each sample site, a quadrat (10×10 m) was established, located at least 10 m from its margin. The soil was collected from five points randomly, and mixed into one sample. Plot age was determined based on historical data supplied by the forest station. The soil samples were passed through a 2-mm sieve, and stored at 4°C before microbial analysis. Microbial biomass carbon (MBC) and T-RFLP analysis at the different growing years of Chinese pine forest succession were conducted within four week after sampling. Subsamples were air-dried and sieved through an 80-μm wide screen for soil physicochemical analyses.

**Soil physicochemical properties analyses**

Soil pH was measured using a glass electrode. Soil samples were mixed in water (the volume ratio of soil to water was 1:1). Soil moisture was determined gravimetrically by drying soils at 105°C for 24 h (Institute of Soil Sciences, Chinese Academy of Sciences, 1978) and the water content was expressed as a percentage of the dry weight (Jia et al., 2005). Soil organic carbon (SOC) was determined by the Walkley-Black method (Nelson and Sommers, 1982) and total N by the Kjeldahl method (Bremner and Mulvaney, 1982).

**Extraction of microbial biomass carbon in soil**

Microbial biomass carbons were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987). Six portions equivalent to 25 g of dry weight soil were taken from each soil sample. Three portions were fumigated for 24 h at 25°C with CHCl₃ (ethanol-free).

Following fumigant removal, the soil was treated with 100 ml of 0.5M K₂SO₄ by horizontal shaking for 1 h at 200 rpm, and then filtered. The three non-fumigated portions were extracted simultaneously at the time fumigation commenced. Organic carbon in the extracts was measured using dichromate oxidation method (Jia et al., 2005). MBC was calculated as follows:

\[
MBC = \frac{C_{\text{org}} (\text{fum}) - C_{\text{org}} (\text{non} - \text{fum})}{0.38}
\]

Where, *C_{\text{org}} (\text{fum})* means the soil organic carbon content after fumigated and *C_{\text{org}} (\text{non-fum})* means the difference between the non-fumigated and fumigated.
Figure 1. The location of Zhongwan forest region in China (shaded yellow point).

Shannon-wiener index analyses

We computed genetic diversity ($H$) of soil microbial-community according to T-RFs composition determined by T-RFLP (Zhang et al., 2009), which incorporates both species richness and evenness, as a way to assess the variation among soil of Chinese pine forests with different ages. The genetic diversity ($H$) was assessed by calculating values for the Shannon-Wiener index (Shannon and Weaver, 1948):

$$H = - \sum P_i \ln P_i$$

Where, $P_i$ is the ratio of species $i$.

The evenness ($E$) of the soil microbial-communities of stands with different ages was assessed by $H$:

$$E = H / \ln H$$

Where, $H$ is Shannon-Wiener index abstained from above.

Differences between mean values for diversity, evenness, and richness were analyzed by ANOVA, with the type of forest ages as the discriminating variable.

Terminal restriction fragment length polymorphism analyses

T-RFs composition was determined by T-RFLP.

Total DNA extraction

Total DNA of each soil sample was extracted from 0.25 g (dry weight) soil by using Ultraclean soil DNA isolation Kits (MO BIO Laboratory, Inc., Solana Beach, CA). Extracted DNA was checked via agarose gel electrophoresis (0.8%) and stored at -20°C.

Amplification of 16S rRNA Genes

16S rRNA genes of soil samples and isolates with expected length of 527 bps were amplified with a pair of bacterial universal primers; fluorescent labeled forward primer BSF 8/20 (6-FAM-5’-AGAGTTTGATCCTGGCTCAG-3’) and reverse primer BSR 534/18 (5’- ATTACCGCGCTGCTGGC-3’). Each 50-μl reaction mixture contained: 33.6 μl PCR water, 5μl of 10x ThermoPol Reaction Buffer, 5 μl of 2 mM dNTP (dATP, dCTP, dGTP, dTTP), 1 μl each of 20 μM 6-FAM-5’-BSF8/20 and BSR534/18, and 0.4 μl of 5 U/μl Taq DNA polymerase. Amplified reactions were carried out in the Bio-rad I Cycler thermal cycler with following cycling conditions: three minutes of denaturation at 94°C, 35 cycles of 75 s at 94°C, 45 s at 55°C for annealing, and 45 s at 72°C for extension, and a final cycle of extension at 72°C for 10 min. Multiple PCR reactions from a single sample were pooled together to minimize PCR-induced random biases. PCR products were purified with the QiAquick Nucleotide Removal Kit (Dingguochangsheng Biotech Ltd., Beijing,
Table 1. Soil physicochemical and microbial properties at the different ages of natural Chinese pine forest secondary succession.

<table>
<thead>
<tr>
<th>Forest age</th>
<th>pH</th>
<th>Moisture content (%)</th>
<th>MBC (g·Kg⁻¹)</th>
<th>SOC (g·Kg⁻¹)</th>
<th>Total N (g·Kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>7.10±0.01 a</td>
<td>18.64±0.34 a</td>
<td>157.45±16.30 a</td>
<td>12.90±0.29 a</td>
<td>1.33±0.02 a</td>
</tr>
<tr>
<td>25a</td>
<td>7.09±0.02 ab</td>
<td>19.98±0.34 a</td>
<td>424.53±23.21 bc</td>
<td>15.30±0.85 b</td>
<td>2.83±0.02 b</td>
</tr>
<tr>
<td>40a</td>
<td>7.05±0.01 bc</td>
<td>22.20±0.81 c</td>
<td>596.77±31.50 c</td>
<td>16.73±0.17 b</td>
<td>3.10±0.03 c</td>
</tr>
<tr>
<td>75a</td>
<td>6.92±0.02 d</td>
<td>16.95±0.03 b</td>
<td>365.25±14.47 cd</td>
<td>14.16±0.12 b</td>
<td>2.28±0.03 d</td>
</tr>
</tbody>
</table>

Values are mean of three replicates (SE). Values in the same column followed by the same letter are not significantly different at p < 0.05 according to ANOVA and Duncan's multiple range comparison (P=0.05).

Digestion of 16S rRNA Genes with restriction endonucleases

Purified PCR product was digested with 20 μl of the following restriction endonucleases: BsuII;2μL 10×Buffer, 4 μl DNA, 0.5 μL BsuII,13.5 μL ddH₂O; MspI:2 μL 10×Buffer, 4 μL DNA, 1 μL MspI,13 μL ddH₂O; HaeIII:2 μL 10×Buffer R, 4 μL DNA, 1 μL HaeIII, 13 μL ddH₂O; HinfI: 2 μL 10×Buffer, 4 μL DNA, 1 μL HinfI, 13 μL ddH₂O. The reaction mixtures were incubated at 60°C for 24 h for BsuII and 37°C for 24 h for MspI, HaeIII and HinfI. For each restriction digestion, three replicates were set up and pooled together to minimize the artificial biases. Digested PCR products were then purified with the QIAquick Nucleotide Removal Kit (Dingguochangsheng Biotech Ltd., Beijing, CHN) (Zhang et al., 2009).

Gene scan analysis on T-RFs

6-FAM labeled terminal restriction fragments (6-FAM-TRFs) of digested amplicons were separated and recoded by a model ABI Prism 377 DNA Sequencer (Dingguochangsheng Biotech Ltd., Beijing, CHN). The fragment length in nucleotides, the peak height at apex and the area under the peak in fluorescence units (FU) of each TRF in a given pattern were calculated by GeneScan Analysis Software Version 3.1. B value of peak detection in the software was set from 15 to 120 (B value represents fluorescence signal) (Zhang et al., 2009).

Data analysis

All TRF profiles within a data set were standardized by the application of the variable percentage threshold method reported by Osborne and Rees, (2006) before further analysis. Following normalization, derivative TRF profiles within a data set were aligned and TRFs which have synonymous fragment sizes were identified and binned together based on the function of Bin table report in the GeneMarker V-1.4 software. All TRFs within a bin just represented the peak which was assigned the average of the sizes of them. A single composite list of the binned peaks (fixed within ±0.4bp) was found among all samples within a data set. For each sample, the present or absence of the binned peaks in the composite list was represented by a binary vector: present (1), and absence (0). The data set was transformed into a binary matrix whose rows represented binned peaks and columns represented samples (Zhang et al., 2009). A dendrogram of hierarchical cluster on different samples (natural Chinese pine forests with different forest ages) was used to generate a matrix with between-groups linkage measured by Euclidean distance of hierarchical cluster of SPSS 16.0 for windows, which represented the similarity and dissimilarity between TRF profiles of different samples.

Statistical analysis

All statistical work was done using the SPSS16.0 for Windows, including one-way ANOVA and Duncan’s multiple range comparison (P=0.05). They were used to analyze means, to least significant difference at the 5% level.

RESULT

Variation of soil physicochemical and microbial properties

Soil pH slightly decreased with extension of succession ages (Table 1). SOC, moisture content, MBC as well as total N exhibited an increase before decreasing trend with succession gradient (Table 1). All soil properties were varied significantly according to ANOVA. Meanwhile, for SOC, there was no significant difference among 25a, 40a and 75a; for moisture content, there was also no significant difference between 10a and 25a according to Duncan’s multiple range comparison (P=0.05).

The moisture content in soil exhibited a positive correlation with total N, soil MBC (p<0.05) and SOC (p<0.01). SOC also exhibited a positive correlation with soil MBC and total N (p<0.01). There was also a significant correlation between MBC and total (p<0.01). The soil pH exhibited a negative correlation with moisture content, total N, soil MBC and SOC (p<0.05) (Table 2).

Shannon-wiener, evenness and richness index analyses

Soil Shannon-wiener index, evenness, and richness in the studied fields showed a stationary trend during the succession stages of 10a, 25a and 40a, but a downturn trend after that (Figures 2 and 3) according to T-RFs peaks composition determined by T-RFLP. The Shannon-wiener index showed a trend of asymptotic increase from10a (4.11±0.11) to 40a (4.32±0.02), but no significant difference between each of the soil samples (P>0.05). It
Table 2. Pearson’s correlation coefficients among soil properties.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Moisture content</th>
<th>SOC</th>
<th>MBC</th>
<th>Total N</th>
<th>pH</th>
<th>Shannon-wiener</th>
<th>Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>0.714**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBC</td>
<td>0.676*</td>
<td>0.706**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td>0.792*</td>
<td>0.809**</td>
<td>0.907**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>–0.812*</td>
<td>–0.655*</td>
<td>–0.782*</td>
<td>–0.933**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shannon-wiener</td>
<td>0.611*</td>
<td>0.258</td>
<td>0.457</td>
<td>0.679*</td>
<td>0.816**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evenness</td>
<td>0.588*</td>
<td>0.250</td>
<td>0.448</td>
<td>0.666*</td>
<td>0.797**</td>
<td>0.977**</td>
<td></td>
</tr>
<tr>
<td>Richness</td>
<td>0.700*</td>
<td>0.362</td>
<td>0.596*</td>
<td>0.791**</td>
<td>0.873**</td>
<td>0.898**</td>
<td>0.899**</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (two-tailed); ** correlation is significant at the 0.01 level (two-tailed).

Figure 2. Variation of Shannon-wiener and evenness indexes with succession gradient; values are mean of three replicates (SE). Values in the same column followed by the same letter are not significantly different according to ANOVA and Duncan’s multiple range comparison (P=0.05).

exhibited the lowest index at 75a (3.42±0.14) which was significantly different from previous three soil samples (15a, 25a and 40a) (p<0.05). A similar trend was observed for evenness and richness indexes in different soil samples, it also showed trend of asymptotic increase from 10a (2.91±0.02 and 78.00±5.03, respectively) to 40a (2.96±0.01 and 83.33±1.76, respectively), however, there was no significant difference between the soil samples (P>0.05). It also exhibited the lowest indexes at 75a (2.78±0.02 and 38.33±0.88, respectively) which was significantly different from the previous three soil samples (15a, 25a and 40a) (P<0.05). All indexes varied significantly according to ANOVA and Duncan’s multiple range comparison (P=0.05). The moisture content in soil exhibited a negative correlation with indexes of Shannon-wiener, evenness, and richness (p<0.05). A similar trend was observed for total N, which also exhibited a negative correlation with indexes of Shannon-wiener (p<0.05), evenness (p<0.05) and richness (p<0.01), respectively. MBC also exhibited a negative correlation with richness (p<0.05). The soil pH showed a positive correlation with indexes of Shannon-wiener, evenness, and richness (p<0.01). The Shannon-wiener index had a positive correlation with evenness and richness (p<0.05); evenness and richness also had significant correlation (p<0.05) (Table 2).
Assay of TRF profiles of different soil samples

The clustering dendrogram based on between-groups linkage measured by Euclidean distance shown in Figure 3 illustrates the similarities between TRF profiles (three replicates of each marked with A, B, C and D) of different samples. Rescaled distance cluster was combined from 14.28 to 17.42, and all soil samples were first divided into two large groups: Group X and Group Y. Group X included all samples derived from the soil collected from natural secondary *P. tabulaeformis* Carr. forest at 10a, 25a, and 40a. Group Y included all the samples collected from natural secondary *P. tabulaeformis* Carr. forest at 75a. For rescaled distance cluster combined from 17.42 to 19.30, Group X was then further divided into two sub-groups: Group X1 and Group X2. Group X1 included all samples derived from the soil collected from natural secondary *P. tabulaeformis* Carr. forest at 10a. Group X2 included all the samples collected from natural secondary *P. tabulaeformis* Carr. forest at 25a and 40a. Finally, for rescaled distance cluster combined from 19.30 to 19.38, Group X2 was completely divided into two sub-groups, 25a and 40a.

DISCUSSION

The goal of this study was to understand the soil microbial-community structure during natural secondary vegetation succession of Chinese pine forest. The results show that soil microbial-structure of secondary vegetation succession of Chinese pine forest appeared to regularly change during the succession.

In this study, comparisons between young secondary, old secondary and old-growth forests allow us to describe regeneration dynamics over stand development based on a conceptual model documented by Oliver (1980), and Denslow and Guzman (2000). This model demonstrates a three-construction process from establishment, thinning, and transition to the steady state (Yasuhiro et al., 2005). Wehenkel et al., (2011) have indicated that genetic diversity should be higher in earlier successional stages than in later stages of tree communities, because high environmental predictability in later successional stages favours low genetic diversity. This agrees with our results. Suo et al., (2008) also indicated that aboveground and belowground components of terrestrial ecosystems have been found to be closely linked, and microbial processes ensure a large part of ecosystem functioning through the decomposition of organic matter.

In our study, the soil shannon diversity index ($H'$), which incorporates both richness and evenness of all soil microspecies observed in the plots according to T-RFs peaks composition determined by T-RFLP, provided another way to evaluate changes in microbial-community composition through successional time (Figures 2 and 3).
They showed an asymptotic increase from 10a to 40a but no significant difference among them. It exhibited the lowest at 75a and was significantly different from previous three soil samples (15a, 25a and 40a) (P<0.05). The trend changes may be related to some factors, which may include forest litter and community understory species, sunlight, soil physicochemical and microbial properties.

Generally, the aboveground and belowground components of a terrestrial ecosystem are considered to be dependent on each other, and residues and roots of different plant species are colonized by different decomposer communities (Wardle et al., 2006). A mechanistic view may lead to the expectation that increased plant species diversity should be reflected in a high diversity as well as activity of soil organisms (Gomoryova et al., 2009). In contrast to our hypothesis, like indexes of soil microbial-diversity, evenness and richness, with extension of secondary forest succession, the indexes asymptotically increased from 10a to the 40a, after that it decreased, indicating that accumulation of plant and soil microbial communities mainly occurred in the early and mid-succession stages (10a–40a) in our study. This also demonstrates that increased plant species diversity should be reflected in a high diversity and activity of soil organisms (Gomoryova et al., 2009) as shown above.

The young secondary succession community (10a in this study) significantly affected soil organic matter accumulation since there were fewer herbs (Thalictrum ssp., Phlomis umbrosa, Epimedium brevicolorum, Polygonatum cirrhifolium and Patrini heterophyua) under the young Chinese pine in the study site 10a than in the two study sites (25a and 40a). This led to severe loss of soil nutrients because of the lack of vegetation cover on surface soil and rain leaching. Meanwhile, the vegetation cover can have fundamental effects on soil properties (Rutigliano et al., 2004; Singh et al., 1989), mainly due to its contribution towards soil organic matter by supplying carbon and energy sources from root exudates and plant remains. In addition, it is well known that SOC and total N in soil have a positive correlation with secondary forest succession; for instance, the SOC exhibited a positive correlation with total N (r=0.01) in our study (Table 2). As a result, there was relatively low SOC and total N at young Chinese pine secondary succession of 10a. Also, soil MBC could reflect the degree of immobilization of carbon and nitrogen. A decrease in soil MBC could result in mineralizing of nutrients (McGill et al., 1986). Soil with low SOC usually has lower MBC, and vice versa (Guggenberger and Zech, 1999); this was also shown in our study where the SOC exhibited a positive correlation with MBC (r=0.01) (Table 2). In our study, soil MBC gradually increased during the early and mid-succession which is partly due to a similar trend with SOC. Moreover, changes in belowground soil properties were relative to changes in aboveground plant species. With secondary forest succession, the vegetation cover increased which further demonstrated that mineralizing of nutrients was improved because the high level of community understory species supplied enough carbon, nitrogen and energy for microbial growth at the mid stage of Chinese pine secondary succession. Consequently, amount of soil microbes exhibited the peak during the succession stages from 25a to 40a. The result of dendrogram of hierarchical cluster shows that microbial-community of 10a was more similar to 25a and 40a (Figure 4), indicating that succession stage at 10a in this study is a transition stage to the mid-succession in our study.

Lack of water in the soil could also represent an important limiting factor for the development of microorganisms across a successional sequence (Chabrierie et al., 2003). In our study, soil moisture appeared to decrease along our successional gradient (Table 1). Bauder (1999) reported that dry soils generally had low organic matter, because organic matter decomposition was faster than accumulation. Jia et al. (2005) also showed that there were positive relationships between organic carbon and soil moisture, which further suggested that the soil moisture had a significant influence on organic matter accumulation or decomposition; this was in agreement with our result as shown in Table 2. The abundance of microorganisms mainly depends on nutrient and water availability. Therefore, soil micro-diversity exhibited the peak during the mid-succession (25a and 40a). After that, with the extension of succession, Chinese pine has been the dominant species during the late succession of 75a, vegetation cover had also reached its peak, and Wilson (1998) indicated that light also represents the main driving mechanism of secondary successions (Chabrierie et al., 2003). Light not only helps the forest plant community but also is beneficial for the forest soil microbial-community, for instance, the soil moisture exhibited a positive correlation with total N, MBC and SOC (P<0.05 and P<0.01) in our study (Table 2). Compared with the initial succession stage (10a), the mid-late succession showed the high level of community understory species and developed root systems are helpful to the moisture holding capacity. Therefore, we suggest that light and soil moisture are two important factors that influence the soil micro-composition during the Chinese pine forest natural secondary succession.

Howard and Lee (2003) showed that species diversity increases in early succession, peaks at mid-succession, and decreases in late succession. Other studies have shown the existence of a mid-succession diversity peak. However, because of the different objectives of these studies and nature of their methods, a specific range of years corresponding to peak succession diversity has proved elusive. The sample site of the present study is typically natural secondary succession in the forest region of Loess Plateau, China, which is rich in calcium and has calcareous soil with a pH ranging from 7.05±0.01 to 7.10±0.01 as shown in Table 1. Consequently, the alkali abiotic environment would be helpful to the
Figure 4. Dendrogram of hierarchical cluster on natural Chinese pine forests with different forest ages. The samples are indicated by letter codes at the branch termini and the same capital letter represented the three replicate samples: A1, A2 and A3 (soil samples were collected from natural secondary *P. tabulaeformis* Carr. forest at 10a); B1, B2 and B3 (soil samples were collected from natural secondary *P. tabulaeformis* Carr. forest at 25a); C1, C2 and C3 (soil samples were collected from natural secondary *P. tabulaeformis* Carr. forest at 40a); D1, D2 and D3 (soil samples were collected from natural secondary *P. tabulaeformis* Carr. forest at 75a).

The development of soil bacteria and actinomycoses and this is probably one of the reasons for the increasing trend of the indexes of soil micro-diversity, evenness and richness during the early and mid-succession (10a to 40a). Furthermore, as the early natural secondary succession developed, sampling site environment at 10a was complicated, with abundant sunlight and dry and wet synchronization (environmental factors that are all beneficial to plant growth). According to our results, 10a community is a transitional type between the shrubland and forest plant habitats. The soil within the top 0-10 cm is a region heavily occupied by grass roots. Consequently, the effect of grassland species quantity and density on soil properties in this layer is very significant. Thus, changes in grass plant species quantity and density may also play a role in determining soil properties and microbial community structure. During the mid-succession (25a–40a) in this study, Chinese pine had been the dominant species in the sampling site from 25a to 40a. Moreover, *P. tabulaeformis* Carr. has a strong adaptability to dry and barren habitat. It grows quickly and possesses a strong ability to develop roots. It also has the function of conserving soil and water, enriching headwaters and improving soil fertility (Zhang et al., 2006). The process of secondary succession is a self-fertilizing process and nutrient supplies from degraded litter are high in the last stages of succession. However, in our study, nutrient supplies from degraded litter were high in the middle stages of succession, partly due to understory species in the mid-succession in our study which was more complicated (that is, *Ostryopsis davidiana*, *L. bicolor*, *Rose xanthina*, *Rosa primula*, *Cotoneaster acutifolius*, *Cotoneas termultiflorus*, *Spiraea pubescens*, *Prunus tomentosa*, *A. ginnala*, *Viburnum schensianum*, *Syringa pekinensis*, *Lonicera ssp.* and *Euonymus alatus*) than that in early and late succession. The highest richness of understory species represents the highest soil micro-diversity, advanced nutrient cycling and soil fertility in this stage, since the aboveground and belowground components of a terrestrial ecosystem are considered to be dependent on each other (Wardle et al., 2006). The dendrogram of hierarchical cluster showed that there was the most similar relationship between 25a and 40a (Figure 4), indicating that the mid-succession, involving 25a and 40a, represented the most similar micro-structure.

As for the late succession, soil micro-diversity, evenness and richness exhibited the lowest of all succession stages. Vegetation cover reached the peak but understory species was the lowest of all stages; there was surprisingly only one kind of understory shrub in our field site (*Quercus liaotungensis*), which is partly due to the sunlight limitation. Chabrerie (2003) indicated that light also represents the main driving mechanism of secondary successions since light not only helps the forest plant community but is also beneficial to the forest soil...
microbial-community. During the late succession (75a) in this study, soil pH was at its lowest at 75-year-old vegetation (6.92±0.02) as shown in Table 1, indicating that lower pH has restricted the development of micro-composition, especially the soil bacteria and actinomyces (Yao and Huang, 2006). In our study, soil MBC, moisture content, SOC as well as total N exhibited an increase before decreasing trend with succession gradient (Table 1), which indicates soil fertility at its lowest during the late succession. Consequently, the indexes of soil micro-diversity, evenness and richness were also at their lowest during the late succession. The dendrogram of hierarchical cluster shows that micro-structure of 75a was distant to the other three sample sites (Figure 4), indicating that succession stage at 75a in this study is probably a specific stage to the succession of Chinese pine forest.

Conclusion

Vegetation succession is a complicated process which is closely related with its belowground components. In our study, we compared the micro-composition of four Chinese pine forest natural secondary succession stages, including, 10a, 25a, 40a and 75a. We found that there was a hump shaped trend during the succession. The peak plant diversity and soil micro-composition appeared at the mid-succession (25a and 40a), and 10a was a transition stage to the mid-succession. Moreover, we found that soil MBC, moisture content, SOC and total N also exhibited an increase before decreasing trend with succession gradient and that sunlight is also a key factor influencing the plant composition and soil micro-structure.

Soil indexes of the soil shannon diversity index (H’), which incorporates both richness and evenness of all soil micro-species observed in the plots according to T-RFs peaks composition determined by T-RFLP, also exhibited a similar trend with soil physicochemical properties during the succession.

As for the late-succession of 75a, its soil fertility and micro-structure was distant from other three stages, indicating that the succession stage at 75a in this study is probably a specific stage to the recession succession. Vegetation cover at 75a reached the peak but understory species was the lowest of all stages; there was surprisingly only one kind of understory shrub in our field site (Q. liaotungensis). Therefore, we infer that natural secondary succession of P. tabuliformis Carr. forest will be replaced by the Q. liaotungensis in the coming centuries in the forest region of Loess Plateau, China.

REFERENCES


Suo AN, Ju TZ, Ge JP (2008). Relationship between species richness...


