西北农林科技大学

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枯落叶中碳化合物在分解过程中的变化及其与叶际微生物群落的关系

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摘要

黄土高原植被恢复后提高森林生态系统生态服务功能的需求越来越大。作为森林碳循环的重要组成部分，其中一个重要目的是加深对从枯落物到土壤的碳返还过程的理解。为提高森林落叶层的固碳能力，迫切需要明晰微生物在落叶层分解过程中的作用。叶际微生物群落在森林生态系统的有机质分解和养分循环中起着重要作用。从微观生态学角度看，枯叶凋落物分解是黄土高原地区生态恢复的核心和基础。据此，本论文将研究主要集中在:1)典型森林系统枯落物的营养、物质化学计量比和结构碳水化合物的分解变化；叶际微生物群落结构与凋落物养分分解过程的关系。试验在黄土高原子午岭森林区进行。选择了三种阔叶树:CL(刺槐)、LL(辽东栎)和 SL(山杨)的凋落物作为研究对象。采用传统分解袋法，在野外布设实验样区后，定期测定凋落物养分(C、N、P)变化、(C:N:P)比值及碳水化合物动态变化。同时，采用 Illumina 平台的高通量测序技术进行叶际微生物群落分析。研究结果如下: 1)枯落物 C 在凋落物分解的前 6 个月显著减少，随后在分解过程中缓速下降；氮在整个分解过程中波动，磷在分解结束时的损失率较高；计量比随分解逐渐降低。2)分解时间对营养物质和结构碳水化合物的影响均大于树种；3)刺槐和辽东栎叶际微生物群落多样性和丰富性在分解 10 个月和 12 个月是分别达到最大值。变形杆菌和放线菌是最主要的细菌门。在真菌群落中，子囊菌门和担子菌门是最大的优势门；4)微生物群落与凋落物化学养分(主要营养元素 C、N、P 和结构性碳水化合物)呈显著正相关；5)碳水化合物和氨基酸代谢在枯落物分解到 8-10 个月时达到最强，其次是外源性生物降解代谢、脂质代谢和能量代谢。

关键词：枯落叶分解；微生物群落；主要营养物质；结构性碳水化合物；16s rRNA

ABSTRACT

After vegetation restoration on the Loess Plateau, there is an increasing need for improving the ecological service function of forest ecosystem. One of the essential target is to deepen the understanding of C turn over from leaf litter into soils, which contribute to important C cycle in forest. In order to develop the carbon sequestration capacity, the act of microorganisms in the forest leaf litter during decomposition process is urgently required. Phyllosphere microbial community of leaf litter decomposition plays a significant role in organic matter decomposition and nutrient cycling in forest ecosystem. From micro-ecology pint of view, leaf litter decomposition is the core and basis for the ecological restoration in the area of the Loess Plateau. In order to go through all the above mentioned points we designed a proper research plan and have been doing the investigation. The investigation mainly focus on: i) The change of typical forest communities in leaves during the decomposition of nutrients stoichiometric ratio and structural carbohydrates; and ii) The relationship between phyllosphere microbial community structure and leaf litter nutrients during decomposition. The experiment was carried in Ziwuling forest, Loess Plateau, China. Three broad leave tree: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) were selected as targeting leaf litter. The decomposing litter bag method was implemented and leaf litters were analyzed for the nutrients (C, N, P) changes, (C:N:P) ratio and carbohydrate compounds dynamics. In addition, high throughput sequencing based Illumina platform was used for microbial community analysis. The overall results of the study are summaries below:

1) Substantial leaf litter C losing was detected at the first 6 months and then it decline constituently and nitrogen fluctuated throughout the decomposition process. However, phosphorus had high losing rate at the end of decomposition. And the nutrients ratios declined with decomposition.

2) Decomposing time was more influential factors for both primary nutrients and structural carbohydrate than tree species and it shows a significant difference over the decomposition periods.

3) In terms of microbial community diversity and richness, locust and oak leaf litter at the 10 and 12 months decomposing were outnumbered comprising with the rest samples. Proteobacteria and Actinobacteria are the most dominant bacterial phyla. Fungal community

diversity indices illustrate that samples decomposed around one year (10 to 12 months) had very high trends in both; richness and diversity. Moreover, Ascomycota and Basidiomycota were the largest dominator phyla among fungal communities.

4) There are a significant positive correlation between microbial communities and leaf litter's nutrients (primary nutrients and structural carbohydrates).

5) Carbohydrate and amino acid metabolism were the highly stimulating functions during 8-10 months leaf litter decomposition, followed by up by the xenobiotics biodegradation and metabolism, lipid metabolism and energy metabolism.

For further study in this area, it is suggested in order to deeply understand and find out more reasonable results for scientific evidences, the likewise analysis should be done in soil from the same plats. It will provide more accurate and comparative data.

KEY WORDS: LEAF LITTER DECOMPOSITION; MICROBIAL COMMUNITY; PRIMARY NUTRIENTS; STRUCTURAL CARBOHYDRATES; 16S RNA

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第一章 文献综述

Chapter 1 Introduction

1.1 碳和主要养分

1.1 Carbon and primary nutrients

Leaf litter decomposition is one of the main processes that recycle nutrients back into the soil. And this process affects both structural and none-structural feature of terrestrial ecosystem [1]. Contribution of leaf litter in term of turnover of organic substance to the soil structural matrix of the ecosystem is a natural process. This environmental state leads to form soil organic matter source by the regularly procedure of nutrient cycle in biomass. Moreover, the reprocessing of litter nutrients is an important for ecosystem dynamics. The regulation of rate and timing of nutrient release also plays vital roles in leaf litter decomposition. Discovering the variation in "C: N: P" stoichiometry of any plant species is the main portion of the investigation. The regulation and balance of these nutrients can influence all the ecological aspects. It could be inspected stoichiometry mechanisms regulating the "C: N: P" ratios of ecosystem compartments and of nutrient dynamics across different tree species.

Stoichiometry allows for spanning various stages of biological organization, from primary metabolism to ecosystem compound's structure and nutrient cycling, and is therefore specifically worthwhile for establishing linkages between different

ecosystem compartments [2]. All organisms, from single cell organisms to multicellular organisms, communities, and ecosystem and even to the biosphere, require energy and nutritional resources in stoichiometric ratios, and so could be simplified and featured into some elementary ratios [3]. Decomposition of living things makes available the products of primary photosynthesis to detritus-based food web and releases inorganic matters into the habitat. For instance, leaf litter decomposition and it plays a vital role in nutrient dynamics and other relevant environment such as forest, grassland and cropland. Decomposers such as soil microorganism can sequester carbon and other nutrients including N and P from organic substrates and exchange inorganic nutrients with the environment to maintain their stoichiometric balance. Additionally, physical losses of organic compounds from leaching and other processes likewise, weathering such as freezing, thawing, drying and erosion may alter litter nutrients.

Generally, there is evidence that leaf litter decay is slowed by cool temperatures in tropical forests [4]. In contrast that climate warming in the tropics is occurring faster at higher elevations [5]. This indicates that various physical factors also influence the decomposition process. The initial rapid decrease of nutrients concentration observed may be due to the loss of the soluble forms of nutrients at the initial stages of decomposition and a slower release of nutrients at the later stages of leaf litter decomposition governed by microbial oxidation of refractory components and physical and biological fragmentation [6]

Carbon (C), nitrogen (N) and phosphorus (P) are three main elements that exist in living organisms as three main biogenic elements. Their interactions are carriers mediating mass and energy flow in ecosystems. Thereby, deciphering C, N and P distribution patterns, C, N and P stoichiometric characteristic and their impacting aspects would contribute to recognize links among ecological biogeochemistry and structures, procedures and functions in various plant species.

The ratios of "C: N: P" in environments and organisms are intimately connected with ecological processes, from the structure of communities [7] to biodiversity. These connections drive a further investigation into environmental factors that determine the "C: N: P" ratios of terrestrial plants [8]. Moreover knowledge about "C: N: P" ratios deepen our understanding about resource allocation of organisms under nutrient dynamic conditions. Terrestrial organisms interact with the ambience and emulate themselves to the environmental "C: N: P" ratios such as soils.

Furthermore, our understanding about C, N and P relationships in terrestrial ecosystems is still limited [9]. On average, atomic "C: N: P" ratios in global soil (186: 13: 1) is well-constrained [10]. But these ratios varied greatly from local to regional scales or among ecosystems [9]. It seems that C: N ratios could keep relatively steady within a narrow range, while C: P and N: P ratios varied violently, often strictly depending on ecosystems. However, the C, N and P changes and "C: N: P" ratios distribution patterns and affecting factors are still poorly constrained.

In the field of forest ecology, understanding the importance of C, N and P in leaf litter

and how they vary during decomposition and among litter types will shed insights on the mechanisms driving litter decomposition, and how leaf litter decomposition and controls the source of C, N and P to basic microbial and chemical control on the biogeochemical cycle. We aimed to i) discover the biogeochemical regulation of C, N and P cycle and ii) to investigate an enlarging imbalance of element stoichiometry exists. Specifically, we will investigate the C, N and P changes and their "C: N: P" ratios changes during leaf litter decomposition within three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) of tree in Ziwuling, Shaanxi, China.

1.2 碳水化合物

1.2 Carbohydrates compounds

Litter decomposition plays a vital part in the nutrient budget of a forest ecosystem, where flora is influenced most significantly by nutrient recycling from leaf litter decomposition. Litter decomposition in terrestrial ecosystems has a major role in the biogeochemical cycling of elements in the environment. Climatic features, like temperature, rainfall, humidity, and seasonal variations affect the rate of litter decomposition [11]. Nutrients are effectually driven within a certain ecosystem throughout leaf litter decomposition and the leaf litter species based, has more complex carbon compounds concentration different decay rate [12]. In most studies focus on the dynamics of leaf litter nutrients are scaled by the length decomposition

process. Leaf litter chemicals and their dynamics throughout decomposition process have been considered as essential controlling factors, that can indicates approximately 60–70% of litter decay rates globally [13] [14]. Recalcitrant component of litter contents are considered as the most vital biopolymer and polysaccharide in leaf litter and it was found out that most of them typically constitutes 20–30% of origin litter mass [15]. The decomposability of recalcitrant component are vary depends on the type of elements for instance in previous study it was hypothesized that lignin is to protect labile litter components from microbial attack because lignin resists degradation and influence cellulose, hemicellulose, and protein in plant cell walls [16]. In addition, leaf litter decomposition rates vary widely among species that decompose in identical ecological situations and environmental condition. Furthermore, because of the various decay rates of its numerous compounds, litter dynamics changes over time in terms of NDF (neutral detergent fiber), ADF (acid detergent fiber), ADL (acidic detergent lignin), semi-cellulose, cellulose and lignin and continuously affecting and changing the decomposition process. In this study we will focus on i) the change of structural carbohydrates ii) and their association with microbial community.

1.3 叶际微生物群落

1.3 Phyllosphere Microbial communities

The phyllosphere is an ecologically and economically important ecosystem that hosts

a large and diverse microbial community [17]. On the earth the gigantic habitats of phyllosphere, the microbial habitat found on the surface of leaves, , with terrestrial leaf surface area expected to reach around 108 km^2 globally [18]. The phyllosphere, known as herb foliage as a microbial habitat – is considered a hostile environment for survival and colonization by microorganisms that fulfill the function of rapid bio-physical fluctuation in solar radiation, temperature, humidity, and heterogeneous availability of nutrients [17] At 10^5 to 10^7 cells/g plant material, such as residues or litters bacteria are normally the most abundant colonizers in the phyllosphere and constitute approximately 10^{26} cells globally [19] Microorganisms are highly diverse and ubiquitous in all kind of ecosystems; they participate in a variety of key ecosystem functions of decomposition process such as nitrogen cycling, mineralization, contribution and preservation of soil organic matter, feeding back responses to climate change and biomass production in order to keep the process cycle [20].

In this study, our aim was to identify and compare the bacterial diversity and dynamics of leaf litter of decomposition in three various tree species phyllosphere at a single geographical site over certain time during one year. This data and survey are needed to analytically assess whether specific species microorganisms or microbial community contribute to the differential leaf litter decomposition process. In recent years, the structure and function of phyllospheric microbial communities have attracted worldwide attention [21, 22] High-throughput technologies have provided

comprehensive datasets portraying microbial life in the phyllosphere by employing transcriptomics [23] and metaproteogenomics, a combination of shotgun metagenomics and proteomics [23]

The phyllosphere of leaf litter is inhabited by diverse microorganisms, though the aspects formulating their community composition are not fully investigated. The tree leaf litter represents the initial contact surface between microorganisms and the plant. We thus aimed to investigate whether mutations in the different leaf litter would affect the diversity of the phyllosphere microbial community during a certain period of decomposition. The leaf litter associated bacterial communities of the three tree species were examined by high throughput second generation amplicon pyrosequencing. We present findings that suggest that plant leaf tree species and environmental aspects are the important factor shaping the community composition of phyllosphere bacteria during leaf litter decomposition. The aim of this study was to find out the changes in phyllosphere bacterial community time in three site and between three tree species, with indicators of bacterial abundance, diversity, and community composition in one year period of time, followed by investigation of the composition of bacteria microbial community. Although the 3 tree treatment leaf litter encompassed different bacterial communities, the bacterial community compositions displayed inconsistent changes in response to the addition of different leaf litter decomposition in across various leaf litter samples. The result that the addition of leaf litter altered leaf litter microbial community was consistent with our

hypothesis and some previous findings [20]. Apart from the community composition, whether the leaf litter microbial abundance, diversity, and function, all respond similarly to decomposition of leaf litter, remains unknown. In this study, we investigated phyllosphere bacterial communities in CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) of tree in Ziwuling, Shaanxi, China. We provide detailed data on the diversity and compare the bacterial community among mentioned tree species.

第二章 材料与方法

Chapter 2 Method and materials

2.1 样区设置和元素化学分析

2.1 Plot set up and nutrient chemical analysis

The leaf litter of CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) of trees were collected from Ziwuling, Shaanxi, China.The leaf litters were collected from the top layer of litter so as to primarily use leaves fallen during this autumn. Then the leaf litters were air-dried before incubating them in litter bags, and oven-dried all litter at 65ºC before conducting analyses. To compare the initial air-dry weights to final oven-dry weights, It was weighed separate

air-dried samples of litter and oven-dried them, to create an air-dry to oven-dry ratio for the initial leaves. This allowed to calculate an initial oven-dry weight for the litter that. The purpose was to compare the final oven dry weight after incubation. Before measuring carbon, nitrogen content, and phosphorus content, the subsamples sorted of litter using a Wiley Mill. Litter Bag Incubation was placed 3-5 g of air-dried litter from each species in approximately 400 cm2, 1 mm-mesh bags, sewn on three sides and closed on the fourth side using staples. Each species had three replicate bags for each of four time periods at each of two sites. Then the due to the instruction it we arranged bags within a time period 2 months distance between every sampling time. The samples' bags on October 3rd, and collected sets of bags from 23th to 28th of every two months. After collection, the leaf litter bags were stored in individual plastic zip-top bags at -20ºC until processing. Afterward it was oven-dried at 65ºC and weighed it to calculate a change in mass during incubation. After enzyme analysis, the litters were dried and weighed the subsamples taken for enzyme assays, in order to obtain a total final mass for the incubated litter.

Carbon and Nitrogen were weighed 4-6 mg of finely ground litter (mesh size of at least 40 in a Wiley Mill, or WigLbug) and folded it into tin vials to be combusted and measured using a CHN elemental analyzer (Perkin-Elmer), which measured the fractions of carbon and nitrogen in the litter. The following step was conducting CHN analysis on composites of two replicate bags, so there were two composite samples analyzed for each species at each site for each time period. The method for my

phosphorus analysis was based on a protocol for measurement of total phosphorus in soils and sediments (Harwood et al. 1969). Later on we measured approximately 0.1 g finely ground litter into glass scintillation vials etched with numbers for identification. To each vial I added 0.5 mL 50% weight by volume magnesium nitrate solution and allowed the litter to absorb the liquid and then ashed the vials at 550ºC to remove organic material. After war it was re-suspended the remaining ash in 10 mL 1 N hydrochloric acid and shook the vials for 16 hours. Finally, diluted this solution 20x and measured phosphate concentrations colorometrically using a method adapted from Murphy and Riley (1962).

2.2 碳水化合物含量测定方法

2.2 The experimental principle of carbohydrate content elements

There several ways to follow in order to get recalcitrant elements result from leaf litter decomposition. Below the steps are explained that in every stage we get values.

(1) Applying a neutral detergent (3% sodium dodecyl sulfate) to dissolve most of the cellular contents of the plant material, removing neutral detergent solute (NDS), including fat, sugar, starch and Protein and so on. The insoluble residue is called neutral detergent fiber (NDF), mainly cell wall components, and including impurities such as cellulose, hemicellulose, lignin, and other impurities.

(2) The components of the neutral detergent fiber (NDF) can be further separated by applying an acid detergent. Soluble in the acidic detergent group, the acid detergent

solute (ADS), including the neutral detergent solute (ADS) and semi-cellulose; the remaining residue is called acid detergent fiber (ADF), including fiber, lignin and laurate. The difference between neutral wash fabric fiber (NDF) and acid wash paint fiber (ADF) is the semi-cellulose content.

(3) When the acidic wash fiber (ADF) is digested with 72% sulfuric acid, the cellulose is dissolved and the residues thereof are lignin and silicate. The residue after sulfuric acid digestion was subtracted from the acid wash fiber (ADF), which is the cellulose content.

(4) The residue after digestion of 725 sulfuric acid is washed out, the ash is silicate, which is called acid insoluble ash, while the part that escapes in the ash is acidic detergent lignin (ADL).

2.3 微生物群落 DNA 提取

2.3 Microbial community DNA extraction

Leaf litter DNA was extracted from 0.5 g soil samples using the MoBioPower Soil DNA isolation kit (12888) following the manufacturer’s instructions. The extracted leaf litter DNA was stored at −20 ℃ until further processing. The V4 hyper variable region of the bacterial 16S rRNA gene and fungal r18RNA were amplified using the forward primers 502F (5'-AYTGGGYDTAAAGNG-3') and the reverse primer 802R (5'-TACNVGGGTATCTAATCC-3'). PCR reactions were accomplished in triplicate in a 25-μL combination covering 0.25μL Q5 high-fidelity DNA polymerase,5 μL of

5XReaction buffer, 5 µL of 5X High GC buffer, 0.5 µL of dNTP mix (10mM strength), 1µL of template DNA (~10 ng), 1 µL of each primer (10 µM) and 11.25 µL of DNase free water. The 98 ℃ for 30s, followed by 25-27 cycles at 98 ℃ for 15s, 50 ℃ for 30s, with a final extension step at 72 ℃ for 5.5 min with the thermal program was implemented . The 2% agarose gel was counted as products of PCR. Marker used was DL 2000 DNA Marker takara 3427A and the obtained DNA ladder sizes were 100 bp, 250 bp, 500 bp, 750 bp, 1000 bp and 2000 bp.

The Illumina MiSeq sequencing - Amplicons were dig out from 2 % agarose gels and distilled using the Axygen Axy Prep DNA Gel extraction kit (AP-GX-500) following the manufacturer's instructions and quantified on Micro plate reader (BioTek, FLx800) using Quant-iTPico Greends DNA Assay Kit, Invitrogen (P7589) and it was done oftter sampling sort out.. Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) using MiSeq Reagent Kit v2 (600-cycles-PE) (MS-102-3003), on an Illumina MiSeq platform (Personalbio, Shanghai) according to the standard protocols. Bioinformatics' processing of the Illumina results - Raw FASTQ files were de-multiplexed and quality-filtered using QIIME (Quantitative Insights Into Microbial Ecology, v1.8.0, http://qiime.org/) with the following criteria: (i) The 300-bp reads were truncated at any site that obtained an average quality score of <20 over a 10-bp sliding window, and the truncated reads shorter than 150 bp were removed and reads containing ambiguous characters were discarded; (ii) the FLASH software (v1.2.7, http://ccb.jhu.edu/software/FLASH/) (Magoč and Salzberg, 2011)was used to allign

the overlapping sequences that passed the quality screening with ≥10 bp. Mismatched reads were discarded. The residual sequences were clustered into operational taxonomic units (OTUs) using UCLUST. OTUs with 97% similarity cutoff were grouped on the basis of an open reference by searching reads against the Green genes database (Release 13.8, http://greengenes.secondgenome.com/) (DeSantis et al., 2006), OTUs with the abundance of lower than 0.001% of total amount from the sample sequenced were not considered in final analysis(Bokulich et al., 2012). OTUs assigned to same taxonomy were combined at various taxonomic levels. Phylogenetic diversity of the whole tree (PD, whole tree) was used as the estimation of α-diversity, which incorporates the phylogenetic breadth across taxonomic levels (Faith, 1992; Faith et al., 2009). A cut-off value of 45,000 sequences (per sample) was used for sub sequent community analysis, in order to minimize the survey effort (number of sequences analyzed per sample). The variances in the general bacterial communities between each pair of samples were resolute consuming the UniFrac space metric analysis (Lozupone and Knight, 2005). This UniFrac analysis is based on assessing branch length of the tree as a measure of phylogenetic distance between the taxonomical groups under question leading to their posterity from either one or the other environment. Both weighted (quantitative) and unweighted (qualitative) variants of UniFrac (Lozupone et al., 2007) are broadly used in microbial ecology, where the former accounts for abundance of observed organisms (Lozupone et al., 2007), while the latter only considers their presence or absence

(Lozupone and Knight, 2005). The analyses mentioned above were accomplished using the MOTHUR program (http://www.mothur. org). The primary sequence data is deposited in Sequence Read Archive (SRA) database of NCBI.

Photos from outdoors and indoors activities

第三章 结果

Chapter 3 Result

3.1 枯落物分解过程中化学养分变化

3.1 Leaf litter chemical change and nutrient dynamic during decay

3.1.1.1 不同分解时期碳元素变化

3.1.1.1 The Carbon change within different sampling time

At the global scale, the dynamics of C, N and P and other nutrients in leaf litter varies due to tree species and other biophysical factors. Annual decay rate of the leaf litter based on one year was investigated and all leaf litter showed distinct various changes in decomposition rates. The decomposition carbon rates were also highly variable, with significant annual variation along all 6 sampling times. Carbon remained constant in Cihuai litter or Locust litter (CL) from first till third sampling time, there was not any significant difference in the early stage of leaf litter decomposition. It dramatically fall down from 470 g/kg which was the top rage to 380 g/kg in forth sample. Litter-C was decreased more in CL in 5^{th} and 6^{th} sampling time 350 g/kg and 330 g/kg, respectively and they were very significant different from all other sampling time (Fig.1).

Likewise, carbon in Liaodong Oak (LL) tree species the first stage of leaf litter

decomposition was with high rate of Carbon (range of 480 g/kg) and there was not significant difference between 1st and 2nd samples. The highest rate in Shaoyang litter or Aspen leaf litter (LL) tree species showed a peak at the 3rd sampling time (500 g/kg) and it intensely decreased to 470 g/kg and it was very significant different comparing with the previous sampling time and it had not significance difference with 5th sampling time. Then it leveled off till 5th sampling time and we observed a slight change between 4th, 5th and 6th sampling times. 6th sampling time was had the lowest rate 360 g/kg and showed the significant differences with the rest of sampling times (Fig.1).

The verity and change of carbon over sampling time was observed in SL and all the sampling time were significantly different from each other. It slightly decreased from 500 g/kg from first sample to 470 g/kg in second sample and again it rose to the same level (500 g/kg) in third sample. It fall down to 320 g/kg in 4th sampling time which was the lowest rate along all sampling time and it showed the significant differences in comparing with other sampling time (Fig.1). It had a steeper increase in 5th sample and drop to 300 g/kg at the last sampling time.

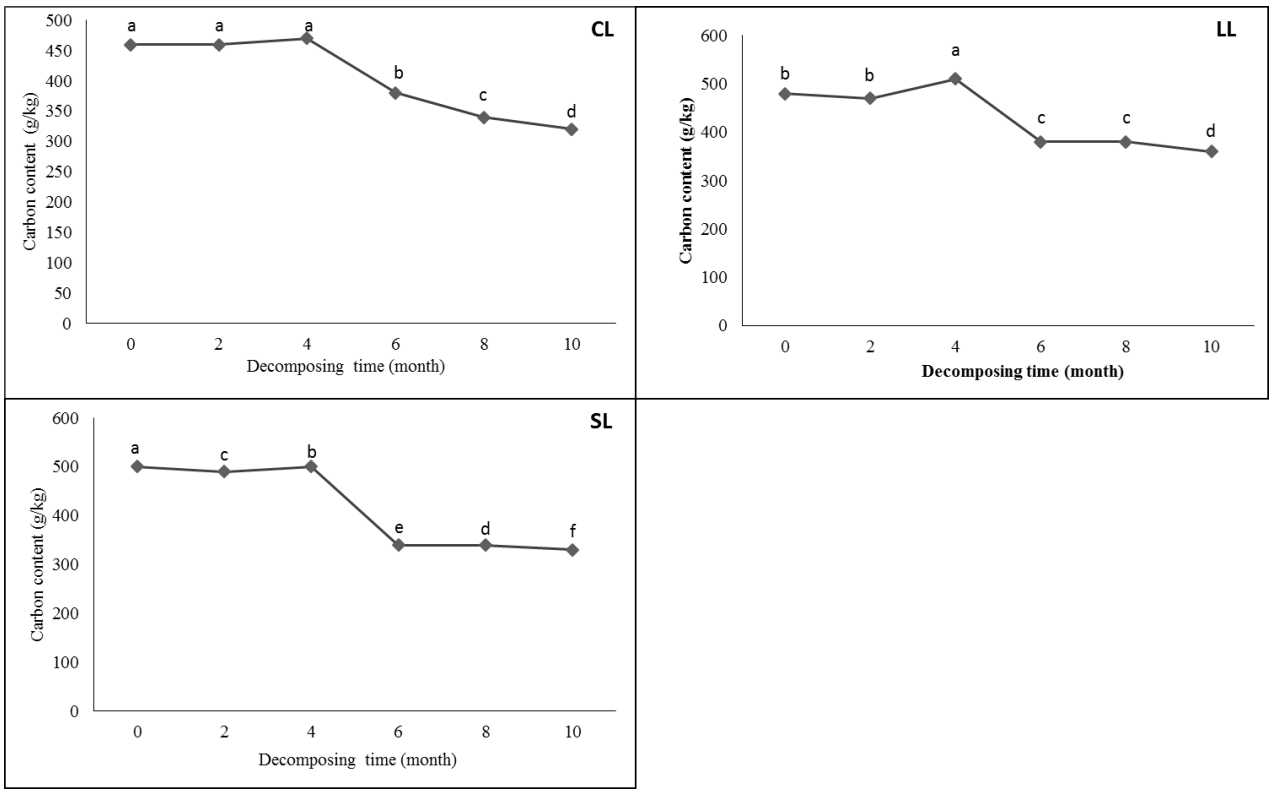

图 1 不同分解时期碳元素变化。 CL（刺槐）、LL（辽东栎）、SL（山杨）

Figure 1. Changes of carbon concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.1.1.2 不同分解时期氮元素的变化

3.1.1.2 The Nitrogen change within different sampling time

Verity in decomposition trends within the three leaf litter tree species are more likely

to be related to the direct special effects of substrate feature such as nitrogen concentration and dynamics (Fig 2.). The poorer initial nitrogen dynamic of early samples of leaf litter LL (7.5 g/kg) and SL (8.5 g/kg) may explain its lower decomposition rate; by contrast, initial nitrogen concentrations were 20 g/kg for CL leaf litter. The Percentage of nitrogen in leaf litter decomposition increased steadily over 3 samples in CL and it reaches 25 g/kg in the 3rd sample. Except 5th and 6th samples there was very significant difference within all samples (Fig 2.).

In LL species the rate of nitrogen was gigantically low (6.5 g/kg) with very different significant rate it sharply rose to just under 12 g/kg and keep leveled off with next sampling time. The trend decline in 4th sample by 10 g/kg and it slightly increased in the following sample and then reach the top rate to 14 g/kg in final stage (Fig 2.). The two last samples had a wide range of significant differences in compare with rest of samples.

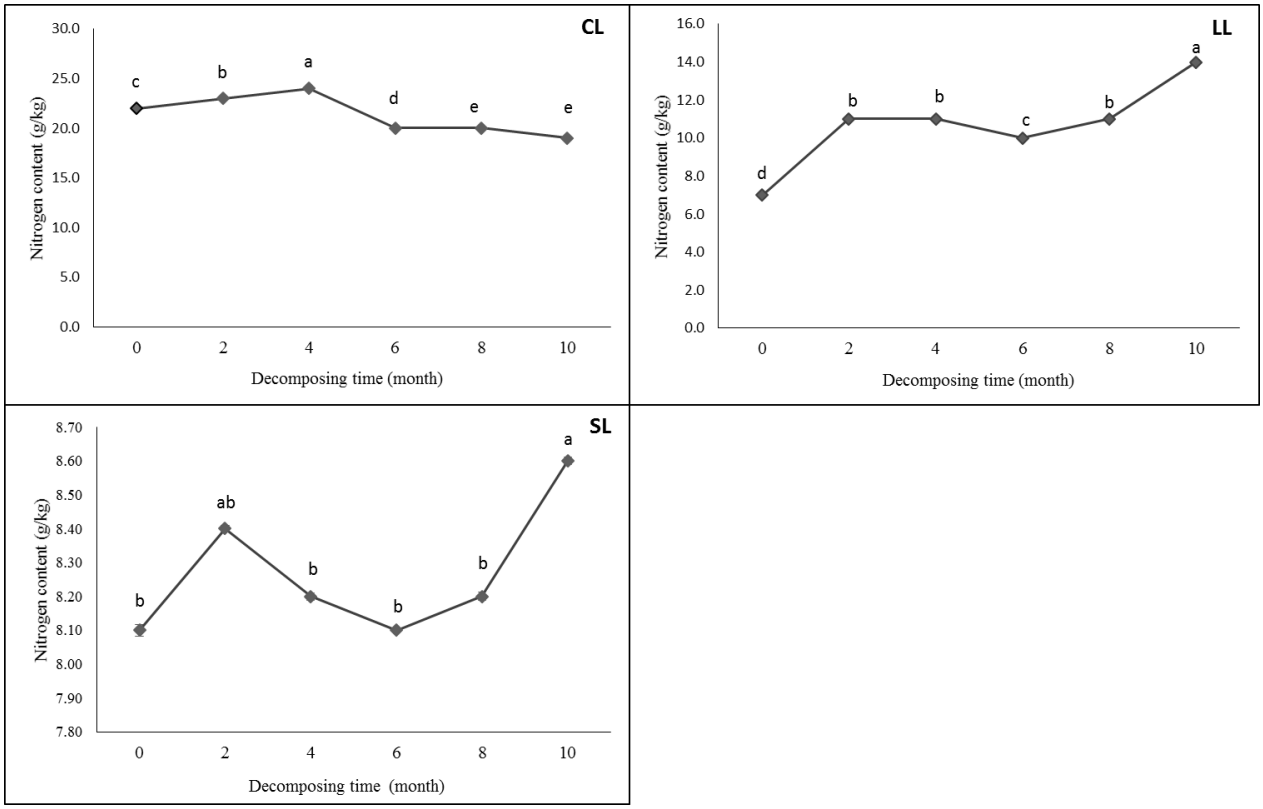

图 2　不同分解时期氮元素的变化

Figure 2. Changes of nitrogen concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.1.1.3 不同分解时期磷元素的变化

3.1.1.3 The phosphorus changes within different sampling time

Phosphorus dynamic and concentration from decomposing leaf litter were

significantly higher and straight upward in all leaf litter decomposition sampling time. In CL tree species the trend Phosphorus leaf litter decomposition concentration and dynamics were significantly different within all sampling times. It slowly started to growth form 0.7 g/kg and kept the trend upward and it reaches it high pint at 0.8 g/kg in the last sapling times (Fig 3.). However, in LL the initial stage of decomposition was in high rate (0.9 g/kg) and it slightly decreased in the second and third samples 0.8, 0.7 g/kg, respectively. The trend remained constant within 4th and 5th samples and there was not significant differences among these two sampling time. Increase of phosphorus concentration and remaining stocks in the following samples it had a peak at 6th sampling time by 1 g/kg litter decomposition rates and the last sampling time had a great significance differences with all samples.

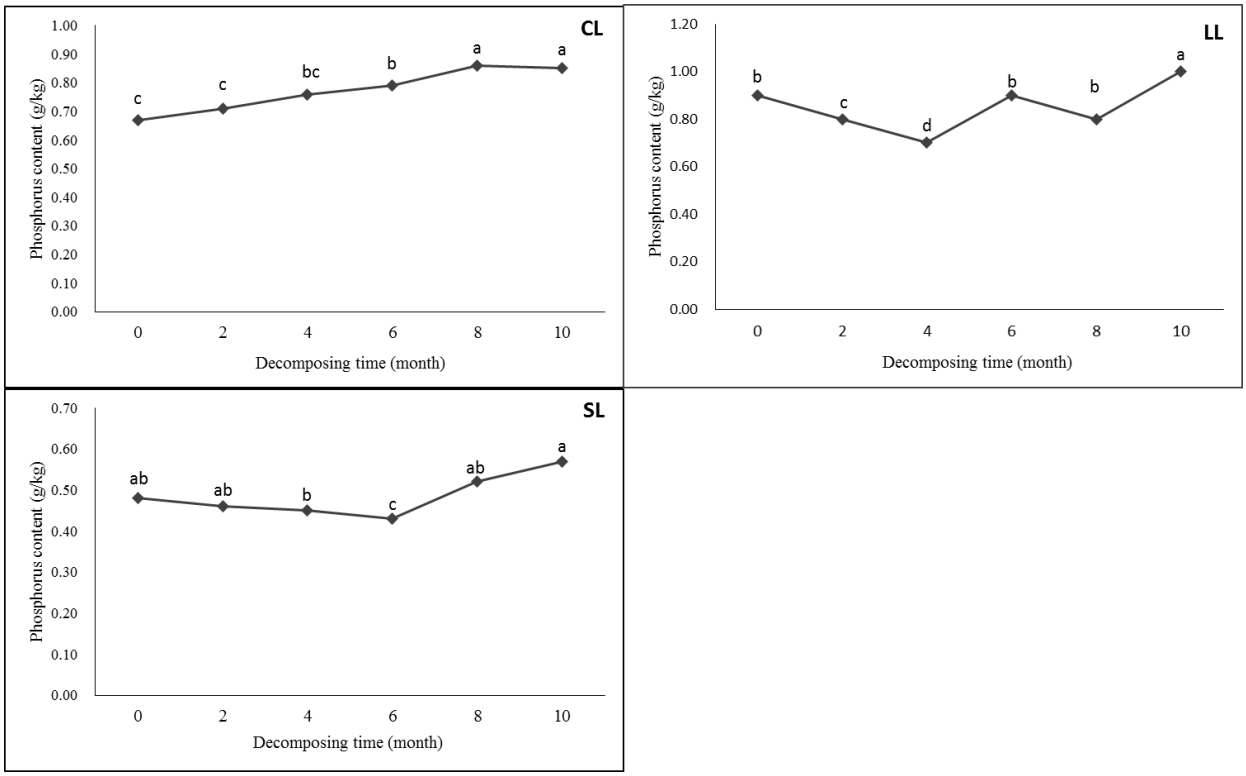

图 3 不同分解时期磷元素的变化

Figure 3. Changes of phosphorus concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.1.2.1 C: N: P 计量比变化

3.1.2.1 Changes of the ratios of C: N: P

Beyond the focus on the leaf litter primary nutrients such as C, N and P dynamics and the concentration, the C:N:P ratio stoichiometry is also a vital indicator of chemical change of leaf litter in a certain time of decomposition. The C:N:P ratio on various sampling time for leaf litter of three various tree species were tested by considering mean and standard deviation and have presented in table 1. In addition, across-sampling time significant differences were also tasted for C:N, C:P, and N:P ratios ($P < 0.05$). C:N ratios decreased throughout the one-year leaf litter decomposition investigation. In CL specie the initial stage of C:N ratio started from 21.13 ± 0.40 (considering mean $\pm$ standard deviation) and it end with 16.67 ± 0.04 at the last sampling time. The mean trend goes down however the standard deviation fluctuated throughout the sampling time. Except 3rd and 4th sampling time, significant differences was observed among other samples. The C:N ratio of LL leaf litter,

which was initially 69.66 ± 0.99, dropped triple time to 26.26 ± 0.16 by the end of year 1. There was not observed significance differences at 2nd and 3rd sampling time, while the rest of samples had great significant differences. SL leaf litter, had an almost constant trend and no significant differences of C:N ratio at 4th and 5th samples 41.57 ± 0.23, 41.91 ± 0.52, respectively. In general the C:N ratio for SL begin form 62.37 ± 1.20 at 1st, decline to 58.10 ± 0.66 at 2nd sample and again rose to 61.00 ± 0.89 the following sample and finally decreased to 38.35 ± 0.56 at last end of year (Table 1).

The trend of C:P ratio in CL specie also steadily decreased throughout the investigation. Presenting in details, the rate of C:P for CL were 883.15 ± 7.80, 651.48 ± 13.48, 616.37 ± 7.55, 483.72 ± 11.07, 399.67 ± 3.87, 376,45 ± 1.29, 1st, 2nd, 3rd, 4th, 5th, and 6th sampling time, respectively. Moreover, the significant differences were observed among all sampling times. The C:P ratios in LL leaf litter decomposition did not exhibit this trend. It initially started from 535.1 ± 9.83 and peaked to 718.10 ± 34.65 at 3rd sample and again slightly remained constant at the two following sampling time and finally dropped to 359.25 ± 7.13 at the end of survey. Despite the 4th and 5th sampling time the significant differences were presented in all other sampling time (Table 1). Similar to the LL specie, the C:P ratio of SL specie increased from 1st (1047.02 ± 32.14) to 3rd (1106.81 ± 36.13)sampling time then it dramatically reduced at 6th (585.54 ± 27.5) sampling time.

N:P ratio in CL specie somewhat declined over all the sampling times. In begun

from 32.34 ± 0.97 and decreased to 22.58 ± 0.08, 1st and 6th sampling times, respectively. The significant differences were not seen in the first 3 samples, just the 3 last sampling time had significant difference. N:P ratios in LL were initially quite low (7.68 ± 0.19) and tended to fluctuate between 14.15 ± 0.40 and 13.68 ± 0.35 throughout the study. The N:P ratios of SL species leaf litter also was vary somewhat during decomposition. There was not significant differences among 2nd, 3rd, 4th (a) and 5th, 6th (c) sampling times and their trend showed quite steady rates (Table 1).

表 1 不同分解时期枯落叶元素计量比变化

Table 1. Changes in mean and standard deviation of C:N:P ratios throughout 6 sampling times of decomposition of three tree leaf litter leaf litter.

Items	species	1st	2nd	3rd	4th	5th	6th
C/N	CL	21.13 ± 0.40 a	20.21 ± 0.13 b	19.24 ± 14.56 c	18.95 ± 0.07 c	17.53 ± 0.14 d	16.67 ± 0.04 e
	LL	69.66 ± 0.99 a	43.36 ± 0.45 b	44.42 ± 0.75 b	38.69 ± 0.69 c	35.29 ± 0.26 d	26.26 ± 0.16 e
	SL	62.37 ± 1.20 a	58.10 ± 0.66 c	61.00 ± 0.89 b	41.57 ± 0.23 d	41.91 ± 0.52 d	38.35 ± 0.56 e
C/P	CL	883.15 ± 7.80 a	651.48 ± 13.48 b	616.37 ± 7.55 c	483.72 ± 11.07 d	399.67 ± 3.87 e	376.45 ± 1.29 f
	LL	535.18 ± 9.83 c	630.70 ± 11.06 b	718.10 ± 34.65 a	435.77 ± 9.49 d	448.54 ± 7.09 d	359.25 ± 7.13 e
	SL	1047.02 ± 32.14 b	1048.24 ± 23.48 b	1106.81 ± 36.31 a	775.75 ± 12.39 c	662.48 ± 8.98 d	585.54 ± 27.5
N/P	CL	32.34 ± 0.97 a	32.23 ± 0.84 a	32.03 ± 0.46 a	25.52 ± 0.54 b	22.80 ± 0.06 c	22.58 ± 0.08 c
	LL	7.68 ± 0.19 e	14.55 ± 0.40 b	16.18 ± 1.04 a	11.26 ± 0.20 d	12.71 ± 0.29 c	13.68 ± 0.35 b
	SL	16.79 ± 0.34 b	18.05 ± 0.60 a	18.15 ± 0.81 a	18.66 ± 0.33 a	15.81 ± 0.40 c	15.27 ± 0.59 c

Notes: Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the sampling time after the placement of the leaf litter bag. (1st -first

sampling after 2 months of decomposing, 2^{nd} – 2^{nd} sampling after 4 months of decomposing, 3^{rd} – 3^{rd} sampling after 6 months of decomposing, 4^{th} – 4^{th} sampling after 8 months of decomposing, 5^{th} – 5^{th} sampling after 10 months of decomposing and 6^{th} – 6^{th} sampling after 12 months of decomposing). The sample was taken every after two months.

3.2 枯落物分解过程中碳水化合物的变化

3.2 Change of structural carbohydrates during leaf litter decomposition

3.2.1.1 中性 (NDF)、酸性洗涤纤维(ADF)、酸性洗涤木质素(ADL)变化

3.2.1.1 Nutritional fractions: neutral detergent fiber (NDF), acid detergent fiber (ADF) and Acid detergent lignin (ADL)

Nutritional fractions might contribute to the leaf litter decomposition process, but the effect could be differ along the sampling times. A two – way analysis of variance tested the change of nutritional fractions such as neutral detergent fiber (NDF), acid detergent fiber (ADF) and Acid detergent lignin (ADL) in three various tree species including CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) along six sampling time within 2 months interval. Sampling time as a main factor showed a great significant rate in NDA changes ($F = 103668$, $P = 0.001$). Leaf litter species also performed a significant level ($F = 941$, $P =$

0.001) and the interaction of sampling times and leaf litter species revealed significant number (F = 21187, P = 0.001) (see Table 1). Likewise, the change of ADF had the sampling time as leading influencer of leaf litter decomposition process and presented significant rate (F = 102421, P = 0.001). All the various sampling times were significantly affected by interaction of the different kind if leaf litter species during decomposition process (Table 1, P < 0.05). The leaf litter species exhibited a normal significant amount (F = 256, P = 0.001). The interaction between the above mentioned factors are similarly had a gigantic significant trend in ADF change. The dynamic of ADL in leaf litter decomposition also had significance at both factors; sampling time (F = 344520, P = 0.001) and species based (F = 233827, P = 0.001). The interaction between sampling time and species was significant (F = 59886, P = 0.001). Individually, in all species along the six sampling time the significant was observed between all of them. The NDF had a great high concentration in SL comparing with CL and LL species. Overall, the mean starts from 43.04 % and reached to 59.62 % for CL, from 48.30 to 55.37 % for LL and 48.66 % to 65.88 % for SL species. As it obvious there was a great significant differences of nutritional fractions change among the three leaf litter species in decomposition process.

表 1 枯落物在不同分解时期的中性洗涤纤维(NDF)、酸性洗涤纤维(ADF)、酸性洗涤木质素(ADL) 的动态变化。

Table 1. Nutritional fractions: neutral detergent fiber (NDF), acid detergent fiber (ADF) and Acid detergent lignin (ADL) in three various three species and two-way ANOVA (T-sampling time and

S- tree species).

Leaf litter species (S)	Sampling time (T)	ADL (%)	ADF (%)	ADL (%)
CL	1st	43.04 (0.02) f_A	38.09 (0.02) f_A	22.18 (0.01) f_A
	2nd	55.09 (0.02) d_A	53.17 (0.02) c_A	33.73 (0.01) d_A
	3rd	56.27 (0.02) c_A	52.74 (0.02) e_A	32.77 (0.01) e_A
	4th	55.02 (0.02) e_A	53.11 (0.02) d_A	46.46 (0.01) b_A
	5th	58.86 (0.02) b_A	57.31 (0.02) b_A	39.36 (0.01)c_A
	6th	59.62 (0.02) a_A	57.09 (0.02) a_A	40.42 (0.01) a_A
LL	1st	48.30 (0.02) f_C	43.39 (0.02) f_B	21.79 (0.01) f_B
	2nd	54.65 (0.02) d_C	54.57 (0.02) c_B	29.05 (0.01) d_B
	3rd	54.84 (0.02) c_C	52.45 (0.02) e_B	28.40 (0.01) e_B
	4th	56.67 (0.02) e_C	54.14 (0.02) d_B	28.29 (0.01) b_B
	5th	54.50 (0.02) b_C	53.53 (0.02) b_B	35.98 (0.01) c_B
	6th	55.37 (0.02) a_C	54.90 (0.02) a_B	38.90 (0.01) a_B
SL	1st	48.66 (0.02) f_B	48.01 (0.02) f_C	26.70 (0.01) f_C
	2nd	53.16 (0.02) d_B	51.41 (0.02) c_C	28.64 (0.01) d_C
	3rd	52.21 (0.02) c_B	50.34 (0.02) e_C	26.19 (0.01) e_C
	4th	49.35 (0.02) e_B	49.04 (0.02) d_C	33.56 (0.01) b_C
	5th	57.51 (0.02) b_B	55.56 (0.02) b_C	31.29 (0.01) c_C
	6th	65.88 (0.02) a_B	59.15 (0.02) a_C	35.06 (0.01) a_C
Two-way ANOVA	Leaf litter species (S)	F = 941	F = 256	F = 233827
		P < 0.001	P < 0.001	P < 0.001
	Sampling time (T)	F = 103668	F = 102421	F = 344510
		P < 0.001	P < 0.001	P < 0.001
	Interaction S x T	F = 21187	F = 12897	F = 59886
		P < 0.001	P < 0.001	P < 0.001

Notes: Reported values shown are means of three replicates with standard deviation (SD) in brackets. Leaf litter species are as follows: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) The sample taking is indicated as follows: 1^{st} -first sampling after 2 months of decomposing, 2^{nd} - 2^{nd} sampling after 4 months of decomposing, 3^{rd} – 3^{rd} sampling after 6 months of decomposing, 4^{th} – 4^{th} sampling after 8 months of decomposing, 5^{th} – 5^{th} sampling after 10 months of decomposing and 6^{th} – 6^{th} sampling after 12 months of decomposing. For leaf litter species, the values sings with upper-case letters indicate

significant difference. For sampling time, the values sings with the various lower-case letters indicate the significant difference.

3.2.1.2 中性洗涤纤维的变化

3.2.1.2 Change of neutral detergent fiber

The decomposition of leaf litter by change of neutral detergent fiber (NDF) presented significant differences along the six sampling times in CL (Cihuai litter or Locust litter) species. The mean and standard deviation (SD) rate of (NDF) has increased from the early stage of decaying process the end of one year cycle. It sky rocked from 1st sampling time to the second sample which was the greatest significant trend along the one year of leaf litter decomposition. Interestingly, the rate of NDF concentration was not significant differences within 2nd and 4th samples and the rate kept leveled off. As it is seen the trend of NDF rose up at the last stage of leaf litter decomposition in CL (Figure 1A). The NDF content of LL (Liaodong Oak) fluctuated during the leaf litter decomposition. The initially rate was 483 g/kg and it dramatically rose up to 546.5 g/kg in second sampling time which present the greatest significant difference among the rest of sampling times. Then it slightly increased from second to third sample and again it peached to 566.7 g/kg at fourth sample that also had significant difference with all sampling time. The change of NDF in LL leaf litter indicated that the rate wend down at the last stage of decaying process. However, the concentration of NDF had an upward trend at SL (Shaoyang litter or Aspen leaf). Still

the on the NDF content and a significant difference ($P < 0.05$) among all sampling time (Figure 1B). It had the least concentration at 1st and 4th 486.6 g/kg and 49.45 g/kg, respectively. Surprisingly, huge significant differences were observed at the two last samples in comparison with the rest of sampling time. The concentration of ADF greatly increased at the end of leaf litter decomposition study (Figure 1C).

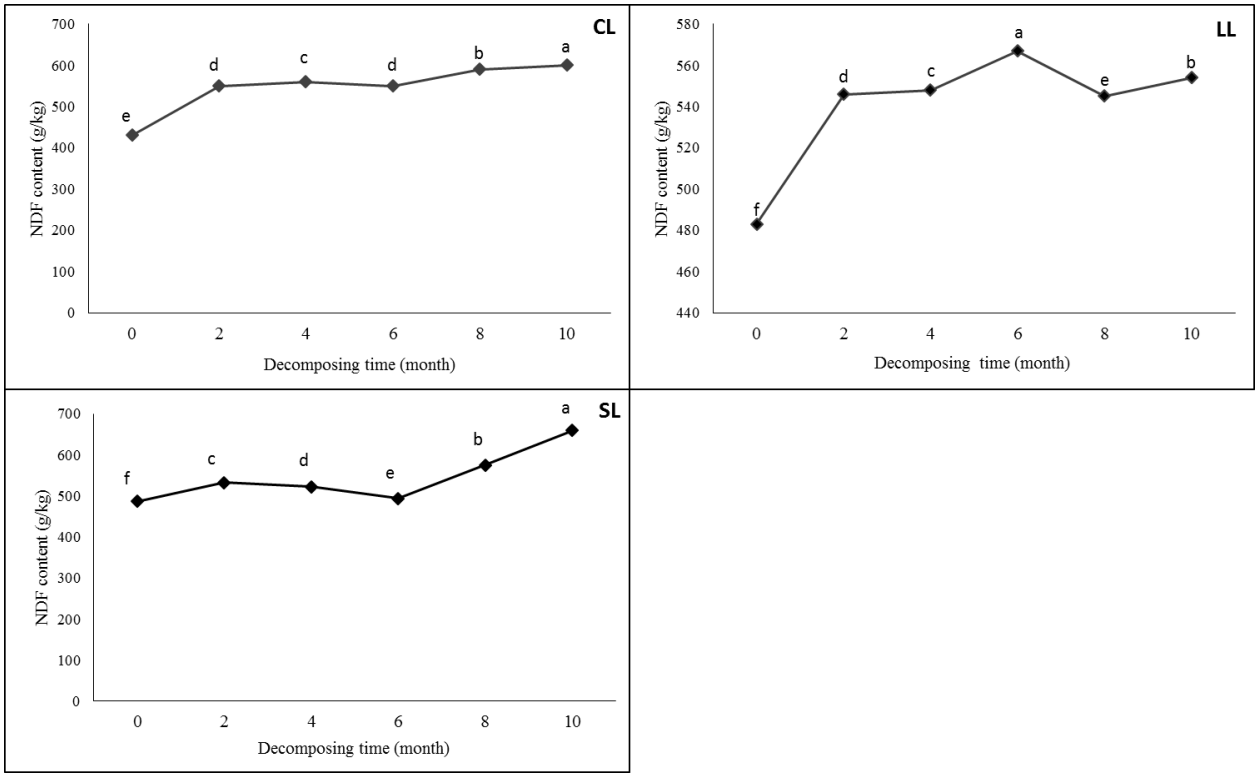

图 1 枯落物不同分解时期中性洗涤纤维的变化

Figure 1. The change of neutral detergent fiber (NDF) concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the

decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.2.1.3 酸性洗涤纤维的变化

3.2.1.3 Change of acid detergent fiber

Analyses of the acid detergent fiber (ADF) change data revealed significant differences ($p < 0.05$) at all sampling times. The concentration of ADF in CL (Cihuai litter or Locust litter) species at the first sample was very low just around 390 g/kg. It vividly rose to 531.7 g/kg at the second sampling time. There was a light reduction at 3rd sample and again it kept constant rate with 2nd sample and there was not significant difference between 2nd and 4th sampling time. The highest change of ADF in CL specie occurs at the end of our study in leaf litter decomposition (Figure 2A). Significant differences ($P < 0.05$) was observed while we was comparing the initial stage and final stage of the investigation. The dynamic of ADF in LL (Liaodong Oak) specie had fluctuated throughout the leaf litter decomposition and the significant differences were noticed within all sampling times. The number of ADF percentage was just above 400 g/kg at the beginning of leaf litter decay then it sharply rose to approximately 550 g/kg in the following sample and it had abundant significant difference. Even tough, the rate of ADF at 3rd, 4th, 5th and 6th sampling times were very close to each other (around 540 g/kg) the significance was presented among them. The composition of ADF peaked to 549 g/kg at the last sampling time which was the top and had the significant differences ($P < 0.05$) with all other sampling time

(Figure 2B). The concentration of ADF in SL (Shaoyang litter or Aspen) quite a bit constant but still it presented the significant differences at all samples. The first and fourth samples had the low rate (480.1 g/kg, 490.4 g/kg), the second and third samples had moderate rate (514.1 g/kg, 503.4 g/kg), fifth and sixth samples had high rate (555.6 g/kg, 591.5 g/kg). Despite the small gap in the change of ADF in LL species, the significant differences were noticed in comparison with each other (Figure 2C). All in all, the change of ADF had great trend at the end of sampling time however, the rate was remarkably low at the initial stage of leaf litter decomposition.

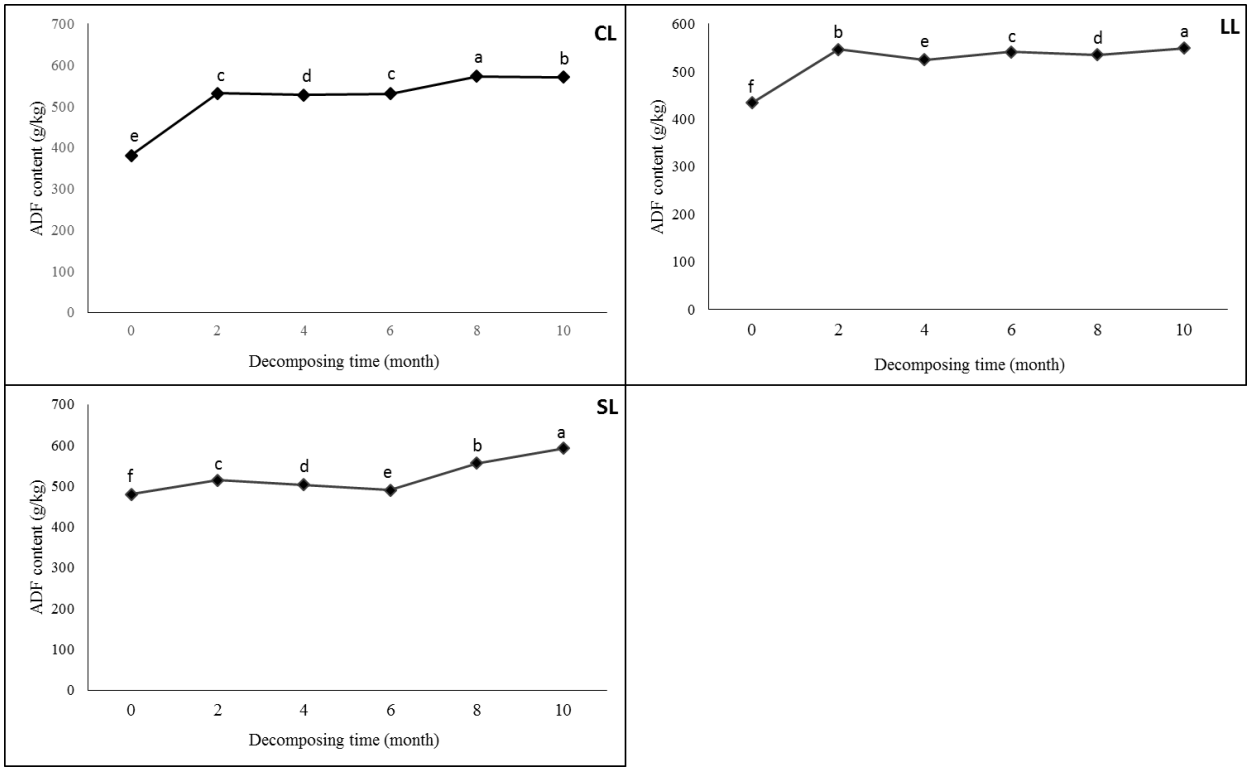

图 2 枯落物不同分解时期酸性洗涤纤维的变化

Figure 2. The change of acid detergent fiber (ADF) concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and

standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.2.1.4 酸性洗涤木质素的变化

3.2.1.4 Change of acid detergent lignin

Here it could be shown that acid detergent lignin (ADL) variation was fluctuated over 6 sampling times in CL (Cihuai litter or Locust litter) leaf litter decomposition. The start point level of ADL concentration was just above 200 g/kg and it drastically growth to approximately 340 g/kg at the next sample which exhibited a huge significant difference. The decomposition process from 2nd to 3rd sampling time indicated slight reduction in ADL change. Lately, the trend gigantically reached to its top (464.6 g/kg) at 4th sampling time that had great different significant with the rest of samples. Although there was noticeable significance among 5th and 6th samples their rate of ADL value was greatly close to each other. Overall the 1st and 4th samples performed vividly significant difference in compare with the all other samples (Figure 3A). In LL (Liaodong Oak) leaf litter specie the change of ADL revealed differently throughout the decomposition process. As it was seen the initial stage of leaf litter decomposition had very low composition of ADL, 217.9 g/kg, 290.5 g/kg, 284 g/kg and 282.9 g/kg, 1st, 2nd , 3rd and 4th sampling times, respectively. Although,

the number of ADL dynamics were at very close scale, the significant difference was perceived. At the end of leaf litter decomposition study, the ADL content considerably increased to 359 g/kg (5^{th} sample) and 389 g/kg (6^{th} sample). The two last sampling time had an excessive significant differences ($P < 0.05$) within all sampling times (Figure 3B). The ADL content in SL (Shaoyang litter or Aspen) leaf litter species presented stable trend but there significant difference between them. It begun with approximately 260 g/kg at the first sample then slightly grown to 281.9 g/kg at the next sample and it reduce to 261.9 g/kg at 3^{rd} sampling time. There was a big gap in change of ADL within 3^{rd} and 4^{th} sampling time which revealed significant difference that it reached to 335.6 g/kg at 4^{th} sample. The rate of ADL somewhat dropped to 312.9 g/kg and again it sky rocked to 350.6 g/kg at the last sapling time which had the enormous significant differences.

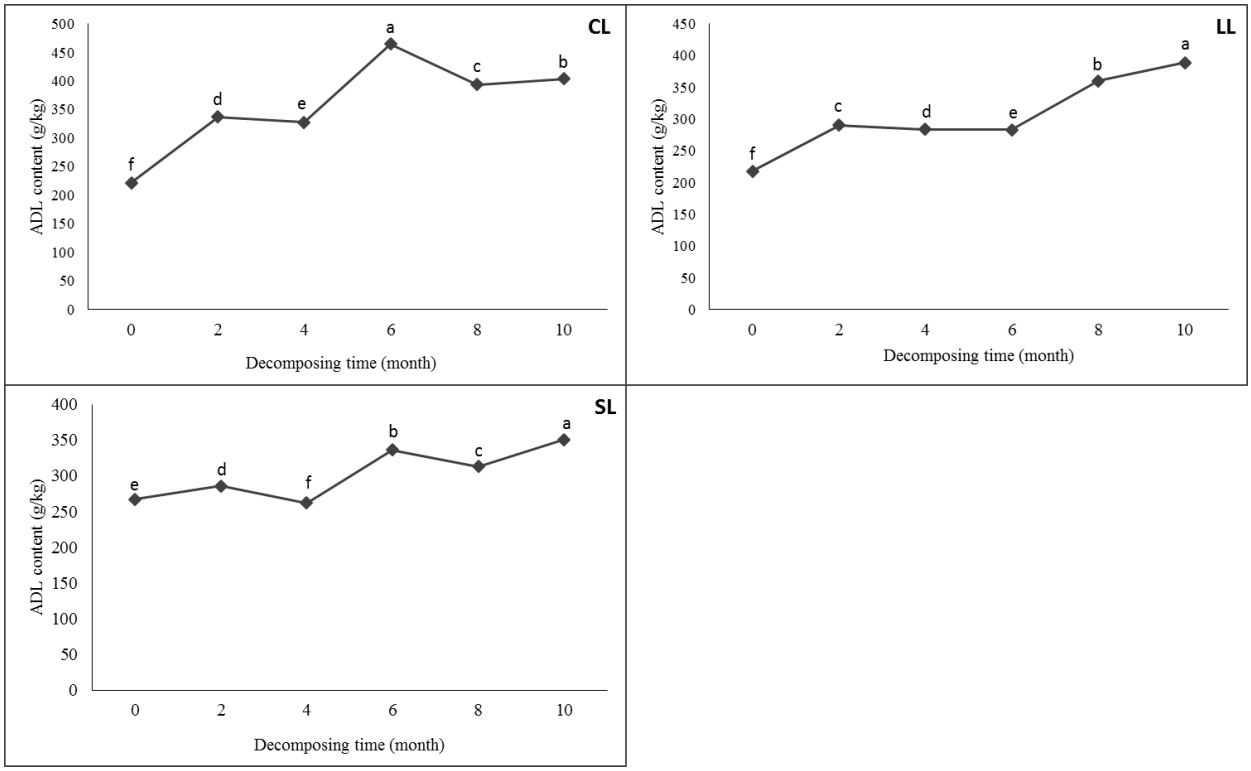

图 3 枯落物不同分解时期中性洗涤木质素的变化

Figure 3. The change of acid detergent lignin (ADL) concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.2.2.1 纤维素、半纤维素和木质素方差分析

3.2.2.1 Two way ANOVA for Semi-cellulose, Cellulose and Lignin

The two way ANOVA was run to figure out the change of structural carbohydrates.

The easily decomposable carbohydrates: semi-cellulose, cellulose and lignin in leaf litter were significantly influenced by sampling times, leaf litter species and their interaction ($P < 0.05$; Table 2). The structural carbohydrates showed significant differences along both: six sampling time and various tree leaf litter decomposition. In details, the semi-cellulose concentration throughout the sampling time presented significant differences and the rate ($F = 240215$; $P < 0.001$) indicated that sampling time can be considered as a main influential factor. In comparison with the sampling time the leaf litter species also presented significant differences. However, the interaction of bother factors ($F = 314019$; $P < 0.001$) had greater influence in the decomposition process. The rate of hemicellulose was quite outnumbered in CL (Cihuai litter or Locust litter) specie than LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter). Cellulose content of leaf litter also revealed a remarkable significances in litter species as main factor ($F = 584231$; $P < 0.001$), sampling time ($F = 164529$; $P < 0.001$) and the interaction ($F = 147823$; $P < 0.001$). The concentration of cellulose in CL and LL species was low in the first sample and then it dramatically rose to high rate in the next 2 sampling time. However, the content of cellulose in SL leaf litter performed high trend at the end of our investigation, there was great significant within all sampling times ($P < 0.05$; Table 2). The dynamic of lignin in leaf litter decomposition process were influenced by sampling time as a main factor ($F = 348980$; $P < 0.001$), various tree species ($F = 237012$; $P < 0.001$) and the interaction of these two factors ($F = 60672$; $P < 0.001$) with a significant differences

(Table 2). The trend of lignin content in all leaf litter species over the sampling time was down to up scales. In terms of litter species based the numbers were high in CL, middle in LL and low in SL.

表 2 不同分解时期纤维素、半纤维素和木质素方差分析

Table 2. Structural carbohydrate: Semi-cellulose, Cellulose and Lignin in three various three species and two-way ANOVA (T-sampling time and S- tree species).

Leaf litter species (S)	Sampling time (T)	semi-cellulose (%)	cellulose (%)	lignin (%)
CL	1st	4.94 (0.003) a_A	15.92 (0.011) d_C	22.17 (0.016) f_A
	2nd	1.92 (0.003) f_A	19.44 (0.011) b_C	33.73 (0.016) d_A
	3rd	3.52 (0.003) c_A	19.98 (0.011) a_C	32.77 (0.016) e_A
	4th	1.91 (0.003) d_A	6.65 (0.011) f_C	46.45 (0.016) b_A
	5th	1.54 (0.003) e_A	17.94 (0.011) c_C	39.36 (0.016) c_A
	6th	2.53 (0.003) b_A	16.67 (0.011) e_C	40.41 (0.016) a_A
LL	1st	4.91 (0.003) a_C	21.60 (0.011) d_B	21.78 (0.016) f_B
	2nd	0.08 (0.003) f_C	25.52 (0.011) b_B	29.04 (0.016) d_B
	3rd	2.39 (0.003) c _C	24.05 (0.011) a_B	28.40 (0.016) e_B
	4th	2.53 (0.003) d_C	25.85 (0.011) f_B	28.29 (0.016) b_B
	5th	0.97 (0.003) e_C	17.55 (0.011) c_B	35.97 (0.016) c_B
	6th	0.48 (0.003) b_C	16.00 (0.011) e_B	38.88 (0.016) a_B
SL	1st	0.66 (0.003) a_B	21.31 (0.011) d_A	26.69 (0.016) f_C
	2nd	1.75 (0.003) f_B	22.78 (0.011) b_A	28.63 (0.016) d_C
	3rd	1.87 (0.003) c_B	24.15 (0.011) a_A	26.19 (0.016) e_C
	4th	0.32 (0.003) d_B	15.47 (0.011) f_A	33.55 (0.016) b_C
	5th	1.95 (0.003) e_B	24.27 (0.011) c_A	31.28 (0.016) c_C
	6th	6.72 (0.003) b_B	24.10 (0.011) e_A	35.05 (0.016) a_C
Two-way ANOVA	Leaf litter species (S)	F = 90284	F = 584231	F = 237012
		P < 0.001	P < 0.001	P < 0.001
	Sampling time (T)	F = 240215	F = 164529	F = 348980
		P < 0.001	P < 0.001	P < 0.001
	Interaction S x T	F = 314019	F = 147823	F = 60672
		P < 0.001	P < 0.001	P < 0.001

Notes: Reported values shown are means of three replicates with standard deviation (SD) in brackets. Leaf litter species are as follows: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen leaf litter) The sample taking is

indicated as follows: 1st -first sampling after 2 months of decomposing, 2nd - 2nd sampling after 4 months of decomposing, 3rd – 3rd sampling after 6 months of decomposing, 4th – 4th sampling after 8 months of decomposing, 5th – 5th sampling after 10 months of decomposing and 6th – 6th sampling after 12 months of decomposing. For leaf litter species, the values sings with upper-case letters indicate significant difference. For sampling time, the values sings with the various lower-case letters indicate the significant difference.

3.2.2.2 半纤维素变化

3.2.2.2 Change of semi-cellulose

The content of semi-cellulose was fluctuated through the leaf litter decomposition process. The initial semi-cellulose concentration was very high at first sample (49.4 g/kg) and then it affectedly dropped to (19 g/kg) in the second sampling time in CL (Cihuai litter or Locust litter) specie. The second top rate was (35.2 g/kg) afterward it start declining until the last fifth samples which had the least number (15.4 g/kg) along one year litter decay study. As it was observed at the last sample it again increased and it revealed that the significant differences was observes among all sampling time ($P < 0.05$, Figure 4A). Interestingly, the dynamic of semi-cellulose in LL (Liaodong Oak) showed almost the same patterns as CL did. The trend was vividly high in 1st sample and it almost went down to zero in the following sample. The content of semi-cellulose increased to (23.9 g/kg) (25.3 g/kg), 3rd and 4th samples,

respectively (Table 4B). afterward the rate stares to decline till the end one year litter decomposition survey. All in all, there was huge significant differences throughout the decomposition process ($P < 0.05$). The SL (Shaoyang litter or Aspen) specie had surprisingly trend over the leaf litter decay in our investigation. The patterns were under 10 g/kg at first and fourth sample which presented great significant difference comparing with other samples ($P < 0.05$). The 2^{nd}, 3^{rd} and 5^{th} samples kept the middle rate with approximately 20 g/kg semi-cellulose content. Surprisingly, the trend reached to 67.2 g/kg at the final sapling time which had the greatest significant difference with the rest of samples (Table 4C; $P < 0.05$).

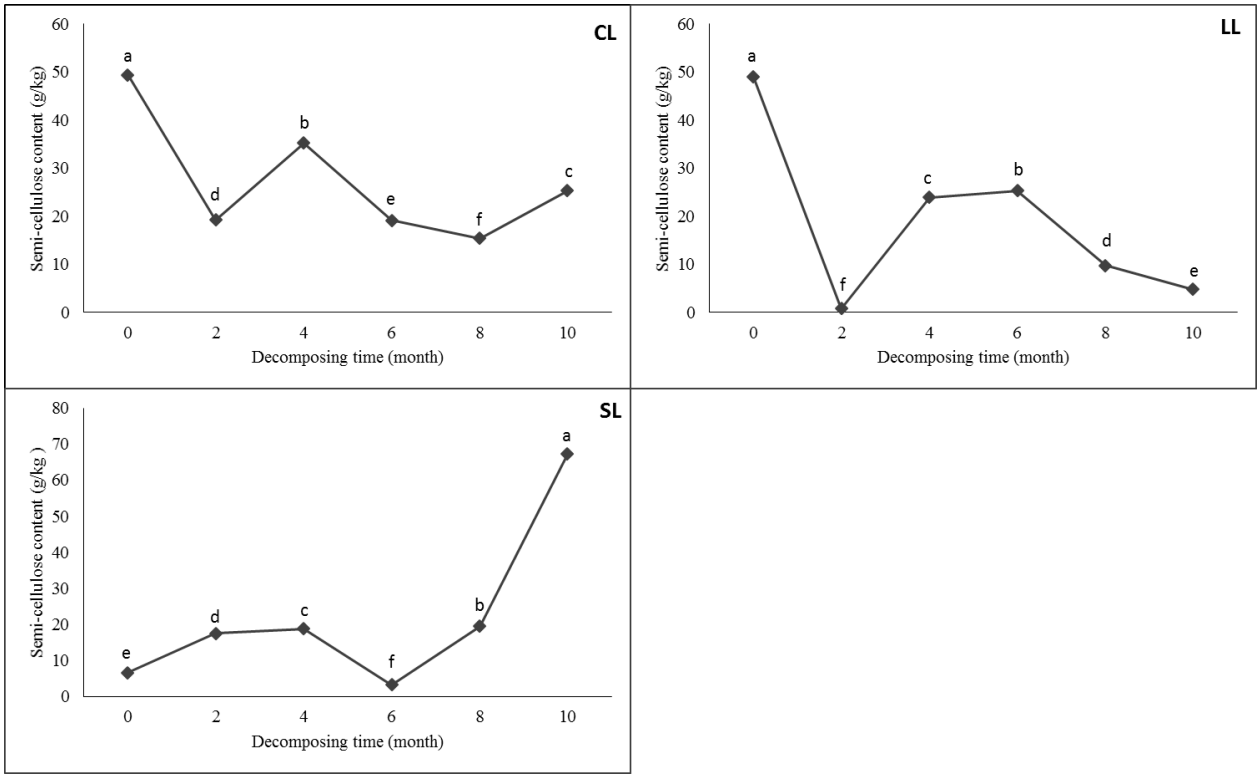

图 4 枯落物不同分解时期半纤维素变化

Figure 4. The change of semi-cellulose concentration from decomposing leaf litter of three

broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.2.2.3 纤维素变化

3.2.2.3 Change of cellulose

The descriptive statistical analysis associate with the cellulose content access six sampling times was reported in figure 5. It can be seen that the concentration of cellulose was increased at the 3 early sampling time and declined at the end of litter study. Our results revealed that cellulose degradation rates were significantly changed throughout the leaf litter decay process in CL (Cihuai litter or Locust) specie. It gradually rose 159.2 g/kg, 194.4 g/kg and 199.8 g/kg, first, second, third sampling time respectively. The content of cellulose sharply collapse to 66.5 g/kg at 5^{th} sapling time that number had very significant difference ($P < 0.05$) with the rest of samples. Afterward the cellulose concentration in CL suddenly augmented to 179.4 g/kg at 5^{th} sample and slightly reduced to 166.7 g/kg at last sample. Among all six sampling time the significances were observed (Table 5A, $P < 0.05$). The result of cellulose in LL (Liaodong Oak) discovered that the level of cellulose was outnumbered at 2^{nd} and 4^{th}

samples, while the lowest point was detected at 6th sampling time. As it seen the content of cellulose showed a significant ($P < 0.05$) reduction at the two final stages. In SL (Shaoyang litter or Aspen) the content of cellulose expectedly increased from 213.1 g/kg at 1st sample to 241.5 g/kg at 3rd sample. Then the number intensely plunged to 152.7 g/kg in the following sample and suddenly it enlarged to 242.7 g/kg. The lignin content showed slight reduction at the final sample in the litter decomposition study. There were significant differences observed throughout the leaf litter decomposition study (Table 5C; $P < 0.05$).

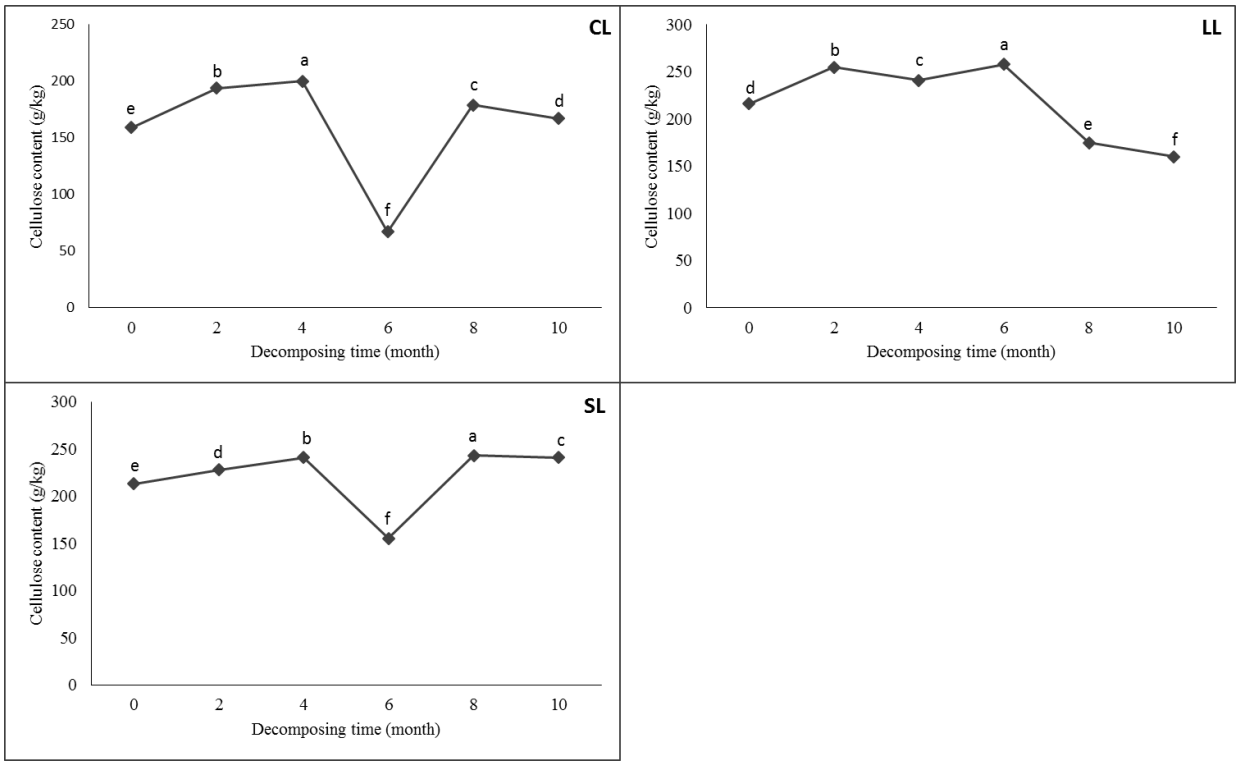

图 5 枯落物不同分解时期纤维素变化

Figure 5. The change of cellulose concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error.

Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.2.2.4 木质素变化

3.2.2.4 Change of lignin

Initial structural carbohydrate results showed that for three of the leaf litter types CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen) there were very significant difference ($P < 0.05$) change in lignin content (Figure 6ABC). The content of lignin in CL was very low at the early stage of litter decomposition. The rate star from just above 200 g/kg (1^{st} sample) and rose to 337.3 g/kg (2^{nd} sample) and slightly reduced to 321.7 g/kg (3^{rd} sample). Then the content of lignin peaked to 464.5 g/kg at 4^{th} sampling time and lately it decreased to approximately 400 g/kg at the end of leaf litter decay study. The significances were presented over the decomposition process ($P < 0.05$; Figure 6A). The concentration of lignin in LL (Liaodong Oak) started from around 220 g/kg at first sample which was the lowest point and it maintained the rate almost at 290 g/kg within 3 following samples. However the numbers at the early stage were very close to each other the significant differences were noticed among them ($P < 0.05$). Interestingly, the content of lignin increased at the two last sampling time that reached to 359.7 g/kg,

388.8 g/kg 5th and 6th sampling time, respectively (Figure 6B). Initial chemical analyses showed that concentration of lignin leaf litter was vary within sampling time in SL (Shaoyang litter or Aspen) species (Figure 6C). Almost the trend of lignin content was fluctuated over the decomposition period and the significant differences ($P < 0.05$) accurately were observed among all the samples. Due to the lignin percentage the samples could be divided into two groups; high and low rate. In the beginning stage of litter decaying lignin almost kept low steady trend around 250 g/kg 1st, 2nd and 3rd samples accordingly. And the last three samples rose to approximately 350 g/kg and had relatively close concentration of lignin in SL specie. Even though, according to the statistical analysis the significant differences were performed within all sampling time ($P < 0.05$; Figure 6C).

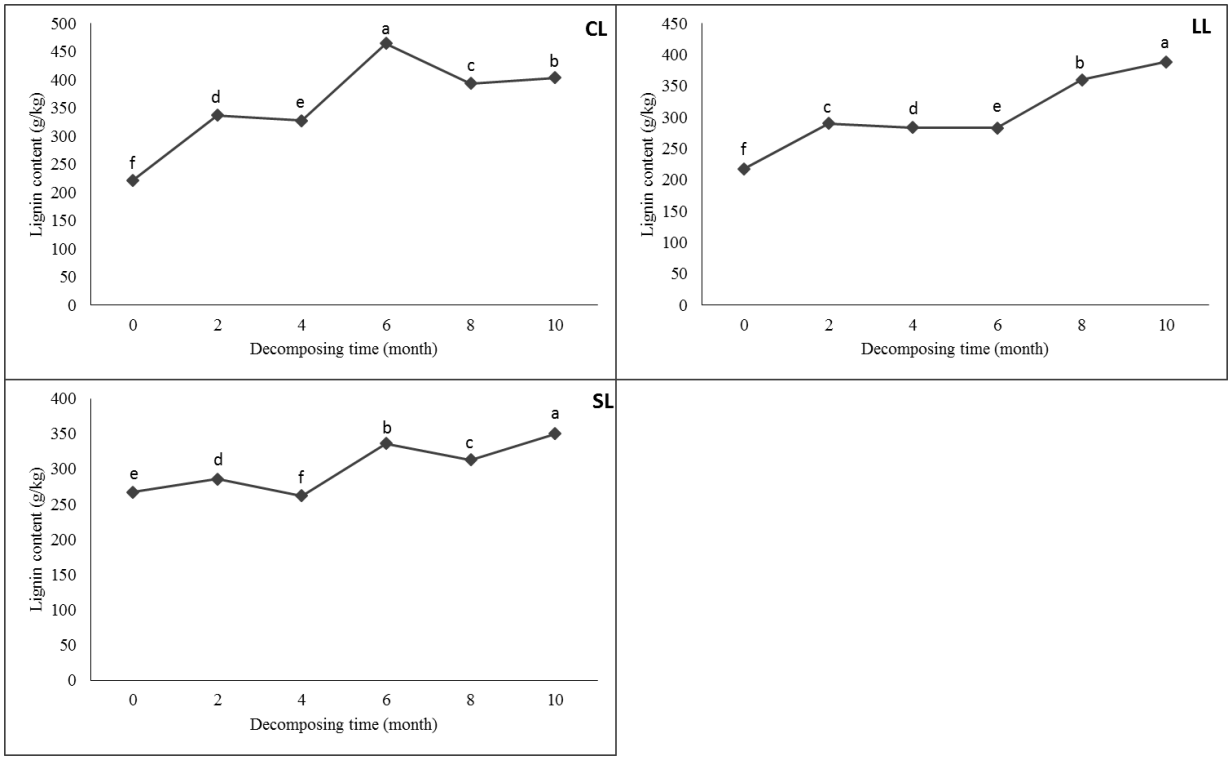

图 6 枯落物不同分解时期木质素变化

Figure 6. The change of lignin Changes of carbon concentration from decomposing leaf litter of three broadleaved deciduous tree species in the Loess Plateau. The line graph indicates mean and standard error. Different small case letters represent a significant difference among 6 sampling times at $P < 0.05$. The capital letters show three species: CL (Cihuai litter or Locust litter), LL (Liaodong Oak) and SL (Shaoyang litter or Aspen). The X-axes number indicates the decomposing time by month after the placement of the leaf litter bag. The sample was take every after two months.

3.3 枯落物分解过程叶际细菌群落动态变化

3.3 Phyllosphere bacterial community dynamics during leaf litter decomposition

3.3.1.1 不同分解时期主要细菌类群

3.3.1.1 Common phyllosphere bacterial communities in various sampling times.

In order to present the common and unique phyllosphere bacterial communities in different sample times, the Venn diagram was presented. (Figure 7) In the early stage of leaf litter the decomposition the dynamics of phyllosphere bacterial community were much more active than second and third ones. In the first and second sample time they harbored 213 and 37 unique OTUs, respectively and they shared 157 OTUs. The pick of OTUs reached to 458 in sixth sample time and it shared 441 OUTs

with the previous (5^{th}) sample time. The second time of phyllosphere bacterial communities shared 157, 66 and 20 OTUs with first, fourth and sixth sample times, respectively, while it had 37 unique OTUs itself. The fourth time sample had medium rate of (161) OTUs comparing with two before and two afterward. It just shared 33 OTUs with sixth time sample and 64 OTUs with fifth sample time however it shared 2 OUTs more in third then the fifth time sample of phyllosphere bacterial communities and just over 30 OTUs shared with the first stage. The lowest OTUs numbers were notice in the third and fifth phyllosphere bacterial communities sample 37 and 88, respectively. Overall, the result exhibited that 588 OTUs were shared among all sampling times and the second largest shared number was 226 OTUs that were common within four sampling times except the last sample time. However, the noticeable rate of OTUs (441) was shared between fifth and sixth sampling time, the lowest trend of OTUs shared were fifth and second sample times (20) (Figure 7).

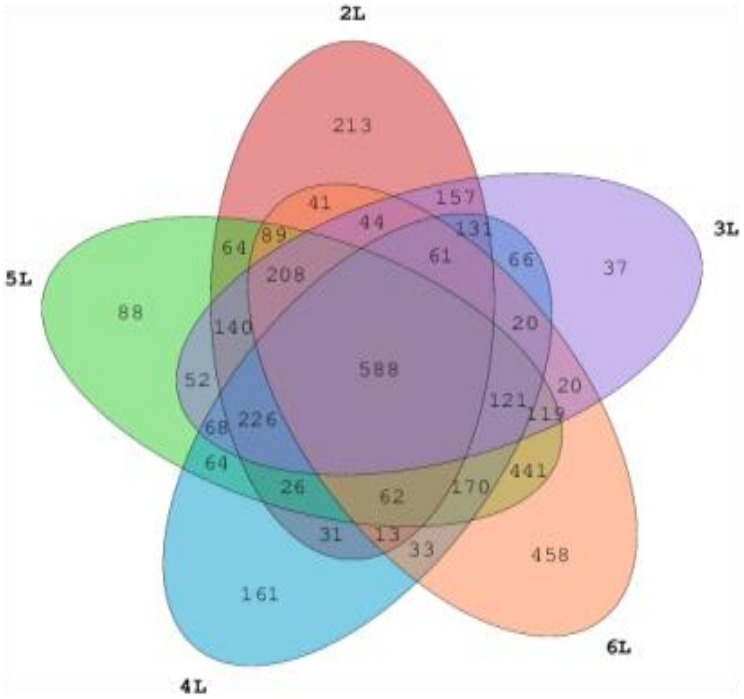

图 7 不同分解时期共有、特殊 OUTs 韦恩图

Figure 7. Venn diagram of the amount of common and unique OTUs in different sampling times. (2, 3, 4, 5 and 6L) refer to the shared and unique OTUs in the first, second, third, fourth and fifth samples of phyllosphere bacterial community inhabiting in leaf litters.

3.3.2 多样性指数分析

3.3.2 Analysis of diversity index

Illumina high-throughput sequencing was implemented to discover bacterial community diversity. As it was revealed in the table 1, alpha diversity was estimated by five indices that includes OTUs in phylum level, Simpson, Chao1, ACE and

Shannon. The biodiversity of 15 leaf litter samples were researched based on the above mentioned indicators. Using a 97% sequence identity cutoff and the number of OTUs ranged from 246 to 1480 (Table 3). The result illustrated that phylum number of these samples ranged from 246 to 1480 based on the effective sequences, while 4LL had the lowest richness (246 OTUs), and 5CL displayed the highest richness (1580 OTUs). However, the 2LL (721 OTUs) and 4LL (246 OTUs) had low richness in the beginning stage of leaf litter decomposition, it dramatically rose to 1217 OTUs (5LL) and 1338 (6LL) at the end of one year sequence of leaf litter decay. It was found that the level of Chao1 was high in 5CL, 6LL, 3CL and 6CL 1576.47, 1473.5, 1398.11 and 1393.7 respectively. Chao1 performed low community richness in 3SL (720.2), 4SL (646.02) and 4LL (252.79). The patterns of ACE accordant with the evaluation of Chao1 and 5CL (1632.17), 6LL (1513.52), 3CL (1460.) and 6CL (1442.3) had noticeable higher rate of community richness. Likewise Chao1' result; the ACE had very low richness in 3SL, 4SL and 4LL that presented as 741.69, 666.03 and 255.12 respectively. According to the Simpson diversity indices, samples 5CL, 4CL and 6LL had the highest community diversity, 0.995329, 0.992242 and 0.99131 respectively, whereas 4LL (0.914098), 3SL (0.927276) and 4SL (0.958237) had low community diversity. Comparatively with the rest of samples, the 4LL (4.94), 2LL (6.9) and 3SL (6.17) samples had the lowest community diversity from the Shannon indicators (Table 3). Overall, all the five indices illustrate that samples 4LL, 4SL and 3SL had very low trends in both; community richness and diversity however 5CL and

6LL had a significant rate in all five parameters.

表 3 叶际细菌群落多样性指数变化

Table 3. Richness and diversity of bacteria community.

Samples	Phylum	Simpson	Chao1	ACE	Shannon
2CL	1111	0.98	1206.06	1249.14	7.88
2LL	721	0.98	811.70	832.73	6.90
2SL	1182	0.96	1288.32	1363.06	7.44
3CL	1284	0.98	1398.11	1460.39	8.04
3LL	1088	0.97	1187.81	1233.98	7.65
3SL	666	0.93	720.20	741.69	6.17
4CL	1244	0.99	1307.24	1350.37	8.62
4LL	246	0.91	252.79	255.12	4.94
4SL	604	0.96	646.02	666.03	6.34
5CL	1480	1.00	1576.47	1632.17	9.09
5LL	1217	0.99	1292.97	1352.19	8.30
5SL	1100	0.98	1196.06	1241.24	7.78
6CL	1266	0.98	1393.70	1442.30	8.14
6LL	1338	0.99	1473.50	1513.52	8.63
6SL	1205	0.99	1304.86	1356.59	8.13

The number represent sampling time, the first letters represents leaf litter of vary tree species as follow: CL- Locust leaf litter, LL - Liaodong oak leaf litter and SL - Aspen leaf litter. The table demonstrates the different parameters of alpha diversity within samples. Sampson and Shannon represent community diversity and the high rate indicates more diversity. Chao1 and ACE are community richness; the high rates illustrate the more richness. The phylum represents the number of OTUs within samples.

3.3.3 细菌群落组成和结构分析

3.3.3 Bacterial community composition and structure change analysis.

We examined the bacterial community compositions of 15 samples from three tree species in 5 times sampling. Most of these bacterial taxa were relatively common across samples at phylum level and around 18 phyla were involved during the leaf litter decomposition in a year. The highest trend was noticed in 4LL (9-100 %), 3SL (24-100 %) and 2SL (25-100 %) that were occupied by Proteobacteria. The second largest dominator phyla were Actinobacteria (average 35%). The difference between these two active phyla was that, Proteobacteria was gigantically active at the beginning stage of leaf litter decomposition, whereas at the last stage of one year cycle decomposition Actinobacteria became active. Bacteroidetes was the third noticeable phyla among other bacterial communities (from 18 to 32 %) and it performed very slowly in the 2SL and 3SL in the beginning of decomposition then leveled off the rest of time (remained 10 %). Accordingly, CL species were dominated by the Cyanobacteria (0-20 %) in the early stage of leaf litter decomposition. At the last stage of one year cycle leaf litter decomposition Saccharibacteria and Gemmatimonadetes (average 4 %) gave started to perform gradually. Surprisingly, Deimococcus-Thermus (0 to 5 %) was appeared in 4CL more visible then the rest of samples. The rest of phyla were least abundant, with an average of 3 % of total bacterial community taxa. (Figure 8)

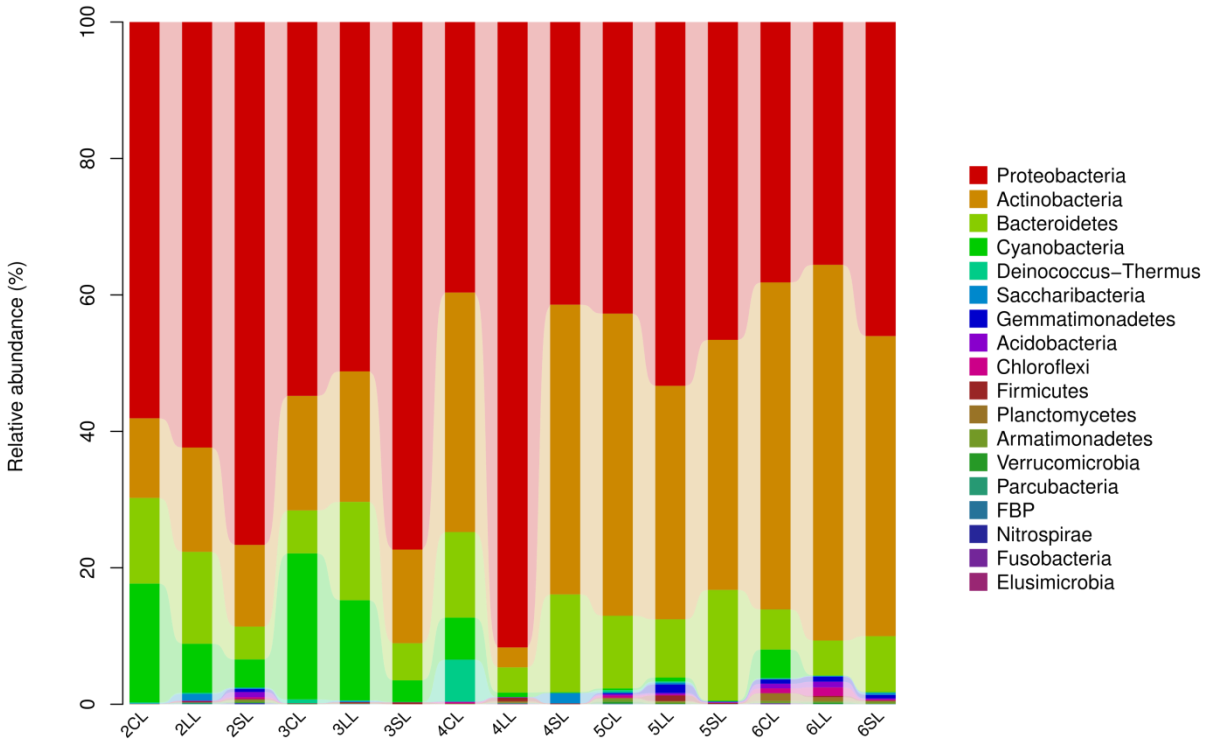

表 8 细菌主要类群（门水平）随枯落物分解的变化

Figure 8. Bacterial community variation during decomposition at the phylum level. Abundances of different phyla are in the analyzed samples. The abundance is presented in terms of percentage in total effective microbial sequences in various samples. Abscissa represents samples, and different color shows different bacterial phyla.

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High-throughput results discovered the presence of huge diverse microorganisms that are involved in leaf litter decomposition. Among the main class

Alphaproteobacteria was the most abundant with average75 % of the total sequences (Figure 9). But it fluctuated all through the leaf litter decomposition process. Except the wide trend of Alphaproteobacteria the following classes Actinobacteria, Gammaproteobacteria and Betaproteobacteria were dominants of the leaf litter decomposition, 22-80 %, 18-63 % and 5-41 % respectively. The above mentioned classes were very well abundant from the second sample time till the fourth sample time and then they dramatically decreased from 60 % to just above 30 %. Chloroplast was with high abundance trend in the first two sampling stages (average range 22 %) and it the completely disappear in further stage of leaf litter decomposition. In contrast, class Sphingobacteria showed more or less constant trend in all samples with average 17 % sequences. Cytophagia Flavobacteria and Deltaproteobacteria were also dominant class with 10-17 %, 3-15 % and 1-14 % of total sequences respectively. The rest minor classes ranged for 0.5 to 2.5 % of the total amount of sequences. (Figure 9)

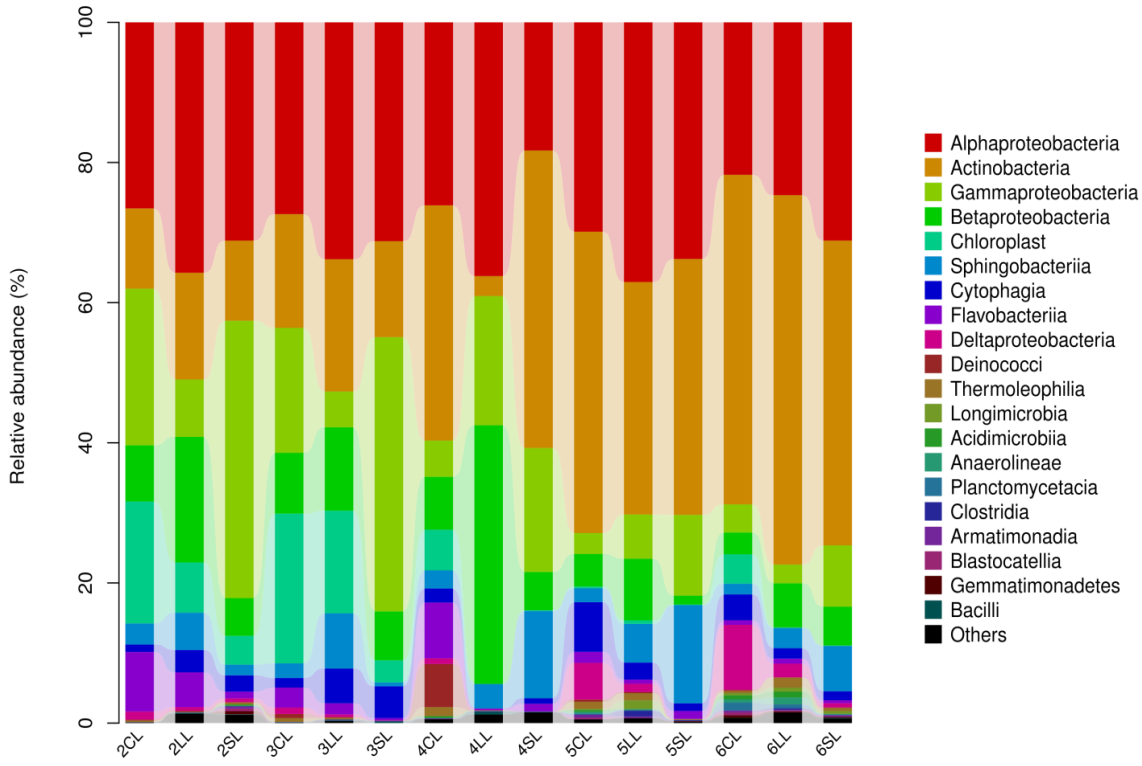

表 9 细菌主要类群（纲水平）随枯落物分解的变化

Figure 9. Relative abundances of the most abundant classes in the analyzed samples. The abundance is presented in terms of percentage in total effective microbial sequences in various samples. Abscissa represents samples, and different color shows different bacterial classes.

3.3.4 Beta 多样性变化

3.3.4 Beta diversity change based on heat map

The samples were clustered based on differences and similarities between the species (Figure 10). The clustering of bacterial community diversity was associated with the

characteristics of the sample. Based on the information concerning the sample bacteria genus (Figure 10), 4LL was consist of Caulobacter, Ochrobacterum, Acitenobacter, Acuabacterium, Limnobacter, Comamonas and Patulibacter had high abundance and clustered as first group. The second category 4Cl, 5Cl and 6Cl were clustered. However 5LL and 6LL were clustered to another semi-category. The leaf litter of 4SL, 5Sl and 6SL were clustered together. 2CL and 3CL with the least low abundance were clustered in a category. Thus, the results of the genus analysis showed that a sample can be clustered into one of the four sub-categories: 2CL 3CL and 2LL, 3LL and 2SL, 3SL with the lowest abundance. Truepera and Skermanella had high relative abundance 4CL sample and remained constant with the rest of the samples. Rhizobium and Aureimonas had lowest abundance in 4LL, 4SL and 3SL but the high abundances have not notice in any samples. The high relative abundances were observed with Devosia, Streptomyces, Dyadobacter, Geodermatophilus and Pedobacter in 5CL sample.

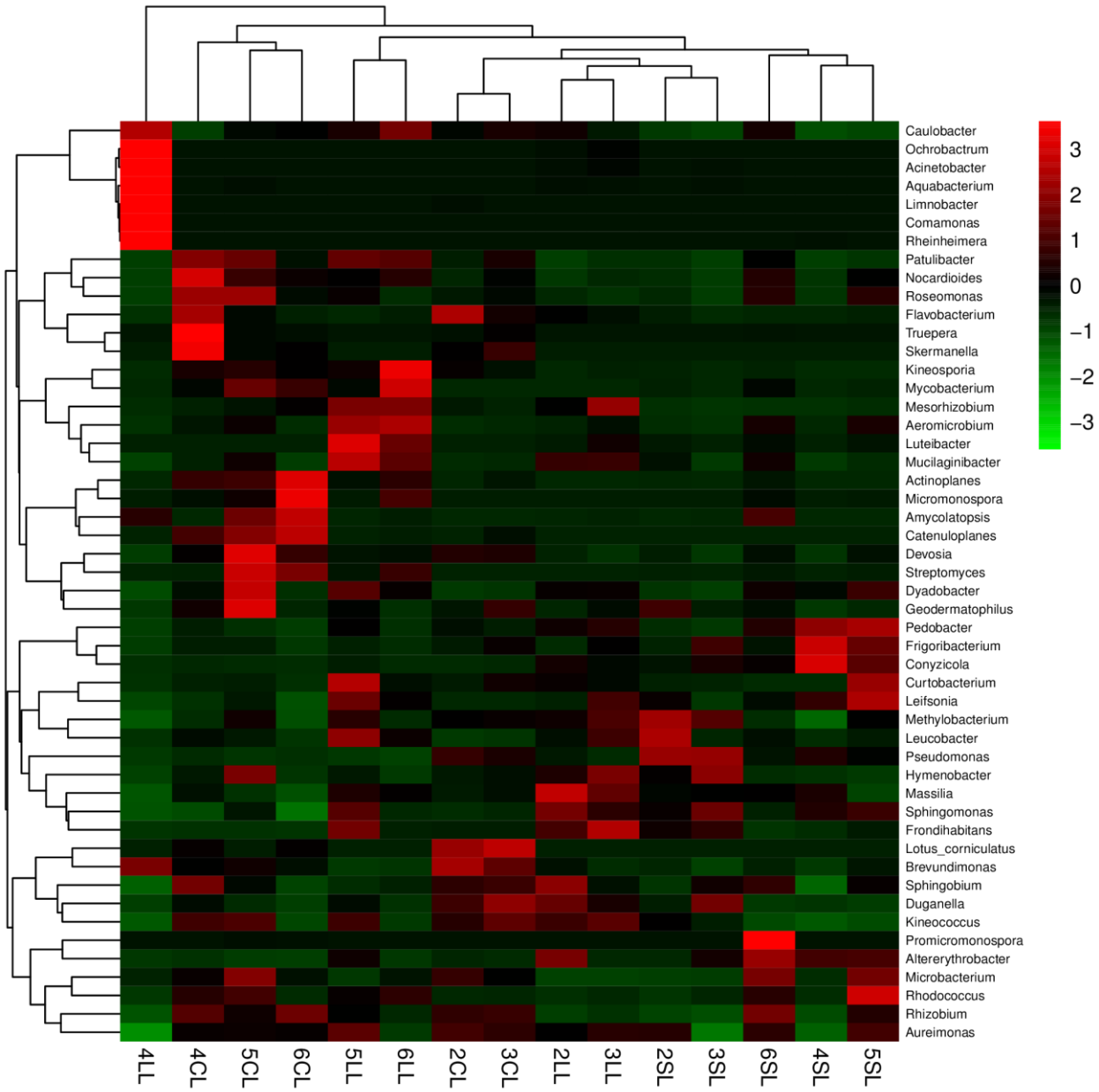

图 10 细菌群落相对分度前 50 的属在枯落物不同分解时期的变化规律

Figure 10. For the level of species and the abundance of information, we collected 50 genuses from the whole abundance sequences to draw a species abundance cluster map. Abscissa for sample information, ordinate for species annotation information, and clustering tree on the left side of the figure as a species tree clustering; above the clustering tree is a sample cluster tree. The

colors; red represents the high abundance and green represents low abundance rate.

3.3.5 细菌群落组成变化

3.3.5 Change of bacterial community composition

Non-metric multidimensional scaling (NMDS) was applied using (A) weighted and (B) unweighted UniFrac distance to identify the community composition of all samples (Figure 11). The percentage in parentheses in the axis represents the proportion of the difference in the raw data that the corresponding principal component can interpret. NMDS1 analysis was performed on Unweighted and Weighted UniFrac distance matrices using R software, and the structure distribution of the community samples were described by two-dimensional or three-dimensional sorting graphs. Totally, 15 samples were observed and with 5 sampling time were signed in these figures. 6CL, 6LL and 6SL had closer distance comparing to other samples and it was noticed in both; weighted and unweighted NMDS1. However, samples; 3CL, 3LL and 3SL had moderate similarity in weighted and a bit different in unweighted NMDS1. Surprisingly, the dissimilarity was observed in 4CL samples. 4CL and 4SL kept the closed distance in weighted NMDS1. 5CL and 5LL were more similarity in diversity parametric of weighted and unweighted NMDS1. However, 5SL were much dissimilar with 5CL in unweighted and it had constant distance rate with 5CL and 5LL in weighted NMDS1. 2CL and 2LL were much more similar in compression with rest of 2^{nd} time samples in weighted NMDS1. In terms of similarities of samples time 2^{nd}, 3^{rd} and 5^{th} samples time were more in closer distance

in weighed NMDS1. Accordingly the same sign were observed in unweighed NMDS1. 4LL were in far huge distance from all samples in A and B NMDS1.

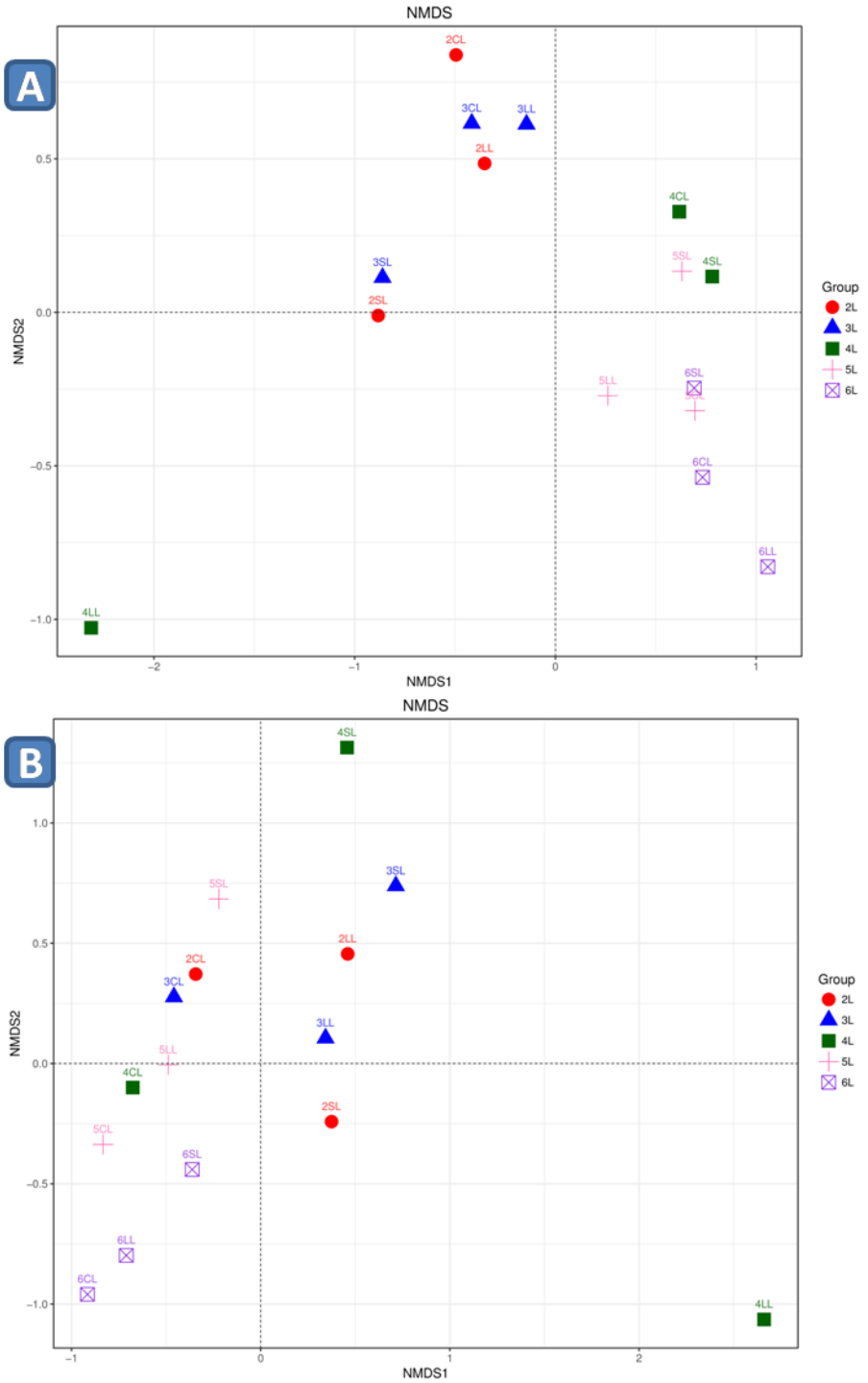

图 11 不同分期时期叶际细菌群落结构的变化

Figure 11. Each point represents a sample. Points of different colors belong to different samples (groups). The closer the distance between the two points, the higher the similarity of microbial community structures between the two samples and the smaller the difference. The number indicates the sampling time and capital letter L represents Leaf litter. A represent weighted and B represented unweighted NMDSs.

3.3.6 细菌（门水平）在不同分解时期的显著性差异

3.3.6 Abundance and significant difference of microbes at the phylum level

After bioinformatics analysis and quality filtering a total of 1802017 bacterial reads and the number of OTUs assigned to bacteria was 15752. Between the 2L, 6L and 3L, 4L, 5L samples groups, there was a significant difference in the abundance of Acidobacteria, which was more abundant in 6L and 2L samples group than in the rest samples ($p<0.01$). 5L were much more abundant by Armatimonadetes, FBP and Gemmatimonadetes, however it were less abundant by Cyanonbacteria, Acidobacteria and Protobacteria. 2L was almost abundant by Acidobacteria , Armatimonadetes and Cyanobacteria kept the middle rate of abundance in the rest phyla except Acitinobacteria. Actinobacteria and Proteobacteria were more abundant in 4L samples compare to the rest of samples ($p<0.05$). FBP and Gemmatimonadetes Fusobacteria and Proteobacteria were significantly less abundant ($p<0.01$) in 5l samples group than

in those from the 2L, 3L, 4L and 6L samples groups, as all indicated in Fig. 5.

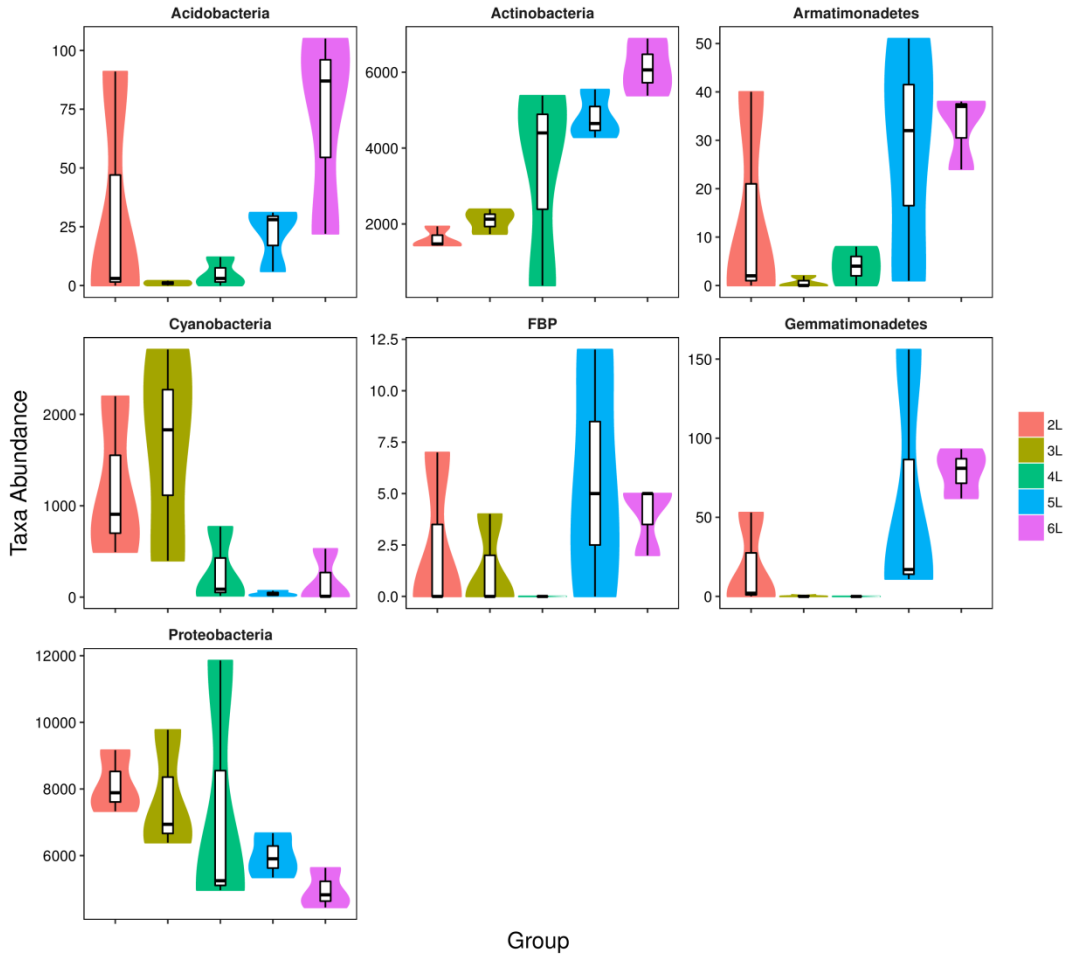

图 12 不同分期时期细菌在门水平上的显著性差异

Figure 12. Taxonomic profiles of the notable significant different bacterium at the phylum level. The most abundant phyla include: Acidobacteria, Actinobacteria, Armatimonadetes, Cyanobacteria, FBP, Gemmatimonadetes and Proteobacteria. The number indicates the sampling time and capital letter L represents Leaf litter.

3.3.7 代谢功能变化

3.3.7 Metabolic function

The differences in the effect of photoperiod on the functional properties across the 5 sample groups were further evaluated. Moderate differences were observed in cellular processes, particularly, in cell motility between all samples except 4L (Figure 13). Cell motility was relatively low in samples from the 6L sample group compared to the motility in samples from the 2L and 3L. However, there were no notable differences in other functions such as transport and catabolism, cell growth and death, and cell communication across the groups. Analysis of the metabolism of the samples showed that carbohydrate metabolism and amino acid metabolism were enhanced in 4L samples and 5L samples were the second top as compared to those in the 2L, 3L and 6L samples. Xenobiotics biodegradation and metabolism, lipid metabolism and energy metabolism performed in moderate level. Other functions did not exhibit any differences across the rest tested sample groups (Figure 14). Similar results were observed in other functional processes, such as genetic information processes and environmental information processes

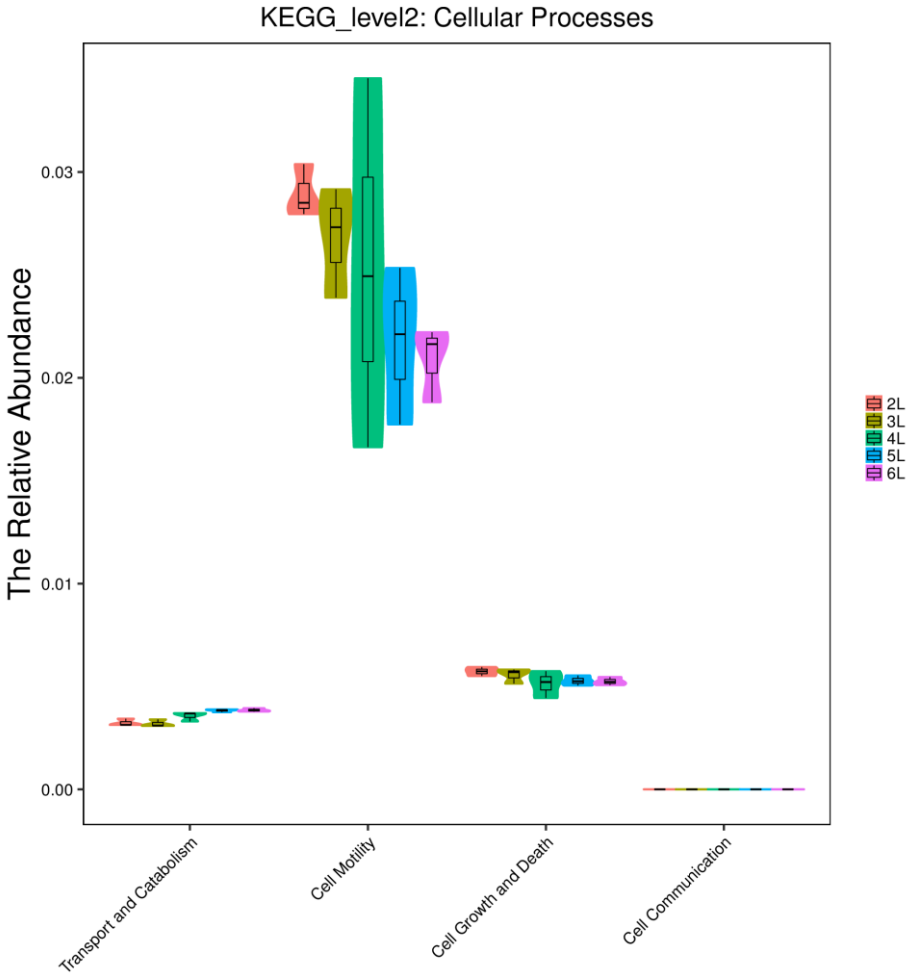

图 12 细胞过程的差异

Figure 13. Functional differences of the cellular processes. Number indicates the sampling time and capital letter L represents Leaf litter.

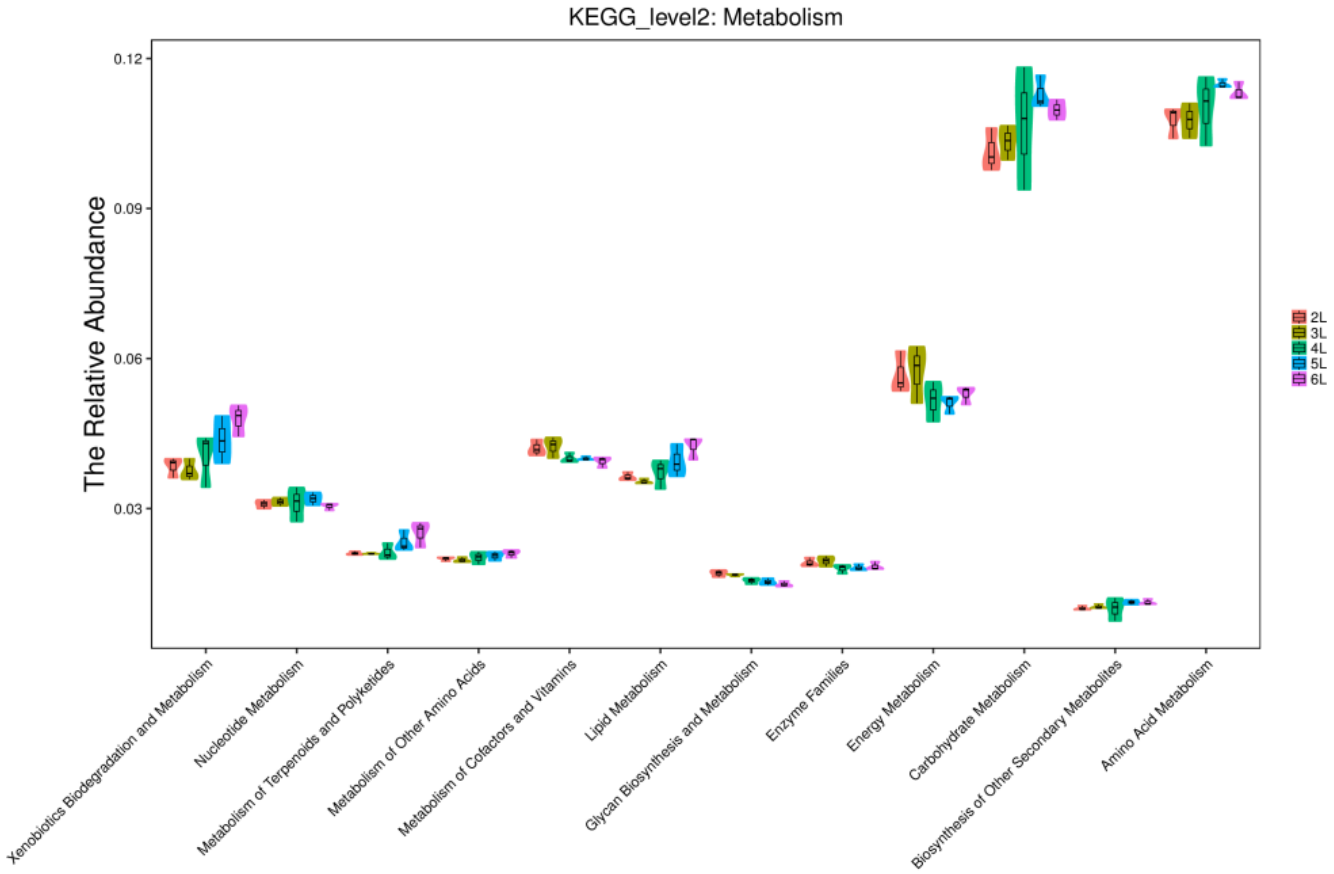

图 14 叶际细菌群落主要代谢功能变化

Figure 14. This figure representing the functional differences at the metabolism level between the different samples time groups. The number indicates the sampling time and capital letter L represents Leaf litter.

3.4 叶际真菌群落动态

3.4 Phyllosphere fungi community dynamics during leaf litter decomposition

3.4.1 不同分解时期叶际真菌类群变化

3.4.1 Common phyllosphere fungal communities by various sample times.

The Venn diagram illustrates the similarity and uniqueness of phyllosphere fungal communities within various samples (Figure 15) 6L and 5L were outnumbered with unique OUTs than 2L and 3L, 157, 54, 23 and 20, respectively. However, 4L had average OTUs unique rates among all of the samples. It shows that the last samples more trended compared to the rest as an instance 5L and 6L had 155 similar OTUs which was the highest shared OTUs. The second top unique OTUs was 110 that was unique for all samples excluding 6L 68 shared OTUs were joined 2L, 3L and 4L for uniqueness. 2L and 3L had 34 OTUs similar with each other which were quite reasonable comparing to their unique OTUs rates. The 3L sample had the least unique OTUs (20) and it shared 14 and 24 OTUs with 4L and 5L samples, respectively. 6L samples that had highest trend among all shared 155, 24, 3 and 5 OUTs with 5L, 4L, 3L and 2L samples. In terms of pairs common OTUs 2L and 3L samples shared 34 OTUs however. It was more than their unique OTUs. 5L sample shared least OTUs with 2l (7) and 12 with 4L and 24, 155 with 3L and 6L samples, respectively. 4L, 5L and 6L samples shared 43 OTUs with each other while the all samples had 5 OTUs in common with the exception of 4L. All in all, we have observed that 97 OTUs were shared within all samples and it was greatly impacted by the last samples time. In this diagram the shared OTUs were less then unique OTUs.

Just it was inversed in case of the first and second samples. The gap was noticed between the early stage of leaf litter decomposition and last stages (Figure 15).

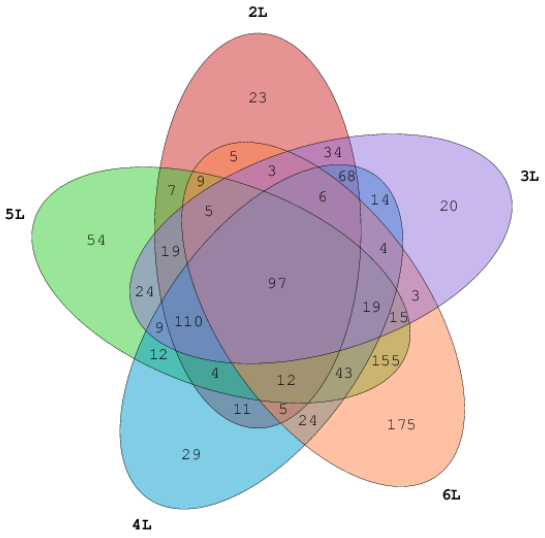

图 15 不同分解时期真菌共有、特有 OTUs 的韦恩图

Figure 15. Venn diagram the amount of common and unique OTUs different sampling time, (2, 3, 4, 5 and 6L) refer to the shared and unique OTUs in the first, second, third, fourth and fifth samples of phyllosphere fungal community inhabiting in leaf litters.

3.4.2 真菌群落多样性指数

3.4.2 Analysis of diversity index

In order to discover the fungal community diversity Illumina high-throughput sequencing was implemented. Above table revealed that, alpha diversity could be studied by several indices that include Simpson, Chao1, ACE and Shannon. Those mentioned indicators were used to discover the biodiversity of 15 leaf litter samples

during certain decomposition period. The result shows that based on the effective sequences 5CL (329) and 6CL (314) had the highest richness comparing to the rest of samples indicating by Chao. Accordingly, 5CL and 6CL were high rated in ACE 334 and 309, respectively. 5SL had the least trend in both: Chao1- 101 and ACE – 105. It was observed that the level of Chao1 were in moderate level at 4CL, 5LL, 3LL, and 4LL 276, 286, 245 and 221 respectively. 5SL (101), 4SL (113) and 3SL (138) had low community richness in comparison with the other samples by Chao1 indicators. The patterns of ACE accordant with the evaluation of Chao1 and 5SL, 4SL and 3SL had noticeable low rate of community richness and 6CL and 5CL had dramatically high trend of richness. According to the Simpson diversity indices, samples 6CL, 5CL and 5LL had the highest community diversity, 0.92, 0.92 and 0.90 respectively, whereas 3LL (0.70), 3SL (0.71) and 2CL (0.73) had low community diversity. 6CL and 5CL had the top trend of Shannon index around 5.12 and 4.97 respectively. Comparatively with the rest of samples, the 5LL (4.68), 6LL (4.25) and 6SL (4.16) samples had the average community diversity from the Shannon indicators (Table 4). Overall, all the microbial-fungal diversity indices illustrate that samples 6CL, 5CL and 5LL had very high trends in both; community richness and diversity.

表 4 真菌群落多样性指数变化

Table 4. The table shows richness and diversity of fungal community.

Microbial diversity index				
Samples	Simpson	Chao1	ACE	Shannon
2CL	0.73	181	182	2.99
2LL	0.78	218	222	3.52
2SL	0.84	158	163	3.56
3CL	0.81	214	217	3.6
3LL	0.7	245	248	3.44
3SL	0.71	138	144	2.55
4CL	0.81	276	283	3.97
4LL	0.66	221	190	2.85
4SL	0.78	113	116	3.05
5CL	0.92	329	334	4.97
5LL	0.9	268	272	4.68
5SL	0.73	101	105	2.75
6CL	0.92	314	309	5.12
6LL	0.87	190	190	4.25
6SL	0.88	206	210	4.16

The number represent sampling time, the first letters represents leaf litter of vary tree species as follow: CL - Locust leaf litter, LL - Liaodong oak leaf litter and SL - Aspen leaf litter. The table demonstrates the different parameters of alpha diversity within samples. Sampson and Shannon represent community diversity and the high rate indicates more diversity. Chao1 and ACE are community richness; the high rates illustrate the more richness.

3.4.3 Alpha 多样性指数变化

3.4.3 Fungi Alpha diversity

In phylum and class level, 15 samples of three tree species in 5 times were researched for discovering fungal community compositions (Figure 16). A vast majority of these fungal taxa were relatively common across samples at phylum level and about 7 phyla were involved during the leaf litter decomposition in a span of year. The top number

was observed in 2CL (almost 100%), 6SL (almost 100%) that were dominated by *Ascomycota. Basidiomycota* was the second largest dominator phyla which recorded average 20%. The only pattern that can be differentiated between these two dominant phyla was that, *Basidiomycota* was more active in 2LL, 5LL and 6LLs samples excluding 3LL and 4LL. *Zygomycota* was the next wide dominant among all phyla while its total rate was just 2% abundance, which was only noticed in 4CL. *Ascomycota* had the least relative abundance in 6LL sample just above 60% and accordingly 2LL (78%), 5LL, 5SL 80% and 81% respectively. Comparing with the above mentioned phyla, in the process of leaf litter decomposition *Chytridiomycota, Cercozoa* and *Ciliophora* had super minimums rate of relative abundance that were not able to figured out the trend. Although, the others phyla had 5% relative abundance in 6LL that was the highest number. 6LL (4%), 5CL (3%) and 3LL (2%) were the abundant rate of the others phyla in total fungal community taxa. (Figure 16A)

Huge diverse microorganisms that were implicated in leaf litter decomposition process, the results were explored by high-throughput. At the class level *Dothideomycetes* was the most abundant from 2% to 97% in all samples during leaf litter decomposition (Figure 16B). However, it abandoned the early stage of decomposition processes. For instance, 2CL, 3CL, and3SL had the highest abundance trend 98%, 96% and 82%, respectively. But it fluctuated in the middle stage of the leaf litter decomposition process. Even though *Sordariomycetes* were

unstable in the early sampling time, it was outnumbered in the end of leaf litter decomposition process comparing with the rest of sampling time. *Sordariomycetes* abandoned 6SL sample from 38% to 98% that was the highest rate. Except the wide trend of these two phyla the following classes *Tremellomycetes*, *Leotiomycetes* and *Agaricommycetes* were dominants of the leaf litter decomposition, average of 22%, 16% and 5% respectively. Those mentioned classes fluctuated all throughout the leaf litter decomposition period of time. 6CL and 6LL had abundant by *Tremellomycetes* and *Leotiomycetes* but 2CL and 3CL almost do not abandoned by these classes. *Microbotryomycetes* and *Eurotiomycetes* had around 5%, 4% abundance in 4SL and 6LL respectively. Others classes that were unknown had high trend in 4SL, 6SL, 2SL, 5SL and 3SL 35%, 30%, 23%, 21% and 20%, respectively. However, 4LL and 2Cl had the least rate of others samples relative abundances. (Figure 16B)

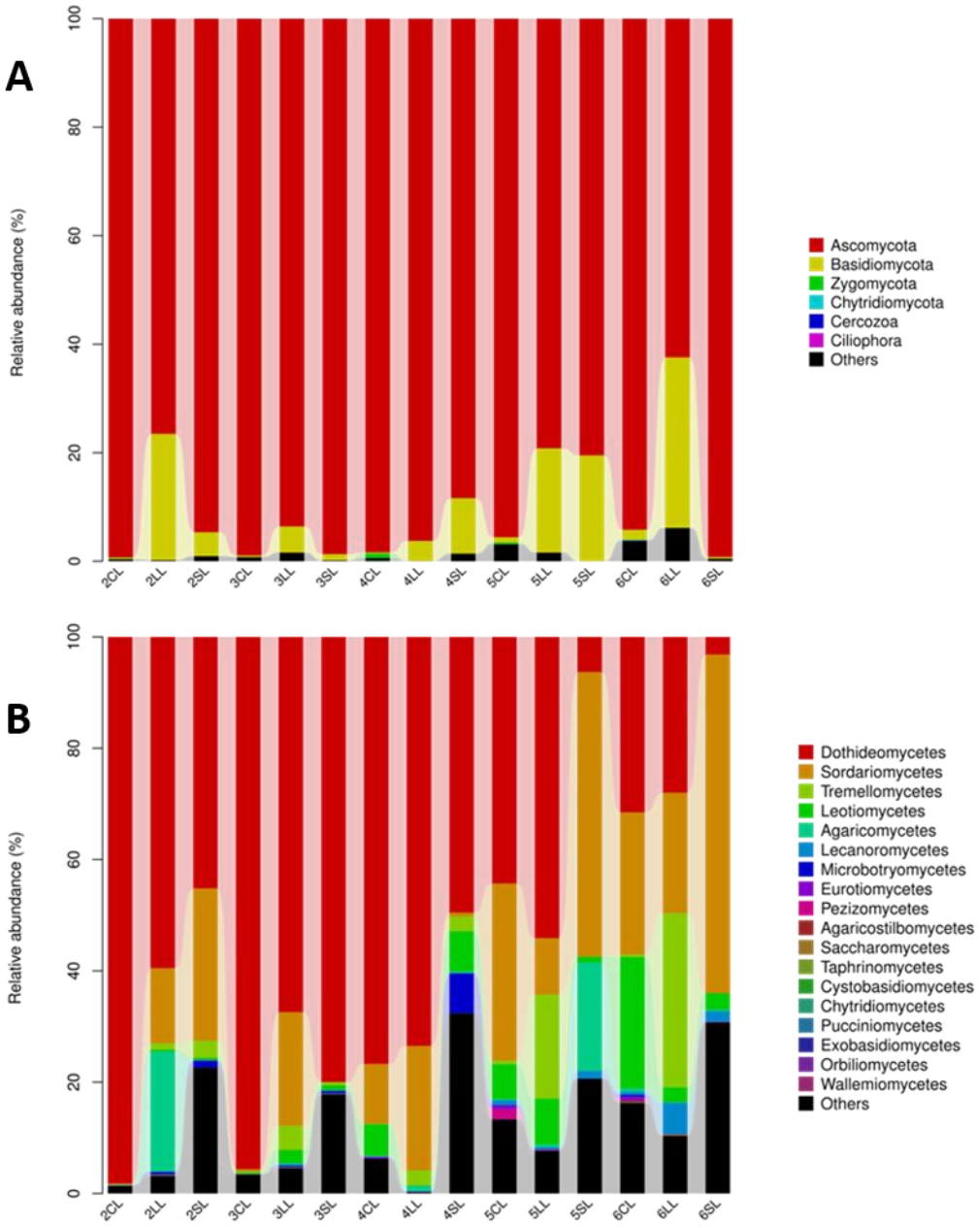

图 16 叶际真菌群落类群在不同分解时期的变化

Figure 16. Fungal community variation during decomposition at different level. A) Abundances of different phyla are in the analyzed samples and B) Relative abundances of the most abundant

classes in the analyzed samples. The abundance is presented in terms of percentage in total effective microbial sequences in various samples. Abscissa represents samples, and different color shows different bacterial phyla.

3.4.4 Beta 多样性变化

3.4.4 Beta diversity change based on heat map

All the samples were generated and clustered based on similarity and dissimilarity among samples (Figure 17). There was a relationship with the patterns of samples and fungal community diversity. According to the fungi genus's samples information (Figure 17), 4CL had high abundance with *Pseudocercospora, Periconia, Dothistroma, Gibberella, Monographella* and *Mortierella* and clustered as first group. 5CL and 6CL another two subgroups of this first group were consist of *Sarcoscypha, aplosporella, Kaemelia* and *Hymenoscyphus, Sclerostagonospora, Recurvomyces, Scleropezicula* and *Coniothyrium* that were clustered to first subgroup and second subgroup, respectively. 6SL one of the second top category that just had high abundance with *Tothia, Stibella, Calcarisporiu* and *Anthostomella* and half of the rest genus had very low abundances. However 5LL and 6LL were clustered to another semi-category which *Dioszegia, Devriesia, Polyscytalum* and *Lophiostoma* and *Paraphaeosphaeria, Lecania, Cylindrosympodium* were with high abundances, respectively. 4LL were clustered in one group with some moderate genus such as *Mycosphaerella, Plagiostoma* and *Cryptococus* and the rest of genus had very low

abundances. Thus, the results of the genus analysis showed that a sample can be clustered into one of the four sub-categories: 2CL 3CL and 4LL, 3LL and had single groups 2SL, 3SL with almost the lowest abundance. Genus such as; *Aureobasidium*, *Sphaerulina* and *Acremonium* apart from 2CL, 3Cl and 5CL had the least abundances with the rest of samples. In 6CL there around 10 genuses had high abundances which were the noticible trend in comparison with the other samples. The high relative abundances were observed with *Rhodotorula*, *Mastigobbasidium* and *Cystofilobasidium* in 4SL sample that was clustred as a single semi- category.

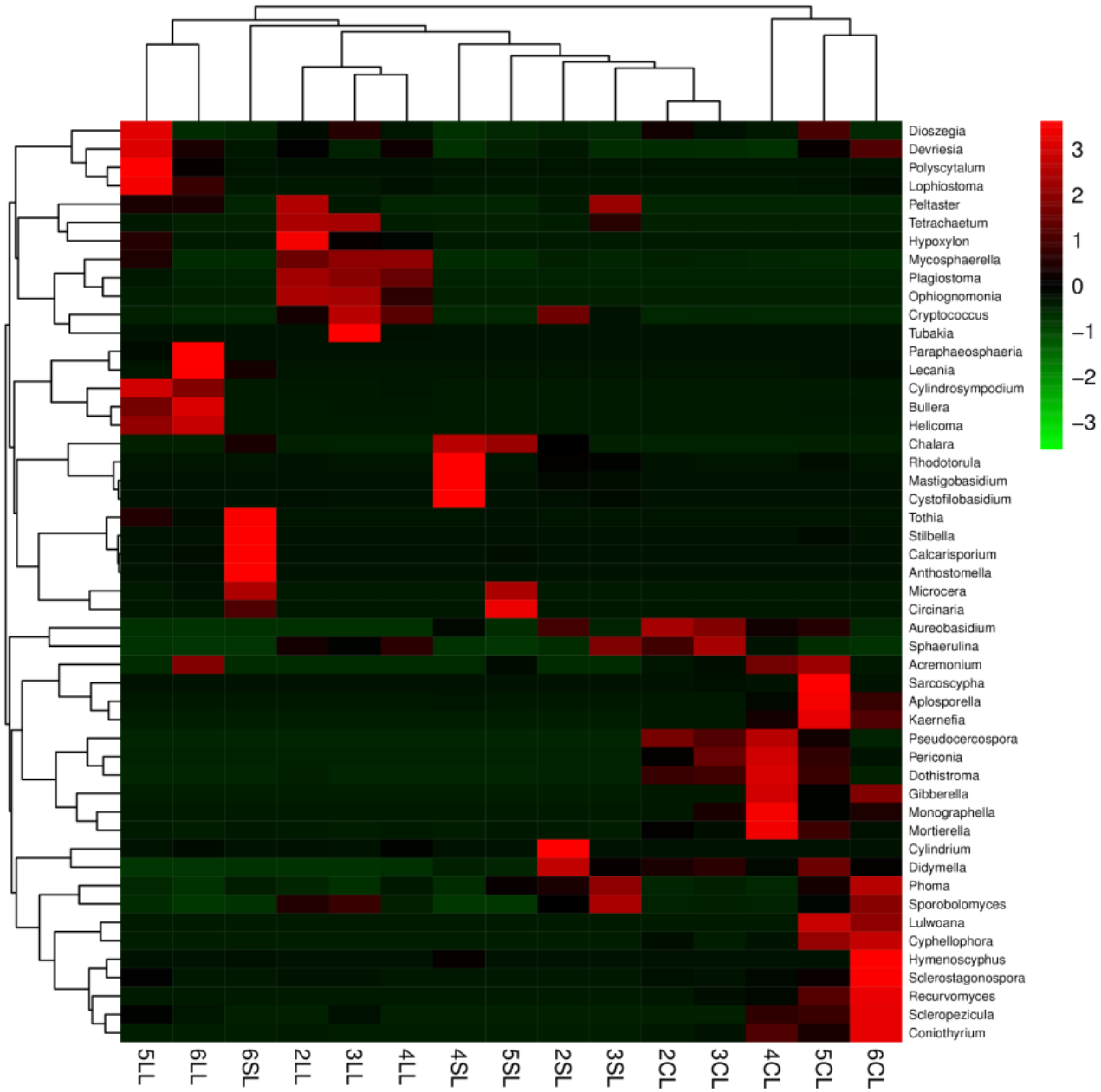

图 17 相对丰度前 50 的真菌属在不同分解时期的变化

Figure 17. For the level of species and the abundance of information, we collected 50 genuses from the whole abundance sequences to draw a species abundance cluster map. Abscissa for sample information, ordinate for species annotation information, and clustering tree on the left side of the figure as a species tree clustering; above the clustering tree is a sample cluster tree. The

colors; red represents the high abundance and green represents low abundance rate.

3.4.5 真菌群落结构的变化

3.4.5 Change of fungal community composition

In order to categorize the community composition of all leaf litter samples the (A) weighted and (B)unweighted UniFrac distance Non-metric multidimensional scaling (NMDS) was applied (Figure 18).The percentage in parentheses in the axis shows the range of the difference in the raw data that the corresponding principal component can interpret. NMDS analysis was performed on Unweighted and Weighted UniFrac distance matrices using R software, and the structure distribution of the community samples were described by two-dimensional or three-dimensional sorting graphs. In the primary stage of the investigation totally 15 samples were observed and with 5 sampling time were figured out in this patterns. 6CL, 6LL and 6SL had closer distance in weighed NMDS however they were placed with more distance in unweighed NMDS (Figure 18A, B). Though, samples; 2SL, 2LL and 2SL had closer similarity in Unweighted NMDS and they had very long distances in weighed NMDS that was the sign of dissimilarity. Surprisingly, the dissimilarity of 4Ls was observed in both weighted and unweighted NMDSs (Figure 18 A, B). 3SL and 3Cl were much more similarity in weighted NMDS but in unweighted NMDS they lose the close distance and 3SL became more similar with 3LL. 5CL, 5SL and 5LL kept the similarity balance in weighted NMDS, while 5CL were dissimilar with both 5LL

and 5SL in unweitghed NMDS. All 5th samples were in optimal rate of similarity and stress level. 2CL and 2LL were much more similar and high stress in compression with rest of 2ndtime samples. In terms of similarities of samples time 2nd, 3rd and 4th samples time were more in closer distance in unweighed NMDS. In terms of single sampling times 2CL and 3CL had the most similarity in both weighted and unweighted NMDSs (Figure 18A, B). 6CL and 6LL were in highest dissimilarity in unweighted NMDS comparing to all samples.

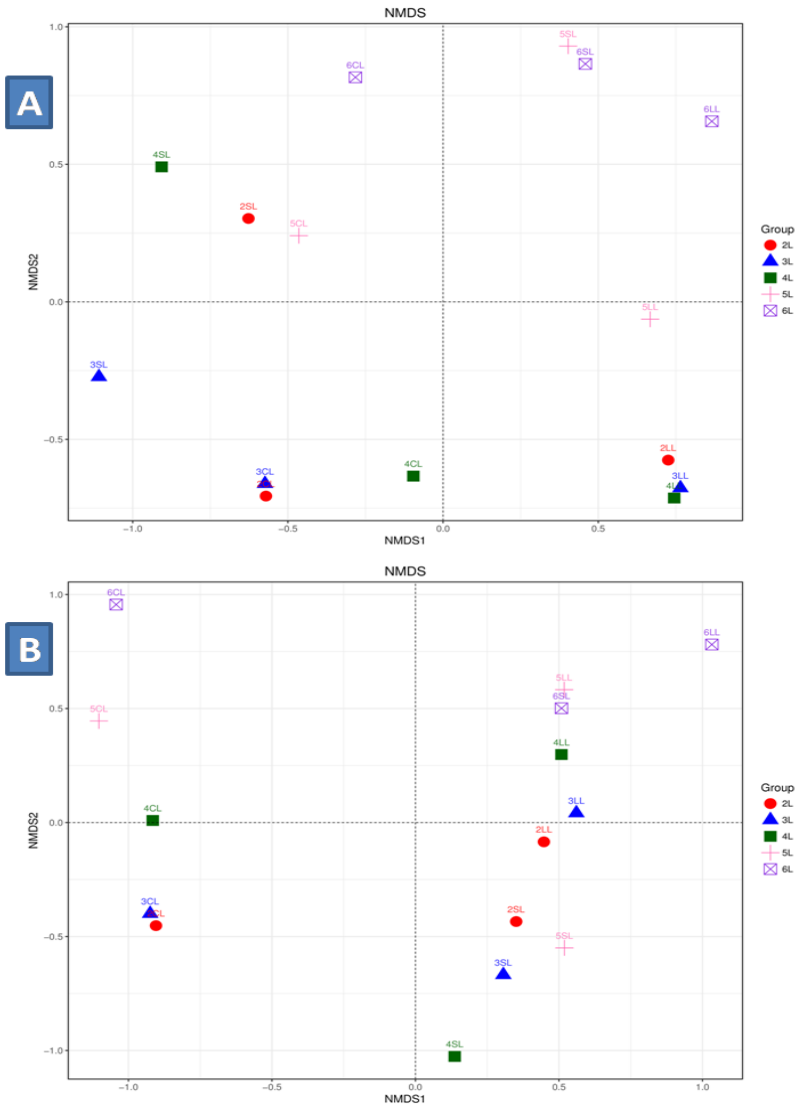

图 18 叶际真菌群落结构在不同分解时期的变化

Figure 18. Each point represents a sample. Points of different colors belong to different samples (groups). The closer the distance between the two points, the higher the similarity of microbial community structures between the two samples and the smaller the difference. Figures A and B represent weighted and unweighted UniFrac scales, respectively. The number indicates the sampling time and capital letter L represents Leaf litter.

3.4.6 真菌群落在门、属水平显著差异的类群

3.4.6 Abundance and significant difference of microbes at phylum and genera level.

A total of 2034356 fungal reads and the 2819 fungal number of OTUs were obtained after bioinformatics analysis and quality filtering throughput of sequencing. Within the 6L, 2L and 5L, 4L, 3L samples groups, there was a noteworthy differentiation in the abundance of Ascomycota phylum, which was more abundant in 6L and 2L samples group than in the rest samples ($p<0.01$), respectively. 5L had moderate taxa abundant while 3L with low and 2L with the least low taxa abundance trend. 5L had huge abundance taxa by Ciliophora. In contrast with Ascomycota, 6L had doubled less taxa abundance in Ciliophora. Surprisingly, 2L, 3L and 4L did not show any taxa abundances in Ciliophora phylum (Figure 19A). Nine genera were significantly ($p<0:01$) different in abundance between the 2L and 5L sample groups. Also more importantly the sample 6L were with high abundance of Austroplaca, Cladonia,

Crocicreas, Gymnomyces, Jahnula, Tomentella and Tuber. Interestingly, Ascochyta, Aureobasidium, Erythrobasidium, Ochrolechia, Sphaerulina and Tetrachaetum were significantly more abundant ($p<0:01$) in 2L sample group. In sample 5L were observed that genera: Ascochyta, Devriesia, Dioszegia, Erythrobasidium and Sarcinomyces were significantly ($p<0:01$) abundant then the rest of samples and genera. Surprisingly, Ascochyta were abundant with all samples groups with the exception of 6L while Cystofilobasidium genera were abandon in samples 4L (Figure 19B).

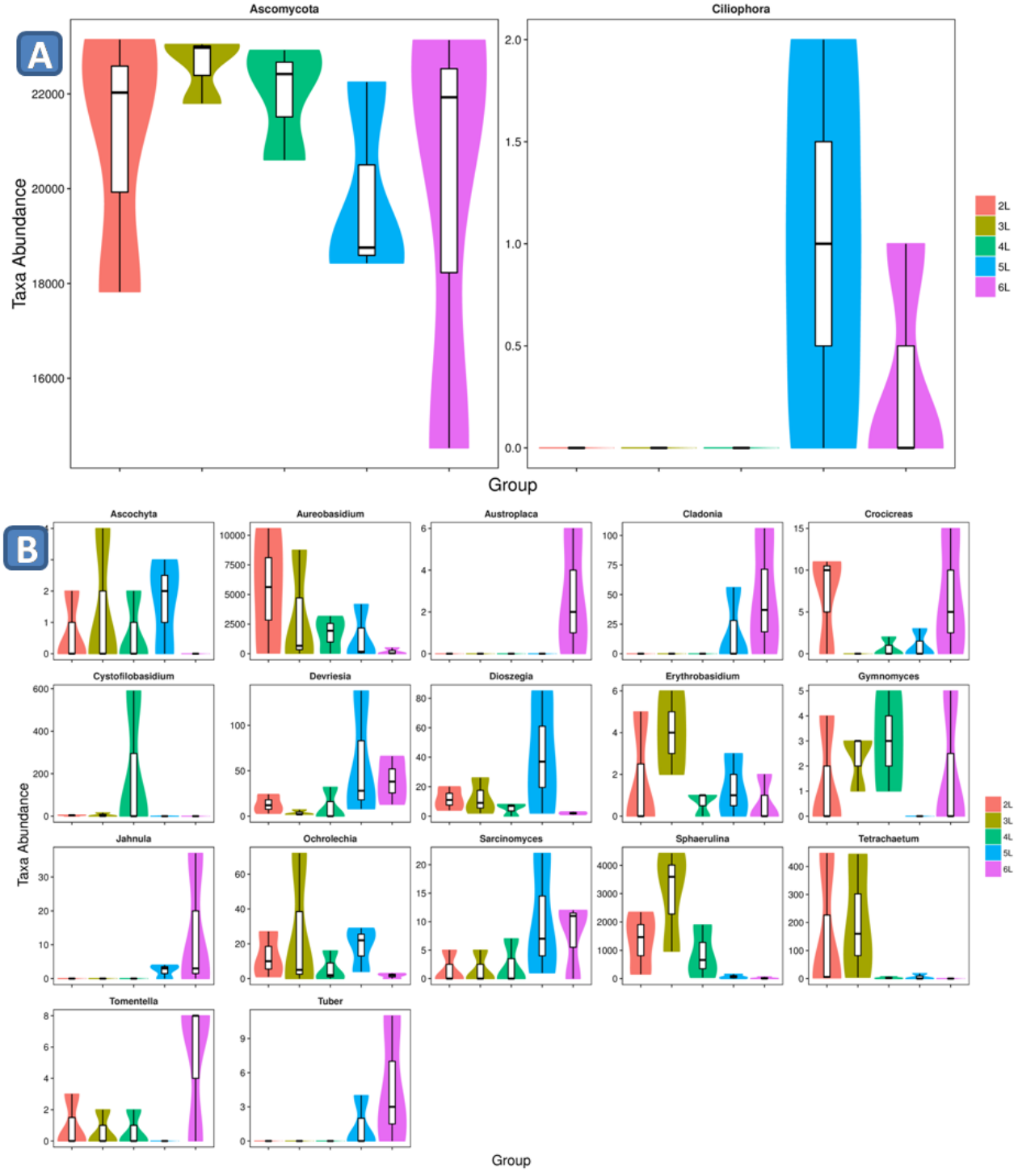

图 19 不同分解时期真菌群落在门、属水平显著差异的类群

Figure 19. Taxonomic profiles of the notable significant different fungal community at the phylum level. A-The most abundant phyla include: Ascomycota and Ciliophora, B is representing the

genera level. The number indicates the sampling time and capital letter L represents Leaf litter.

3.5 叶际微生物和叶片养分的关系

3.5 Relationship between phyllosphere microbes with nutrients during leaf litter decomposition

3.5.1 叶际细菌群落和主要养分的关系

3.5.1 Correlation of bacterial community with the primary nutrients in leaf litter decomposition

In order to assess whether any primary nutrients of leaf litter correlated with bacterial taxa results, a total of 35 variables (6 primary nutrients including C, N, P, C/N, C/P, N/P and 29 bacterial taxa; 13, 16 variables at phylum and class level, respectively Table 5) were regressed with the Pearson correlation coefficient. After generating the correlation analysis, just 3 values were significantly correlated at phylum level and 9 values illustrated significant correlation at class level. Carbon didn't showed any correlation at phylum level however there was a significant positive correlation ($r = 0.630$, $P < 0.011$) with Flavobactriia class. Cyanobacteria as one of the abundant phyla had significant positive association ($r = 0.626$, $P < 0.013$) with N (Nitrogen). And at the class level, Chloroplast and Flavobacteriia ($r = 0.634$, $P < 0.013$), ($r = 0.600$, $P < 0.018$) had a significant relationship with N composition in leaf litter decomposition, respectively. Despite, the no significance relationship at

bacterial phylum level, there were two classes (Thermoleophilia ($r = 0.594$, $P < 0.020$) and Acidimicrobiia ($r = 0.536$, $P < 0.039$)) significantly positive correlated with P (Phosphorus). In terms of nutrients ratios, the C/N ratio showed a positive association with Proteobacteria which one of the most abundant bacterial phyla. In additional, it had positive correlation with Gammaproteobacteria ($r = 0.634$, $P < 0.011$) at class level. Gammaproteobacteria as the abundant class presented the same trend with C/P ratio the association direction and significance were as follow, ($r = 0.855$, $P < 0.001$). The N/P ratio illustrated more correlation with the bacterial taxa. It showed positive correlation with Cyanobacteria ($r = 0.709$, $P < 0.003$) at phylum level and Chloroplast ($r = 0.711$, $P < 0.003$), Flavobacteriia ($r = 0.635$, $P < 0.011$) at class level (Table 5).

表 5 细菌群落与叶片养分的相关性分析

Table 5. Bacterial community correlation with nutrients and their ratios at phylum and class

levels

	Taxon	C		N		P		C/N		C/P		N/P	
		r	P	r	P	r	P	r	P	r	P	r	P
Phylum level	Proteobacteria	0.063	0.824	-0.335	0.223	-0.156	0.580	0.590*	0.021	0.486	0.066	-0.222	0.426
	Actinobacteria	-0.290	0.295	0.049	0.864	0.144	0.608	-0.421	0.118	-0.505	0.055	-0.106	0.707
	Bacteroidetes	0.203	0.467	-0.018	0.950	-0.362	0.184	-0.048	0.864	0.025	0.930	0.111	0.693
	Cyanobacteria	0.478	0.071	0.626*	0.013	0.128	0.649	-0.267	0.337	0.143	0.611	0.709**	0.003
	Deinococcus-Thermus	-0.070	0.804	0.377	0.166	0.169	0.548	-0.352	0.198	-0.186	0.508	0.325	0.237
	Saccharibacteria	-0.120	0.670	-0.361	0.187	-0.241	0.388	0.231	0.407	0.153	0.586	-0.212	0.449
	Gemmatimonadetes	-0.186	0.507	-0.147	0.601	0.324	0.239	-0.085	0.764	-0.344	0.210	-0.369	0.176
	Acidobacteria	-0.195	0.487	-0.043	0.880	0.289	0.297	-0.067	0.813	-0.181	0.519	-0.217	0.437
	Chloroflexi	-0.169	0.548	0.157	0.576	0.503	0.056	-0.360	0.187	-0.474	0.074	-0.141	0.616
	Firmicutes	-0.191	0.496	-0.421	0.118	0.194	0.489	0.300	0.277	-0.100	0.723	-0.585	0.022
	Planctomycetes	-0.137	0.627	0.206	0.461	0.469	0.078	-0.404	0.135	-0.508	0.053	-0.070	0.803
	Armatimonadetes	-0.235	0.399	0.017	0.953	0.264	0.341	-0.178	0.525	-0.281	0.310	-0.149	0.597
	Verrucomicrobia	-0.110	0.698	0.252	0.365	0.447	0.095	-0.351	0.199	-0.361	0.186	0.017	0.952
Class level	Alphaproteobacteria	-0.156	0.579	-0.382	0.160	-0.015	0.958	0.427	0.112	0.076	0.787	-0.497	0.059
	Actinobacteria	-0.290	0.294	0.034	0.904	0.122	0.666	-0.409	0.130	-0.494	0.062	-0.110	0.695
	Gammaproteobacteria	0.192	0.493	-0.272	0.327	-0.552	0.033	0.634*	0.011	0.855**	0.000	0.119	0.673
	Betaproteobacteria	-0.039	0.890	-0.150	0.594	0.359	0.189	0.133	0.637	-0.175	0.532	-0.344	0.209
	Chloroplast	0.479	0.071	0.624*	0.013	0.119	0.672	-0.264	0.343	0.151	0.592	0.711**	0.003
	Sphingobacteriia	-0.121	0.668	-0.488	0.065	-0.558	0.031	0.238	0.393	0.051	0.856	-0.336	0.221
	Cytophagia	-0.207	0.459	0.133	0.637	0.163	0.562	-0.006	0.983	0.058	0.838	0.046	0.871
	Flavobacteriia	0.637*	0.011	0.600*	0.018	0.150	0.594	-0.422	0.118	-0.078	0.783	0.635*	0.011
	Deltaproteobacteria	-0.040	0.889	0.467	0.079	0.443	0.098	-0.580	0.023	-0.499	0.058	0.236	0.396
	Deinococci	-0.070	0.804	0.377	0.166	0.169	0.548	-0.352	0.198	-0.186	0.508	0.325	0.237
	Thermoleophilia	-0.142	0.613	0.399	0.140	0.594*	0.020	-0.559	0.030	-0.573	0.026	0.090	0.749
	uncultured_bacterium	-0.147	0.601	-0.364	0.182	-0.230	0.410	0.241	0.387	0.157	0.576	-0.223	0.424
	Acidimicrobiia	-0.166	0.553	0.248	0.373	0.536*	0.039	-0.422	0.117	-0.487	0.066	-0.053	0.850
	Clostridia	-0.105	0.711	-0.191	0.496	0.217	0.437	0.051	0.856	-0.212	0.448	-0.363	0.184
	Armatimonadia	-0.219	0.433	0.096	0.734	0.267	0.337	-0.230	0.409	-0.285	0.303	-0.066	0.814
	Bacilli	-0.175	0.532	-0.460	0.084	-0.025	0.929	0.483	0.068	0.196	0.483	-0.462	0.083

Note. Variables were used for correlation analysis between bacterial community and primary nutrients of leaf litter during decomposition. Variables were regressed with a Pearson correlation coefficient. The correlation was formulate at phylum and class level.

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.05 level (2-tailed).

3.5.2 叶际细菌群落与碳水化合物的关系

3.5.2 Correlation of bacterial community with the carbohydrates in leaf litter decomposition

Correlational analyses were used to examine the relationship between the phyllosphere bacterial community at phylum and class level structural carbohydrates in leaf litter during decomposition. Results indicated none significance relationship between bacteria taxa and neutral detergent fiber (NDF) (Table 6). Armatimonadia class that was belong to Armatimonadetes phyla had positive association with acid detergent fiber (ADF) ($r = 0.559$, $P < 0.030$), ($r = 0.523$, $P < 0.046$), respectively. Interestingly, the acid detergent lignin (ADL) was highly related with the phyllosphere bacteria taxa and almost 3 phylum and 5 classes were significantly correlated with it. At phylum level Actinobakteria ($r = 0.707$, $P < 0.003$), Deinococcus - Thermos ($r = 0.635$, $P < 0.011$) and Chloroflexi ($r = 0.516$, $P < 0.049$) were positively correlated with ADL. Moreover, Actinobakteria, Deltaproteobacteria, Deinococci, Thermoleophilia and Acidimicrobiia as representative of bacterial class level had great positive and significant correlation with ADL, ($r = 0.693$, $P < 0.004$), ($r = 0.525$, $P < 0.045$), ($r = 0.635$, $P < 0.011$), ($r = 0.782$, $P < 0.001$) and ($r = 0.562$, $P < 0.029$), respectively. Surprisingly, the association of bacteria taxa with semi-cellulose was not observed in this investigation. Cellulose showed a positive significant relationships with Proteobacteria ($r = 0.627$, $P < 0.012$) at phylum level and with Alphaproteobacteria ($r = 0.631$, $P < 0.012$), Bacilli ($r = 0.540$, $P < 0.038$) at class level.

Lignin one of the structural carbohydrates that revealed all positive significant correlation with around 8 bacterial community including 3 phyla and 5 classes. Actinobakteria had the strongest positive significant ($r = 0.707$, $P < 0.003$) association with Lignin and Deinococcus – Thermos ($r = 0.635$, $P < 0.011$) and Chloroflexi ($r = 0.516$, $P < 0.049$) showed moderate positive relationship at phylum level. As ADL performed a positive significant correlation with above mentioned classes of bacterial Lignin did accordingly.

表 6 叶际细菌群落与碳水化合物的相关性分析

Table 6. Bacteria community correlation with structural carbohydrate at phylum and class levels

	0	NDF		ADF		ADL		Semi-cellulose		Cellulose		Legnin	
		r	P	r	P	r	P	r	P	r	P	r	
Phylum level	Proteobacteria	-0.242	0.385	-0.346	0.207	-0.745	0.001	0.015	0.958	0.627*	0.012	-0.745	0.0
	Actinobacteria	0.331	0.228	0.456	0.088	0.707**	0.003	0.008	0.976	-0.529	0.042	0.707**	0.0
	Bacteroidetes	-0.153	0.585	-0.074	0.792	0.065	0.818	-0.235	0.399	-0.109	0.700	0.065	0.8
	Cyanobacteria	-0.125	0.658	-0.252	0.366	-0.151	0.590	0.131	0.641	0.034	0.905	-0.151	0.5
	Deinococcus-Thermus	-0.052	0.853	-0.069	0.806	0.635*	0.011	-0.006	0.984	-0.722	0.002	0.635*	0.0
	Saccharibacteria	-0.398	0.142	-0.330	0.230	-0.119	0.674	-0.379	0.163	-0.042	0.881	-0.119	0.6
	Gemmatimonadetes	0.224	0.423	0.313	0.256	0.309	0.263	-0.002	0.995	-0.172	0.539	0.309	0.2
	Acidobacteria	0.133	0.636	0.249	0.371	0.259	0.350	-0.107	0.705	-0.152	0.589	0.259	0.3
	Chloroflexi	0.160	0.569	0.346	0.207	0.516*	0.049	-0.208	0.458	-0.380	0.163	0.516*	0.0
	Firmicutes	-0.201	0.473	-0.159	0.570	-0.242	0.386	-0.203	0.468	0.179	0.523	-0.242	0.3
	Planctomycetes	0.299	0.280	0.420	0.119	0.398	0.141	-0.007	0.979	-0.214	0.445	0.398	0.1
	Armatimonadetes	0.492	0.062	0.559*	0.030	0.397	0.143	0.213	0.445	-0.141	0.617	0.397	0.1
	Verrucomicrobia	0.199	0.477	0.364	0.182	0.375	0.168	-0.147	0.601	-0.218	0.436	0.375	0.1
Class level	Alphaproteobacteria	0.165	0.556	0.174	0.535	-0.500	0.057	0.095	0.737	0.631*	0.012	-0.500	0.0
	Actinobacteria	0.332	0.226	0.454	0.089	0.693**	0.004	0.015	0.959	-0.515	0.049	0.693**	0.0
	Gammaproteobacteria	-0.433	0.107	-0.604	0.017	-0.624	0.013	0.001	0.997	0.362	0.184	-0.624	0.0
	Betaproteobacteria	-0.067	0.811	-0.072	0.800	-0.381	0.161	-0.038	0.894	0.375	0.168	-0.381	0.1
	Chloroplast	-0.125	0.658	-0.250	0.369	-0.152	0.590	0.129	0.647	0.035	0.902	-0.151	0.5
	Sphingobacteriia	-0.081	0.775	-0.069	0.808	-0.178	0.525	-0.074	0.794	0.157	0.576	-0.178	0.5
	Cytophagia	0.041	0.885	0.143	0.610	0.054	0.847	-0.145	0.605	0.015	0.957	0.055	0.8
	Flavobacteriia	-0.146	0.603	-0.114	0.687	0.325	0.237	-0.152	0.588	-0.411	0.128	0.326	0.2
	Deltaproteobacteria	0.372	0.173	0.509	0.052	0.525*	0.045	0.014	0.961	-0.304	0.270	0.525*	0.0
	Deinococci	-0.052	0.853	-0.069	0.806	0.635*	0.011	-0.006	0.984	-0.722	0.002	0.635*	0.0
	Thermoleophilia	0.173	0.538	0.290	0.294	0.782**	0.001	-0.084	0.766	-0.695	0.004	0.782**	0.0
	uncultured_bacterium	-0.433	0.107	-0.358	0.191	-0.125	0.657	-0.414	0.125	-0.050	0.861	-0.125	0.6
	Acidimicrobiia	0.149	0.597	0.324	0.238	0.562*	0.029	-0.198	0.479	-0.440	0.101	0.562*	0.0
	Clostridia	-0.173	0.538	-0.077	0.784	0.062	0.826	-0.275	0.321	-0.107	0.704	0.062	0.8
	Armatimonadia	0.429	0.110	0.523*	0.046	0.426	0.113	0.127	0.653	-0.191	0.495	0.426	0.1
	Bacilli	-0.070	0.804	-0.165	0.557	-0.579	0.024	0.113	0.687	0.540*	0.038	-0.579	0.0

Note. Variables were used for correlation analysis between bacterial community and structural carbohydrate of leaf litter during decomposition. Variables were regressed with a Pearson correlation coefficient. The correlation was formulated at phylum and class level.

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.05 level (2-tailed).

3.5.3 真菌群落与叶片主要养分的相关性分析

3.5.3 Correlation of fungal community with the primary nutrients in leaf litter decomposition

Table 7 shows the correlation between fungal community and primary nutrients of leaf litter during decay. Moderate perceived control over fungal abundance was associated with the dynamics of the nutrients which revealed positive affect. With one exception, no significance relationship between fungal community and carbon and nitrogen were observed. According to the microbial sequencing data there mots fungal community that was clustered as “other” showed positive significant correlation at phylum level ($r = 0.563$, $P < 0.029$) and class level ($r = 0.563$, $P < 0.029$). Inattentive relationship was notice among fungal taxa and C/N ratio and C/P ratio throughout the leaf litter decomposition. N/P ratio just had a positive significant association with Ascomycota phylum ($r = 0.532$, $P < 0.041$) and Dothideomycetes class ($r = 0.535$, $P < 0.040$). In summary, the relationship between phyllosphere fungal communities was poorly related during the leaf litter study (Table 7).

表 7 不同分解时期叶际真菌群落与叶片主要养分的相关性分析

Table 7. Fungal community correlation with nutrients and their ratio at phylum and class levels

		C		N		P		C/N		C/P		N/P	
	Taxon	r	P	r	P	r	P	r	P	r	P	r	
Phylum level	Ascomycota	0.227	0.416	0.304	0.270	-0.188	0.502	-0.053	0.852	0.296	0.285	0.532*	0
	Basidiomycota	-0.214	0.445	-0.372	0.172	0.101	0.719	0.138	0.623	-0.225	0.420	-0.563	0
	Other	-0.136	0.629	0.126	0.654	0.563*	0.029	-0.363	0.183	-0.531	0.042	-0.206	0
	unidentified	-0.121	0.668	0.300	0.278	0.010	0.971	-0.382	0.159	-0.135	0.632	0.366	0
	Zygomycota	0.012	0.966	0.477	0.072	0.220	0.430	-0.465	0.080	-0.260	0.348	0.402	0
Class level	Dothideomycetes	0.422	0.117	0.482	0.069	0.251	0.366	-0.116	0.681	0.199	0.477	0.535*	0
	Sordariomycetes	-0.299	0.279	-0.342	0.212	-0.213	0.445	0.070	0.803	-0.210	0.452	-0.412	0
	unidentified	-0.241	0.386	-0.336	0.221	-0.411	0.128	0.348	0.203	0.416	0.123	-0.092	0
	Tremellomycetes	-0.134	0.634	-0.140	0.619	0.464	0.082	-0.064	0.820	-0.347	0.205	-0.421	0
	Leotiomycetes	-0.187	0.506	0.223	0.424	0.273	0.324	-0.426	0.114	-0.423	0.116	0.068	0
	Incertae sedis	-0.161	0.567	-0.513	0.051	-0.724	0.002	0.310	0.261	0.243	0.384	-0.234	0
	Agaricomycetes	-0.115	0.684	-0.284	0.305	-0.328	0.233	0.221	0.428	0.030	0.915	-0.277	0
	Other	-0.136	0.629	0.126	0.654	0.563*	0.029	-0.363	0.183	-0.531	0.042	-0.206	0
	Lecanoromycetes	-0.143	0.612	-0.087	0.758	0.279	0.315	-0.177	0.529	-0.391	0.149	-0.317	0
	Microbotryomycetes	-0.096	0.734	-0.316	0.251	-0.398	0.142	0.237	0.396	0.310	0.262	-0.046	0
	unidentified	-0.121	0.668	0.300	0.278	0.010	0.971	-0.382	0.159	-0.135	0.632	0.366	0
	Eurotiomycetes	-0.082	0.772	0.471	0.076	0.477	0.072	-0.612	0.015	-0.565	0.028	0.211	0

Note. Variables were used for correlation analysis between fungal community and primary nutrients of leaf litter during decomposition. Variables were regressed with a Pearson correlation coefficient. The correlation was formulated at phylum and class level.

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.05 level (2-tailed).

3.5.4 叶际真菌群落和碳水化合物的相关性分析

3.5.4 Correlation of fungal community with the carbohydrates in leaf litter decomposition

Correlation were computed among fungal community and structural carbohydrate in decomposition various leaf litter. The results suggested that 11 out of 102 were

statistically significant and most of them were strongly correlated. The correlations of acid detergent lignin (ADL) and lignin were positively and significant related with fungal taxa in litter study. It was shown that Zygomicota had significant positive association ($r = 0.708$, $P < 0.003$) with ADL and lignin (Table 8). Neutral detergent fiber (NDF) had only a positive significant correlation ($r = 0.769$, $P < 0.001$) with Sardariomycetes class. Sardariomycetes class showed the same direction with acid detergent fiber (ADF) correlation ($r = 0.754$, $P < 0.001$). ADF had a positive significant relationship with class Eurotimycetes correlation ($r = 0.541$, $P < 0.037$). Ascomycota phylum presented a positive significant association with semi-cellulose ($r = 0.574$, $P < 0.025$) and Sardariomycetes class ($r = 0.533$, $P < 0.041$) also had a positive significant relationship with it. Again at the class level ADL and lignin showed a positive significant correlation with phyllosphere fungal communities, Leotiomycetes ($r = 0.557$, $P < 0.031$), Eurotimycetes ($r = 0.619$, $P < 0.014$), accordingly. In general, the result suggests that fungal community more correlated with ADL and lignin during leaf litter decomposition.

表 8　不同分解时期叶际真菌群落和碳水化合物的相关性分析

Table 8. Fungal community correlation with structural carbohydrate at phylum and class level

	Taxon	NDF		ADF		ADL		Semi-cellulose		Cellulose		Legnin	
		r	P	r	P	r	P	r	P	r	P	r	P
Phylum level	Ascomycota	0.193	0.490	-0.071	0.801	-0.063	0.825	0.574*	0.025	0.031	0.913	-0.062	0.826
	Basidiomycota	-0.221	0.428	0.029	0.919	-0.033	0.906	-0.568	0.027	0.051	0.857	-0.033	0.906
	Other	0.144	0.610	0.341	0.213	0.449	0.093	-0.239	0.392	-0.309	0.263	0.448	0.094
	unidentified	-0.128	0.649	-0.072	0.797	0.459	0.085	-0.179	0.524	-0.534	0.040	0.459	0.085
	Zygomycota	0.020	0.942	0.042	0.881	0.708**	0.003	-0.023	0.934	-0.744	0.001	0.708**	0.003
Class level	Dothideomycetes	-0.510	0.052	-0.584	0.022	-0.174	0.536	-0.212	0.448	-0.114	0.685	-0.173	0.537
	Sordariomycetes	0.769**	0.001	0.754**	0.001	0.071	0.802	0.533*	0.041	0.313	0.255	0.071	0.803
	unidentified	0.315	0.252	0.146	0.602	-0.008	0.978	0.493	0.062	0.084	0.765	-0.008	0.977
	Tremellomycetes	-0.159	0.571	0.000	0.999	0.200	0.475	-0.373	0.171	-0.216	0.439	0.200	0.476
	Leotiomycetes	0.200	0.476	0.292	0.292	0.557*	0.031	-0.023	0.936	-0.452	0.091	0.557*	0.031
	Incertae sedis	-0.209	0.456	-0.213	0.446	-0.153	0.586	-0.131	0.642	0.055	0.844	-0.153	0.586
	Agaricomycetes	0.014	0.960	0.180	0.521	-0.274	0.322	-0.270	0.331	0.390	0.151	-0.275	0.322
	Other	0.144	0.610	0.341	0.213	0.449	0.093	-0.239	0.392	-0.309	0.263	0.448	0.094
	Lecanoromycetes	0.275	0.321	0.394	0.146	0.305	0.270	-0.017	0.951	-0.126	0.655	0.304	0.270
	Microbotryomycetes	-0.542	0.037	-0.560	0.030	-0.092	0.745	-0.329	0.231	-0.190	0.497	-0.092	0.745
	unidentified	-0.128	0.649	-0.072	0.797	0.459	0.085	-0.179	0.524	-0.534	0.040	0.459	0.085
	Eurotiomycetes	0.392	0.148	0.541*	0.037	0.619*	0.014	0.009	0.974	-0.390	0.150	0.620*	0.014

Note. Variables were used for correlation analysis between fungal community and structural carbohydrate of leaf litter during decomposition. Variables were regressed with a Pearson correlation coefficient. The correlation was formulate at phylum and class level.

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.05 level (2-tailed).

第四章 讨论

Chapter 4 Discussion

4.1 叶片主要养分变化

4.1 Main nutrients change

All leaf litter species indicated various one year cycle changes in decomposition trend. About 1/4 of primary elements change occurred in all study sites during the initial sampling time from October to March 2018 regardless of leaf litter species. In terms of Carbon change our result was agreed with above mentioned findings by Jaeeun Sohng [24]. Due to the seasonal change and environmental factors the variation occurred after 3rd sampling time that we have noticed a huge differences and Carbon dynamics change in our study. Pandey in his research found that, greater decomposition process happens during rainy season, he believes that favorable moisture and temperature are the based condition for microorganism to be active in decaying litter [25]. According to our result we can classify the C leaf litter decomposition into two time period: 1 is warm season another could be harsh season. It can be consist of biotic and abiotic factors.

According to our result that was presented in CL species initial N concentration in leaf litter was a reliable indicator of litter decomposition rates because litter with high N concentrations was more rapidly decomposed than those with low N concentrations. The immobilization phrase is quite different within sampling times, in LL and SL species leaf litter it was with high rate at the end of one year study. Melillo have concluded that this result caused due to where N is sufficiently low in concentration in

the litter material to be limiting to microbes; and a continuous external source of N was available [26]. Percentage changes in leaf litter N in LL and SL showed that N was immobilized during the middle and end of year in all study period. The immobilization was sustained until the beginning of the second summer, 2018 (Figure 2).

Physical impact such as leaching and other on P indicated that the early stage and the overall change of it is not stable throughout the one year study. As previously study discovered, in comparison with other nutrients; the concentration of P is generally slow and less [26]. The result of our research defined that P was shown at the beginning of the study, and then a process of P immobilization started to increase within two months later and continued to peaked top trend until the 6^{th} sampling time. It has been suggested that the P in decomposing leaf litter and its immobilization and uptake by micro-organisms cannot be concluded within one year result because the immobilization phrase starts at the end of year. There existed significant different within various sampling time and concentration and dynamics of P is all tree species ($P < 0.05$) (Figure 3).

The stoichiometry and dynamics of the C:N:P ratio showed remarkable differences within different sampling time and among various ecosystem mechanisms. The previous study revealed that C:N has been shown to be critically important in leaf litter decomposition of most broad leaf forest. The C:N ratio showed greater variation among species than other elements with N, with a more marked trend towards higher

values [26] .The acute C:N ratio at which mineralization begins may different with leaf litter species type and different period of time including season and physical condition of time. In our result the C:N in CL. LL, and SL species leaf litter demonstrated an initial leaching phase. All three species exhibited a -net immobilization phase, which was expected based on their high initial C:N ratios. During the immobilization phase C:N ratios of all three leaf litter types gradually decreased. It appeared that the onset of net mineralization was suitable at the early stage of decomposition had dependent factor from microorganism and environmental aspects.

Except few unstable rate of C:P the most picture of the trend starts from high values and moving downward. The result shows the ratio partially depends of the leaf litter quality. In regression models tested by Zhang, for N (P) and the C:N and C:P effect, nutrient contents sometimes explain a huge portion of variances in decomposition rate[26]. According to our result this may indicate that a critical C:P ratio had not yet been obtained in litter of CL, LL and SL species. Another factor that could influence the variances of nutrients ratio in leaf litter is the microbial activity and resource stoichiometry [7]. For instance, the stoichiometry imbalanced between resource and microbial biomass reveal a shortage of microbial activity by a certain nutrients [2].

The N:P ration had slight verses trend comparing with C: nutrients ratios. It was leveled off in the 6 months of decomposition in CL species. However, in LL and SL

the rate increased till 3rd sampling time and then stared to decline. Overall, the C:N:P ration has a strong linage with moisture, temperature, precipitation [27] and microbial composition along the certain period of time.

4.2 结构性碳水化合物变化

4.2 Structural carbohydrate change

In this research, it was found that nutritional fractions change in leaf litter decomposition significantly varied among leaf litter of tree species types in the loess plateau of China, and that the variations were apparently related to differences along the sampling time in litter quality and species based (Table 1). The average of NDF was from 48 % to 62 % this this result was quite similar with the previous findings [28] [29]. Due to the descriptive and influential analysis, sampling time can be considered as the most important aspect responsible for change of recalcitrant elements in various leaf litter species (Table 1). All of recalcitrant elements: NDF, ADF and ADL' contents were increased from early stage of decomposition to the end of our leaf litter investigation. The sampling time noticed in all leaf litter decomposition as a main influential factor ($P < 0.05$). In terms of the NDF, ADF and ADL concentration in different observed tree species, CL had more contribution of above mentioned elements (Table 1). However there is a close estimation the change of NDF content might affect based on the leaf litter species as Agnihotri did in his research [30]. Most of our ANF, ADF and ADL concentration are in line with

Xiangli Kong except ADL since his result the ADL presented very low rate [31]. Another reason for the high trend of the elements at the endo of study could be the adaption of microbial community for the digestibility of the litter nutrients, since it takes time. However Agbagla-Dohnan found that the digestibility of ANF, ADF and ADL are different along species [31].

The leaf litter decomposition are subordinated with trend of structural carbohydrates in the ecosystem globally. The interaction of sampling time and leaf litter species factors had outnumbered in comparison with the factors separately. Lignin had the low concentration in the all species at the initial stage of leaf litter decay. According to Allin point of view Lignin is the slowest carbon fraction to decompose since of its carbon circle structures, and this is confirmed for our leaf litter decay study [32] [12]. The low rate of lignin at the early period of decomposition could be related to the sensitivity of it to the temperature as it was mentioned and quite agree with Berbeco research [33]. The cellulose concentration in leaf litter de composition is the only carbohydrate that was impacted by the leaf litter species and illustrated great significances in all three litter species (Table 2). As it was observed the rate of cellulose was fluctuated over 6 sampling time and it explains that cellulose mostly will decay within one year decomposing process. But Jennifer M point out that the less the litter has cellulose it will decompose it early and we have observed that SL had high rate of cellulose contend [34]. The content of cellulose was higher at the second, third and fourth sampling time which was in cold temperature season. This

result indicates that most structural and unstructured carbohydrates and elements and concomitant accelerated microbial activity may illuminate quick decomposition rates observed during winter and early spring in cold regions [35]. From all structural carbohydrate semi-cellulose is naturally low recalcitrant than other cell wall materials. Sampling time as a main factor to impact the decomposition process, indicates that semi-cellulose content was high at 1st sample for CL and LL while it was outnumbered at last sample for SL. Berg found out that semi-cellulose easily decompose at the 160 days of initial stage of litter decay and we have seen it is true for CL and LL of our investigation [36]. Overall, all carbohydrate contents of leaf litter were influences by both factors (sampling time and species based) and their nitration which every one of them related with wide range of sub factors.

4.3 叶际微生物群落

4.3 Microbial community

4.3.1 不同分解时期叶际细菌群落变化

4.3.1 Common phyllosphere bacterial communities in various sampling times.

There are numerous fundamental questions still remain in phyllosphere community ecology. Since the phyllosphere is in actual fact a heterogeneous surface environment, most bacteria are constrained in mobility and access to resources needed for growth

and survival as well as interact with other microorganisms. While it is well established that there is significant variation among bacterial composition between them, it is interesting, why and how the number of OTUs fluctuating in a certain span of time. Our results clearly show that, in the prior of leaf litter the decomposition the dynamics of phyllosphere bacterial community gradually increased and leveled off in the middle stage and again rise at the end of one year circle.

It indicate that after the adaptation process that occur in the first sampling time the composition of OTUs started to rise up and it could be the initiation findings of this investigation. In parallel to our work, Williams TR had in-depth investigation of the microbial community dynamics in the phyllosphere and he found out that the phyllosphere is colonized by bacteria that is predominantly distinguished by season and shows significant day-to-day variation[37]. Due to previous studies of environmental factors including ultraviolet light, relative humidity, and temperature likely influence the viability of bacteria community on plants especially on leaf litter [19] In general, our study – and these other previous studies – finds lower representation of OTUs in the harsh cold season and it has similar result with some other investigation that was done previously. Previous studies have discovered that phyllospheric bacteria communities are affected by climate changes whether directly or indirectly[38, 39] . In addition, the specific location of leaves on a plant will also influence bacterial colonists[40] I conclude the habitat of leave would be the initiated for the phylosphere of leaf litter during decomposition.

4.3.2 多样性指数分析

4.3.2 Analysis of diversity index

In order to find out the diversity of phylosphyere microbial community the Illumina high-throughput sequencing was implemented. The way of discovering the diversity we estimated the richness by OTUs, Chao1 and ACE and the diversity within samples Simpson and Shannon indices. The rarefaction figure shows the observed number of OTUs with increasing number of sampled sequences and in illustrates that 5CL has a more species based factors that the bacteria inhabited in high rate in this tree species. This findings is agree with some previous investigated work that was done by Eva E. Reisberg [20]. In terms of richness the CLs samples are the most leading species among all samples. It indicate that the richness of bacterial community was specialized for CL species and actually they were well performed in the last stages of leaf litter decomposition. These were sign of the species based, adaptation SL samples were not suitable for the phyllosphere bacteria community comparing to other species and environmental factors.

Shannon indicators could provide important information about community diversity and could be regarded as a functional indicator (Awasthi et al., 2014). In our study, along with the agreement with other studies argue that environmental parameters have the critic and sensitive impact in the richness and diversity of phyllosphere bacterial community in leaf litter during decomposition[20]. All in all, all the mentioned

indices demonstrate that samples 4LL, 4SL and 3SL had very low trends in both; community richness and diversity however 5CL and 6LL had a significant rate in all five parameters. In this investigation we find out the diversity and richness of phyllosphere bacteria community was outnumbered at the end one year decomposition.

4.3.3 叶际微生物群落类群变化

4.3.3 Community composition and structural change.

Totally in our investigation around 15samples were processed. Studies on the subject of leaf litter decomposition have focused on the composition of bacterial communities and their relation with the phyllosphere community. Bacterial community shows a succession of colonizers over the litter decomposition. Most of these bacterial taxa were relatively common across samples at phylum level and around 18 phyla were involved during the leaf litter decomposition in a year. The most common and highest figures were observed in 4LL, 3SL and 2SL that were occupied by Proteobacteria. The second largest dominator phyla were Actinobacteria (average 35%). The difference between these two active phyla was that, Proteobacteria was gigantically active at the beginning stage of leaf litter decomposition, whereas at the last stage of one year cycle decomposition Actinobacteria became active. Bacteroidetes was the third noticeable phyla among other bacterial. Purahong found the same result has we did and he claimed that bacterial community shows a succession of colonizers over

the litter decomposition[23]. The most abundant taxa found over the entire succession are Proteobacteria, Actinobacteria and Bacteroidetes [41] Accordingly, CL species were dominated by the Cyanobacteria (0-20 %) in the early stage of leaf litter decomposition. In general, phyllosphere bacteria are present almost in the first stage of the decomposition however,at the last stage of one year cycle leaf litter decomposition Saccharibacteria and Gemmatimonadetes gave started to perform gradually. Hartmann almost had the same result comparing with our result, he found that the role(s) of bacteria in coniferous and boreal forest soil processes has received less attention but it has been shown that soil communities are dominated by Actinobacteria, Proteobacteria and Acidobacteria and in lower abundances by Bacteroidetes, Gemmatimonadetes, Firmicutes, Verrucomicrobia and Planctomycetes[23]. Furthermore, to the considerations of bacterial communities and plant species, nutrient limitation and extreme change of water and other materials availability are the most common variables in forming the microenvironments for the more diverse bacterial communities in these phyllosphere[23].

4.3.4 Beta –多样性变化

4.3.4 Beta diversity dynamics

The clustering of bacterial community diversity was linked with the characteristics of the samples. According to the result of the sample bacteria at genus level, the first group was 4LL that were consist of Caulobacter, Ochrobacterum, Acitenobacter,

Acuabacterium, Limnobacter, Comamonas and Patulibacter had high abundance and clustered as first group. The CL samples were the second group that was semi-categorized. At the end of one year circle the leaf litter of 4SL, 5Sl and 6SL were clustered together and it demonstrates that the SL species were more in common in terms of the bacterial community diversity. In comparison with SLs samples CLs samples were species based clustered with the least low abundance. The Streptomyces, Dyadobacter, Geodermatophilus and Pedobacter were in high relative abundance in 5CL sample. In general the samples were categorized based on species and the CL samples were with high abundance though SL performed very less abundance.

4.3.5 叶际细菌群落结构变化

4.3.5 Change of bacterial community composition

Non-metric multidimensional scaling results demonstrate that bacterial community dynamics are driven primarily by variation in leaf litter species (i.e., CL, LL and SL), however a certain effect of geographic distance between the species was exist that can be taken to consider. A biotic and abiotic has a considerable impact in the diversity and composition bacteria in leaf litter decomposition. CL and LL of 5^{th} sample times were more similarity in diversity parametric of weighted and unweighted NMDS1. However, in unweighted 5SL were much dissimilar with 5CL and it had constant distance rate with 5CL and 5LL in weighted NMDS1. In order to take the similarities of samples time 2^{nd}, 3^{rd} and 5^{th} samples to consider they were more in closer distance in weighed NMDS1. Accordingly the same sign were observed in unweighed

NMDS1. 4LL were in far huge distance from all samples in A and B NMDS1. Taken together these results suggest that although environmental and climatic processes may play a significant role in determining bacterial community structure in this plateau, for the locations observed here the influence of local biophysical conditions appears to be the primary impacted influencer. Because sampling was performed specifically to encompass extremes in both spatial distance and soil conditions, this may provide evidence for predominantly local, environmental controls over the distribution of dry valley microorganisms.[23]

4.3.6 枯落物在不同分解时期显著性差异的微生物类群

4.3.6 Abundance and significant difference of microbes at the phylum level

As expected based on previous reports of Weisz and Kamke the diversity of bacterial communities was lower in comparison with the diversity in some habitat[42]. In contrast with the above mentioned findings our result showed that among all samples that we observed there was a significant difference in the abundance of Acidobacteria, which was more abundant in 6L and 2L samples group, In this study, analysis of a similar number of sequences revealed slightly more abundant by Armatimonadetes, FBP and Gemmatimonadetes bacterial phyla in 5L. Only the phyla: Acidobacteria, Armatimonadetes and Cyanobacteria Proteobacteria were represented by many sequences in all investigated sponges and it was also the dominant phylum in a moderate trend. Overall, the bacterial community pattern in leaf litter

decomposition is characterized by low phylum-level diversity with usually FBP and Gemmatimonadetes Fusobacteria and Proteobacteria as the most prominent phylum and the absence of other phyla typical of most sample groups.

4.4 叶际微生物群落和叶片养分的关系

4.4 Relationship of microbial community and leaf litter nutrients during decay.

To recognize how phyllosphere microbial communities on leaf litter are related to nutrients that are available there in leaf litter, in a certain period of decomposition, the correlation between nutrients and microbial community was summarized in a terrestrial forest ecosystem. The result that Carbon had significant positive correlation with Flavobactriia class and with this findings was agree with Bodenhausen N research result [43]. Whenever the decomposition proceeded, bacterial abundance was increased, which is in agreement with other litter investigation [41, 44] consequential in the microbial community will get positive relationship with the individual elements. Cyanobacteria could be one of the bacterial community example that was with an abundant rate and had significant positive association with N. More over N was highly decomposed by several bacterial classes such as Chloroplast and Flavobacteriia that had a significant positive relationship. The adaptation of most bacterial taxa to difference in leaf litter chemistry and microenvironment suggests that leaf chemistry has an essential influence on the development and existence of bacteria

on leaf litter during decomposition process [45]. For instance the significance relationship at bacterial phylum level, Thermoleophilia and Acidimicrobiia were at significantly positive correlated with P. In nutrients ratios, association with Proteobacteria, Gammaproteobacteria, Cyanobacteria and Flavobacteriia was a most significant ad positive correlations (Table 5).

Armatimonadia at class level and Armatimonadetes at phyla level positively association with acid detergent fiber (ADF). Moreover, the ADL with intensive rate of degidration was related to the high abundances of Actinobakteria, Deltaproteobacteria, Deinococci, Thermoleophilia and Acidimicrobiia with great positive and significant correlation. Surprisingly, the association of bacteria taxa with semi-cellulose was not observed in this investigation. In addition, the above ADL correlation performance was likewise true for Lignin accordingly. As we observed semi-cellulose and cellulose was more less significate association with microbial communities and some other researchers believe that, leaf litter decomposition is strongly limited by weather and seasonal variation. However, the limiting activities and the mechanical devastating of some of the microbes , the degradation products of cellulose and lignin are suitable substrates for most fungi and bacteria, which produce extracellular enzymes that analysis these erections into biologically usable forms [46]. Surprisingly after generating the microbial data the result showed that some of the unidentified phyla and classes that compound as others exhibited positive significant correlation at both levels with P. And N/P ratio was the mostly correlated with the

moderate abundant Ascomycota phyla and Dothideomycetes class. Although, the correlation of fungi was a bitter improved with structural carbohydrates. Zygomicota, Ascomycota, Sardariomycetes, Eurotimycetes and Leotiomycetes were significantly positive correlation with structural carbohydrates. It reveals that fungal community was more suitable for decomposing the structural carbohydrates.

第 5 章 结论

Conclusion

All in all, the dynamic of C, N and P and their ratio in leaf litter are discovered and it fully proved that they varied during decomposition. The leaf litter C and N are highly

decomposed at the early stage of decomposition but P start to decompose at the last sage of decomposition during the litter study. Furthermore, leaf litter decomposing rates vary within sampling time and among species in structural carbohydrates but the sampling time has more impact on the decomposition processes. The strong interaction of time and species impacted neutral detergent fiber (NDF), acid detergent fiber (ADF), acidic detergent lignin (ADL), semi-cellulose, cellulose and lignin to cause continuously effect and changes the decomposition process. The bacterial community there diversity and richness of locust and oak leaf litter at the 10 and 12 months decomposing are high rated than the other samples. Proteobacteria and Actinobacteria are mostly active in litter decomposition study. Leaf litter decomposed around one year (10 0r 12 months) exhibited high trends in both; richness and diversity of fungal communities. Moreover, Ascomycota and Basidiomycota were the highly dominated phyla in fungal communities. There are a significant positive correlation between microbial communities and leaf litter's nutrients. Metabolism function analyses indicated that the carbohydrate and amino acid metabolism were the highly stimulating functions and were enhanced during middle-term decomposing (8-10 months). Whereas, xenobiotic biodegradation and metabolism, lipid metabolism and energy metabolism were performed in moderate level.

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