Response of Soil Microbial Biomass Carbon, Nitrogen and Enzyme Activity to *Sophora Davidii* Skeels and *Pennisetum Sinese* Roxb Intercropping Systems

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**Abstract.** To understand the response mechanism of soil microbial biomass carbon content, soil microbial biomass nitrogen content and soil enzyme activities to intercropping pattern, an experiment consisting of three cropping patterns: *Sophora davidii* Skeels monocropping pattern, *Pennisetum sinese* Roxb monocropping pattern and *S. davidii* and *P. sinese* intercropping pattern had established in the southern region of Guizhou Province, China. The results showed that soil microbial biomass carbon content, microbial biomass nitrogen content and enzyme activities were gradually reduced with the depth (0-30 cm) of the soil layer increased under the three cropping patterns. The microbial biomass carbon and nitrogen content of *S. davidii* and *P. sinese* intercropping pattern were significantly higher than those of *S. davidii* monocropping and *P. sinese* monocropping pattern in 0-20 cm soil layer (P <0.05). Five soil enzyme activities were ranked: *P. sinese* monocropping > *S. davidii* and *P. sinese* intercropping > *S. davidii* monocropping in most soil layers. The soil nutrient contents (soil organic matter, total nitrogen, total phosphorus, and total potassium) and soil microbial biomass content (microbial biomass carbon and nitrogen) were highly significant positive correlation (P <0.01), while five soil enzyme activities had a significant positive correlation with each other (P <0.05). However, there was no significant correlation between soil microbial biomass content and soil enzyme activities. Additionally, there was non-significant correlation between the activities of nitric oxide synthetase, the activities of β-N-acetylglucosidase and the activities of soil nitrogenase, the activities of soil Leucine aminopeptidase and the activities of soil glutamine synthetase were showed a significant (P <0.05) or very significant positive correlation (P <0.01). In summary, the intercropping pattern of *S. davidii* and *P. sinese* has a significant effect on soil quality improvement in shallow soil (0-20cm), and the microbial biomass carbon, soil microbial biomass nitrogen and soil enzymes can be used as an important index for evaluating the changes of soil quality.
1. Introduction
Microorganisms in soil were the driving force for soil energy and nutrient conversion and played an important role in the biogeochemical cycle [1], and participated in biochemical processes such as soil organic matter decomposition and humus formation. Soil microbial biomass carbon, soil microbial biomass nitrogen, and carbon and nitrogen nutrient cycling were closely related, which can respond to different agricultural measures and reflect soil fertility status [2-4]. Enzymes were “catalysts” for soil biochemical reactions, secreted by microorganisms in the soil were important components of soil enzymes. The increase in enzyme activity can significantly increased the effective nutrient contents of the soil, which was beneficial to soil fertility and yield. Studies [5-7] had shown that large-scale continuous cropping of a single plant will reduce the microbial species and functional diversity in the soil. This pattern will destroy the stability of the soil ecosystem and hinder the sustainable development of the soil ecosystem. Researchers [8] had found that the intercropping pattern can alleviate the obstacles of continuous cropping of broad bean, and this method can improve the rhizosphere soil ecological environment.

The leguminous grass intercropping pattern had attracted widespread attention due to its obvious advantages in maintaining nitrogen balance in the agricultural system and increasing the yield of the system. However, few studies have focused on the efficient use of nitrogen in the intercropping pattern of legumes. The reports of the interaction between nitrogen and microorganisms were likely rare. *S. davidii* is a leguminous shrub with broad adaptability and high protein content; *P. sinese*, also known as king grass, is a perennial, high-yielding, high-quality mowing type of the grass family *Pennisetum* forage grass. The above two kinds of grass can be used as high-quality forage sources for livestock had found based on previous studies. There is no report on the intercropping cropping patterns of the two, especially the *S. davidii* and *P. sinese* intercropping cropping pattern. The impact was unknown. Therefore, the objective of this study was to better understand the changes in microbial biomass carbon, nitrogen, and soil enzyme activity in different soil layers, and soil microbial biomass carbon, nitrogen and soil enzyme activity under *S. davidii* and *P. sinese* intercropping through field experiments. This pattern will provide scientific support for forage ecological cultivation and continuous soil improvement in karst areas of China.

2. The materials and methods

2.1. The overview of the test site
Field experiments were conducted at the experimental farm of Guizhou Grass Industry Research Institute, Dushan County, Qiannan Prefecture, Guizhou Province (Latitude 25°51′N, Longitude 107°33′E, elevation 980 m). Dushan was a subtropical temperate monsoon climate with four distinct seasons. It has the characteristics of a cold plateau climate, sufficient sunlight, abundant light and heat resources, and an average annual temperature 15°C, annual rainfall is 1429.9 mm, frost-free period was 297 days. The soil in the test area in this area was yellow soil. The physical and chemical properties of the soil in the 0-30 cm soil layer before the experiment were as follow: organic matter content was 18.70 g·kg⁻¹, pH was 7.17, total nitrogen content was 1.21 g·kg⁻¹, total phosphorus content was 0.53 g·kg⁻¹, total potassium content was 13.74 g·kg⁻¹, fast-acting potassium content was 108.0 mg·kg⁻¹, effective phosphorus content was 21.6 mg·kg⁻¹ and alkaline nitrogen content was 50.2 mg · kg⁻¹.

2.2. Experimental design
The experiment was designed as the *S. davidii* monocropping, *P. sinese* monocropping, and *S. davidii* and *P. sinese* intercropping. There were 9 plots, each with an area of 65 m². The soil background data were sampled and measured before the planting in early March 2019. Then the site were deepened and *S. davidii* monocropping pattern was planted with a planting density of one plant per m², *P. sinese* monocropping pattern had a planting density of one plant per m², and the interplanting pattern had a planting density of one plant per m². The test grass species were *P. sinese* and Panjiang *S. davidii* (510) (Guizhou Institute of Praticultural). The ecological conditions and field management measures were the
same. The experiment were arranged in a completely randomized design with four replications per treatment.

2.3. Index determination
A 5-point sampling method to collect samples from three soil layers, 0-10 cm, 10-20 cm and 20-30 cm had used in the maximum biomass period (October 2019). A 2 mm sieves had used after each soil sample was mixed evenly. Then one part was to determine the microbial biomass carbon and nitrogen in the soil, the other was to determine enzyme activities and soil nutrient contents (organic matter, total nitrogen, total phosphorus, total potassium). The microbial biomass carbon and nitrogen had measured by chloroform fumigation-extraction-non-dispersive infrared absorption method [9].

The microbial biomass C and N were calculated according to the following equation.

\[ C = CF \frac{CUF}{K} \] (1)

\[ N = NF \frac{NUF}{K} \] (2)

Where CF and NF were the carbon and nitrogen content of fumigant soil extract respectively, CUF and NUF were the carbon and nitrogen content of unfumigated soil extract respectively, and K (K=0.45) was the conversion coefficient of fumigation extraction method.

The soil nutrients were measured by a nutrient analyzer (TPY-6PC in Zhejiang Top). The absorbance of nanabacteria were measured by Elisa to know soil enzymes (NOS, β-N-NAG, Nitrogenase, LAP, and GS).

2.4. The data processing
Analysis of variance was performed using SPSS version 23.0 software; and data statistics and mapping were performed using Microsoft Office 2010.

3. Results and analysis

3.1. The effect of intercropping patterns on soil microbial biomass carbon and nitrogen
Microbial carbon and nitrogen were considered as the storage of soil active nutrients, which are important sources of available nutrients for plant growth. As shown in Figure 1, the microbial biomass carbon and nitrogen contents were decreased with the increase of soil layer depth under three cropping patterns, and there were significant differences between different soil layer depths (P <0.05). Under S. davidii and P. sinese intercropping pattern, the microbial biomass carbon and nitrogen contents were the highest in the soil layer 0-10 cm exhibiting 260.51 mg·kg\(^{-1}\) and 76.71 mg·kg\(^{-1}\), respectively, while were the lowest in the soil layer 20-30 cm exhibiting 123.50 mg·kg\(^{-1}\) and 26.35 mg·kg\(^{-1}\), respectively. The microbial biomass carbon and nitrogen significantly decreased with the increase of the soil layer depth under different treatments (P <0.05). S. davidii and P. sinese intercropping pattern was significantly higher than S. davidii monocropping in the soil layer of 0-20cm, indicating that the S. davidii and P. sinese intercropping has mutual benefit and promotion in the shallow surface layer of soil.
Note: Different lowercase letters indicate significant differences between different cropping patterns under the same soil layer depth; different uppercase letters indicate significant differences between different soil layer depths under the same cropping pattern, P<0.05. S indicates S. davidii monocropping, P indicates P. Sinese monocropping, and I indicates S. davidii and P. sinese intercropping.

3.2. The effects intercropping patterns on soil enzyme activities
The five soil enzyme activities were decreased gradually with the increase of soil layer depth under three cropping patterns (Fig. 2). Except for Nitrogenase activity in 10-20cm and leucine activity in 10-30cm, the five enzyme activities were significantly higher than other cropping patterns in different soil layers under the P. sinese monocropping (P<0.05). The sequence of enzyme activities including β-N-acetylglucosidase and leucine activities in 0-30cm, nitrogenase and nitric oxide synthase activities in 0-20cm and glutamine synthetase activity was P. sinese monocropping > S. davidii and P. sinese intercropping > S. davidii monocropping. There was no significance between S. davidii monocropping and S. davidii and P. sinese intercropping of β-N-acetylglucosidase activity in 0-10cm and nitrogenase and leucine activity in 0-20cm (P> 0.05), while the other treatments were significantly different (P<0.05).

The above results showed that the soil enzyme activity was significantly higher than the other two cropping patterns under the P. sinese monocropping, which is consistent with the development of the root system. Because a large amount of hydrogen peroxide can be generated by the root density and respiratory intensity, which were one of the important substrates for enzyme conversion, it can enhance soil enzyme activity. In the present study, the result indicated that the monocropping were more effective to enhance soil enzyme than the intercropping of P. sinese.
Figure 2. Changes in soil enzyme activities in different soil layers under the intercropping patterns of *S. davidii* and *P. sinese*.
Table 1. Analysis of the correlation of the measurement indicators

| Index                | Organic matter | Total nitrogen | Total phosphorus | Total potassium | Microbial biomass carbon | Microbial biomass nitrogen | Soil nitric oxide synthase | β-N-acetylaminoglucosidase | Nitrogenase | Leucine aminopeptidase | Glutamine synthetase |
|----------------------|----------------|----------------|------------------|----------------|--------------------------|---------------------------|----------------------------|--------------------------|-------------|-----------------------|---------------------|
| Organic matter       | 1              | -              | -                | -              | -                        | -                         | -                          | -                        | -           | -                     | -                   |
| Total nitrogen       | 0.675**        | 1              | -                | -              | -                        | -                         | -                          | -                        | -           | -                     | -                   |
| Total phosphorus     | 0.684**        | 0.597**        | 1                | -              | -                        | -                         | -                          | -                        | -           | -                     | -                   |
| Total potassium      | 0.703**        | 0.545**        | 0.534**          | 1              | -                        | -                         | -                          | -                        | -           | -                     | -                   |
| Microbial biomass carbon | 0.618**     | 0.599**        | 0.578**          | 0.539**        | 1                        | -                         | -                          | -                        | -           | -                     | -                   |
| Microbial biomass nitrogen | 0.487**     | 0.455**        | 0.507**          | 0.487**        | 0.871**                  | 1                         | -                          | -                        | -           | -                     | -                   |
| Nitric oxide synthase | 0.318*        | 0.339*         | 0.316*           | 0.307*         | -0.126                   | -0.134                    | 1                          | -                        | -           | -                     | -                   |
| β-N-acetylaminoglucosidase | 0.326*      | 0.322*         | 0.319*           | 0.324*         | -0.067                   | -0.038                    | 0.246                      | 1                        | -           | -                     | -                   |
| Nitrogenase           | 0.337*         | 0.325*         | 0.322*           | 0.331*         | 0.239                    | 0.198                     | 0.388**                    | 0.614**                  | 1            | -                     | -                   |
| Leucine aminopeptidase | 0.338*        | 0.319*         | 0.325*           | 0.327*         | 0.158                    | 0.212                     | 0.124                      | 0.546**                  | 0.420**     | 1                     | -                   |
| Glutamine synthetase  | 0.330*         | 0.0338*        | 0.315*           | 0.333*         | 0.298                    | 0.296                     | 0.339*                     | 0.379*                   | 0.460**     | 0.577**               | 1                   |

Note: * indicates a significant difference at the 0.05 level, and ** indicates a significant difference at the 0.01 level.

3.3. The correlation of measurement indicators

The soil organic matter, total nitrogen, total phosphorus and total potassium were extremely significantly positively correlated to soil microbial biomass carbon and microbial biomass nitrogen (P <0.01), and significantly positively correlate to soil enzyme activity (P <0.05, Table 1). However, there were non-significant correlation between soil microbial biomass carbon, soil microbial biomass nitrogen and soil enzyme activity. Except the nitric oxide synthase was non-significant difference with β-N-acetylglucosidase and leucine aminopeptidase (P>0.05). β-N-acetylglucosidase, nitrogenase, leucine aminopeptidase and glutamine synthetase showed a significant (P<0.05) or highly significant positive correlation (P <0.01).

4. Conclusion

1) The microbial biomass carbon and nitrogen content under the S. davidii and P. sinese intercropping pattern were significantly higher than S. davidii monocropping pattern and P. sinese monocropping in the 0-20cm (P < 0.05). The experimental results indicated that the S. davidii and P. sinese intercropping pattern has a phenomenon of mutual benefit and promotion in the shallow surface layer of soil.

2) The sequence of five enzyme activities were P. sinese monocropping > S. davidii and P. sinese intercropping > S. davidii monocropping, which indicated that P. sinese growth promoted soil enzyme activity.

3) The soil organic matter, total nitrogen, total phosphorus, and total potassium has extremely significant positive correlations with microbial biomass carbon and microbial biomass nitrogen (P <0.01), and significant positive correlations with soil enzyme activities (P <0.05); there were non-significant correlation between microbial biomass carbon, soil microbial biomass nitrogen and soil enzyme activity indicators. Soil β-N-acetylglucosidase, nitrogenase, Leucine aminopeptidase and glutamine synthetase were showed a significant (P <0.05) or highly significant positive correlation (P <0.01).

4) The soil nutrients were significant relationship with soil microbial biomass carbon, soil microbial biomass carbon, nitrogen and soil enzymes under the S. davidii and P. sinese intercropping. Therefore, microbial biomass carbon, soil microbial biomass nitrogen and soil enzymes in the soil can be used as important indicators for evaluating soil quality and soil fertility changes. These factors can provide early
warning tips for soil quality changes in the intercropping system and help us to better protect and use land.

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