Intercropping Effects of Sophora davidii and Silage Maize on Soil Physicochemical Properties, Enzyme Activities and Yield

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Abstract. Crops-beans intercropping has the advantages of efficient utilization of light energy, space environmental resources and increasing land productivity. People have done a lot of research on the yield and soil nutrient utilization of grain crops intercropping system, which proves that crops-beans intercropping is of great significance in production practice. However, there is little research on the soil properties and yield affected by intercropping forage grass with shrub legume. In order to explore the ecological value and yield effect in the intercropping mode of shrub legume and forage grass, and to provide the high-quality forage resources in the region, we selected Sophora davidii and silage corn for field intercropping experiment. To study the benefit of intercropping and the feedback regulation between crop yield and soil fertility. The results showed that the land equivalent ratio was 1.501 under intercropping mode, and intercropping silage corn between S. davidii rows could improve the land use efficiency, and compared with S. davidii monoculture system, the land yield increased by 1286.4% in fresh matter and 738.5% in dry matter. At the same time, intercropping increased the content of available N in soil and promoted the sustainable utilization ability of soil. This has important enlightenment for the restoration area of S. davidii planting in karst area, taking into account the restoration effect, improving soil, increasing grass and increasing efficiency.

1. Introduction
Intercropping can make full use of land resources, improve the total yield per unit area, alleviate continuous cropping obstacles [1], and reduce the risk of crop diseases and pests [2]. Crop/soybean intercropping is a common intercropping mode. While making full use of soil fertility to improve land productivity [3], rhizosphere microorganisms associated with legumes can provide a certain amount of nitrogen for the intercropping system [5-10], reducing the demand for soil fertilizer under the same yield. Reducing fertilizer residues in soil [10], maintaining ecological advantages such as soil fertility sustainability. The addition of gramineous grasses increased the demand for N in the intercropping system, thus reducing the "nitrogen repression" effect of legumes [10,14], promoting legume nodules to synthesize more nitrogen, and improving crop yield and nutritional quality [4]. The root twining system formed by Gramineae and Leguminosae can effectively enhance the attachment ability of crop roots to
soil, effectively strive for the ability of water and soil conservation of cultivated land, and play a positive role in controlling soil erosion and desertification control. It is generally believed that intercropping can increase the content of organic matter in the soil, and then increase the content of various elements in the soil, and is conducive to the enrichment of nitrogen, phosphorus and potassium in the surface soil. A large number of studies suggest that legumes can increase the content of nitrate nitrogen in soil by the following three ways: 1. Planting legumes in soil, legumes absorb less available nitrogen, so that the available nitrogen in soil is absorbed by other plants. 2. Nitrogen fixation products released from nodular roots. 3. During the production and development of legumes, root abscission, leaf abscission and N mineralization of root nodules are inevitable due to metabolism [19]. The intercropping system of Leguminosae and Gramineae plays the role of symbiotic nitrogen fixation of Leguminosae plants. 30% to 50% of the nitrogen required by legumes comes from the nitrogen fixed by rhizobia [15-17], which greatly reduces the consumption of soil fertility by crops. Studies have found that intercropping is more conducive to the absorption of phosphorus by plant roots [22]. The physiological activities of one species make the surrounding environment change, but it is conducive to the growth of another species, which is the intermediate promotion [28]. Two species live in the same environment and will exchange with the environment in the stage of production and development, which will inevitably affect the environment to some extent and lead to changes in the environment. If this change of local environment has a negative effect on another species, there is no interspecific promotion, on the contrary, it is interspecific promotion. Compared with competition, promotion is more important [20]. Most of the phosphorus in the soil is organic phosphorus without applying phosphorus fertilizer, and the ability of plants to absorb organic phosphorus directly is very weak. The other necessary phosphorus absorption pathway of plants is the mineralization of organic phosphorus to realize the transformation of inorganic phosphorus. The mineralization process mainly includes the acidification of organic phosphorus by organic acids secreted by plant roots and the degradation of organic phosphorus by soil microorganisms [28-30]. Intercropping can improve soil microbial diversity.

Crop / soybean intercropping can save land resources, make full use of light energy and explore soil fertility, which is more conducive to the protection of ecological environment and the cultivation agronomy of sustainable agricultural development. Guizhou karst landform characteristics, planting traditional corn yield is low, low economic benefits, corn straw treatment is difficult, a large number of burning straw, air pollution is large, at the same time, it is very easy to cause fire and other hazards. Silage corn in production practice can avoid the production of straw waste, forage crops are less affected by the market than food crops, and the economic benefit is more stable. In this study, we selected Sophora davidii, a legume Shrub Forage with strong stress resistance and suitable climatic conditions in Guizhou, and conducted an intercropping experiment with silage maize, which is widely planted in Guizhou and has large market demand. We analyzed the differences and interaction mechanism of crop yield and soil physicochemical properties, as well as the demand of crops for various soil nutrient elements, so as to provide technical support for the study of efficient grass field cropping system of Sophora davidii and silage maize.

2. Material and party

2.1. Overview of test site
The experiment was carried out in the Guizhou Academy of Agricultural Sciences, Jinzhu Town, Guiyang City in 2019, at 26°30'16″N north latitude and 106°39'20″E east longitude. The test site is 1105m above sea level, the annual average temperature is 15.3℃, the annual extreme maximum temperature is 35.1℃, the annual extreme minimum temperature is -7.3℃, the annual average total precipitation is 1129.5 mm, the soil type is Haplic alisol, and the soil background is consistent.
2.2. Experiment design
A field randomized block experiment was adopted in the experiment. Three planting patterns were designed: Sophora davidii monoculture (B|Y), silage maize monoculture (YD) and Sophora davidii/silage maize intercropping (B | Y). Each planting mode is set with five repeated plots, the size of which is 5m*2m, and the plots are separated with a width of 1m. The row spacing and plant spacing of Sophora davidii were 60cm and 60cm; respectively. The row spacing and plant spacing of silage corn were 60cm and 30cm respectively. The row spacing and plant spacing of Sophora davidii and silage corn in the intercrop were as same as in monocropping and the distance between neighbouring rows in intercropping was 0.3 m. The experimental site was sown on May 1, 2019. Cut and harvest the experimental crops on August 11th, 2019. Crop rhizosphere soil is sampled at a depth of 0-20cm.

2.3. Test indicators and methods
2.3.1. Yield and land equivalent ratio. One 1m*1m quadrat was randomly selected in each plot under each planting mode. We weighted fresh crop yield, dried and measured dry matter yield.

Land equivalence ratio (LER) measures intercropping advantage under the same area [16]. If LER > 1, it indicates that the intercropping system has advantages over the two crops, and the higher the value, the more obvious the intercropping advantages are.

The formula for LER is as follows: \( \text{LER} = \text{PLER}_a + \text{PLER}_g = \frac{Y_{bi}}{Y_{bm}} + \frac{Y_{yi}}{Y_{ym}} \)

 \( Y_{bi} \) and \( Y_{bm} \) are the hay yield of Sophora davidii in intercropping system and Sophora davidii in monoculture. \( Y_{yi} \) and \( Y_{ym} \) are respectively the hay yield of silage corn in intercropping system and under monoculture condition.

2.3.2. Soil physicochemical properties. Soil organic carbon content (OC, g/kg) was measured by potassium dichromate volumetric method; The nitrate nitrogen content (NO3-N, mg/kg) was determined by colorimetric method, and ammonium nitrogen content (NH4-N, mg/kg) was determined by ultraviolet spectrophotometry. The available phosphorus content in soil (mg/kg) was determined by NaHCO3-ultraviolet spectrometer [33]. Test soil pH value with PH tester (soil-water ratio is 1: 2.5) [13]. Measuring soil water content (WC) by drying method, randomly selecting points in the quadrat, taking soil with a ring knife, weighing (M0), sealing, and taking it back to the laboratory for drying to constant weight (M1) at 105°C.

\[ WC = \frac{M_0 - M_1}{M_1} \]  

(1)

2.3.3. Soil enzyme activity. We used ELISA® kit (Shanghai Enzyme-Linked Biotechnology Co., Ltd., China) [11-12] to determine C- acquiring enzyme -\( \beta \)-N- acetylglucosaminidase (NAG). N- acquiring enzyme-soil leucine aminopeptidase (LAP); Nitrogen fixation (Nitrogenase); Nitric oxide synthase (NOS); Glutamine synthetase (GS) and P- acquiring enzyme-acid phosphatase (ACP).

2.4. Data processing
Excel 2007 was used for processing and drawing, IBM SPSS Statistics 25 data processing system software was used for single factor variance analysis, and Duncan's test method was used for multiple comparisons. Pearson correlation coefficient comparison method was used to analyze the correlation of variables.

3. Results and analysis
It can be seen from Table 1 that planting patterns and crops can affect the rhizosphere soil properties, but have no significant effect on soil pH and ammonium nitrogen. The yield of silage corn in intercropping mode was significantly lower than that in monoculture (P<0.05), and the yield of Sophora
davidii was slightly lower, but the difference was not significant (P>0.05). The soil water content of Sophora davidii was 14.55% under monoculture and 15.83% under intercropping, and there was no significant difference between them (P>0.05). The moisture content of rhizosphere soil under silage corn monoculture was 18.60%, which was significantly higher than other treatments (P<0.05). The moisture content of rhizosphere soil under silage maize intercropping was 16%, which was significantly higher than that under Sophora davidii monoculture. The fibrous root system of silage corn has a positive effect on the water retention of surface soil, and intercropping can distribute this positive effect to the planting system, which is beneficial to improve the water retention capacity of soil. Compared with monoculture, intercropping significantly increased soil organic carbon, available phosphorus content and soil water content in Sophora davidii rhizosphere. The activities of leucine aminopeptidase (LAP), nitric oxide synthase (NOS), glutamine synthetase (GS) and acid phosphatase (ACP) in the rhizosphere soil of Sophora davidii were improved by intercropping, but the effect was not significant. Intercropping could not affect the available nitrogen content, ammonium nitrogen content, pH, β-N-acetylglucosaminidase (NAG) activity and Nitrogenase activity of Sophora davidii rhizosphere soil. Intercropping reduced the aboveground biomass of Sophora davidii, but there was no significant difference. Compared with monoculture, intercropping significantly reduced the fresh and dry yield of silage corn and the moisture content of rhizosphere soil. The activities of β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP) and nitric oxide synthase (NOS) in rhizosphere soil of silage corn were significantly increased. The nitrate nitrogen content and glutamine synthetase (GS) activity of silage corn rhizosphere soil were increased, but the effect was not significant. Intercropping had no significant effect on organic carbon content, ammonium nitrogen content, available phosphorus content, pH, Nitrogenase activity and acid phosphatase (ACP) activity in rhizosphere soil of silage corn. Organic carbon content, available phosphorus content, soil water content and Nitrogenase activity of silage corn rhizosphere were significantly higher than those of Sophora davidii rhizosphere. The nitrate nitrogen content, β-N-acetylglucosaminidase (NAG) activity, nitric oxide synthase (NOS) activity and glutamine synthetase (GS) activity in rhizosphere soil of silage maize were significantly lower than those in Sophora davidii.

Table 1. Changes of crop yield and physical and chemical properties of rhizosphere soil under different planting patterns

| Plant | Test index       | Way | OC(g/kg) | NH4_N(mg/kg) | NO3_N(mg/kg) | AP (mg/kg) | pH       |
|-------|-----------------|-----|----------|--------------|--------------|------------|----------|
|       |                 | Y   | 29.17±4.53a | 4.49±3.44a | 9.91±2.16b | 29.57±3.31a | 7.11±0.10a |
|       |                 | JY  | 31.7±4.95a  | 3.21±2.37a | 12.47±2.70ab | 27.61±6.41a | 7.10±0.13a |
|       |                 | B   | 17.02±3.47c | 1.56±0.40a | 15.12±2.17a | 11.25±2.59c | 7.10±0.08a |
|       |                 | JB  | 22.72±2.63b | 1.82±0.56a | 15.84±3.06a | 18.16±2.94b | 7.02±0.04a |
|       |                 | Y   | 18.60±1.00a | 0.07±0.01b | 1.31±0.16b | 0.81±0.12b | 3.24±0.33c |
|       |                 | JY  | 16.00±0.71b | 0.10±0.01a | 1.54±0.10a | 0.81±0.02b | 4.00±0.49b |
|       |                 | B   | 14.55±0.92c | 0.10±0.01a | 1.46±0.17ab | 1.02±0.08a | 4.35±0.34ab |
|       |                 | JB  | 15.83±0.42b | 0.10±0.02a | 1.57±0.16a | 0.98±0.11a | 4.63±0.32a |
|       |                 | Y   | 0.43±0.10c  | 0.40±0.10b | 3089.54±419.58a | 853.49±105.7a |
|       |                 | JY  | 0.54±0.09bc | 0.45±0.07b | 1948.71±470.91b | 540.69±136.03b |
|       |                 | B   | 0.58±0.10ab | 0.50±0.12ab | 150.18±16.89c | 86.18±9.29c |
|       |                 | JB  | 0.70±0.09a  | 0.62±0.07a | 133.4±19.45c | 74.76±12.03c |
Note: B stands for Sophora davidii planted under monoculture, JB stands for Sophora davidii planted in intercropping system, Y stands for silage corn planted under monoculture and JY stands for silage corn planted in intercropping system. OC, organic carbon; NH$_4^+$, ammonium nitrogen; NO$_3^-$, nitrate nitrogen; AP, available phosphorus; WC, water content, NAG, β-N- acetylglucosaminidase; LAP, soil leucine aminopeptidase; Nitrogenase, nitrogenase; NOS, nitric oxide synthase; GS, glutamine synthetase; ACP, acid phosphatase.

It can be seen from table 2 that the dry matter yield of silage maize alone is 853.48kg/mu > silage maize intercropped with 540.69 kg/mu > Sophora davidii alone is 86.18kg/mu > Sophora davidii intercropped with 74.76kg/mu. Intercropping inhibited the growth of the two crops, significantly reduced the yield of silage maize (P<0.05), and had no significant effect on the restriction of Sophora davidii yield (P < 0.05) Under intercropping mode, the land equivalent ratio is 1.501. Generally speaking, intercropping silage corn between Sophora davidii rows can improve the land use efficiency, and compared with Sophora davidii monoculture system, the land yield can be increased by 1286.4% in fresh matter and 738.5% in dry matter. This has important enlightenment for the restoration area of Sophora davidii planting in karst area, taking into account the restoration effect, improving soil, increasing grass and increasing efficiency.

**Table 2. Yield and equivalent ration under the intercropping patterns**

| Cropping pattern | Yield(kg/667m2) | PLER | LER  |
|------------------|-----------------|------|------|
|                  | Sophora davidii | Silage corn | PLERa | PLERb |
| BD               | 86.18c          | -     | -    | -    |
| YD               | -               | 853.48a | -    | -    |
| B|Y               | 74.76c          | 540.69b | 0.867 | 0.634 | 1.501 |

Note: BD stands for Sophora davidii; YD stands for oat monoculture; B|Y indicates Sophora davidii/silage corn intercropping.

It can be seen from table 3 that crop dry matter yield is significantly positively correlated with soil organic carbon, total phosphorus content, soil water content and soil ammonium nitrogen content, and negatively correlated with soil nitrate nitrogen content, β - n-acetylglucosidase, nitrogenase, nitric oxide synthase, glutamine synthase and acid phosphatase activity. Soil organic carbon content was positively correlated with soil available phosphorus content and soil water content, and negatively correlated with soil nitrate nitrogen content, nitrogenase activity and nitric oxide synthase activity. There was a significant positive correlation between soil ammonium nitrogen content and soil pH, soil available phosphorus, soil water content, and a significant negative correlation with glutamine synthetase activity. Soil nitrate nitrogen content has a significant negative correlation with soil available phosphorus content and soil water content, and a significant positive correlation with soil nitric oxide synthase and nitrogenase. There is a significant positive correlation between available phosphorus content and soil water content, and a significant negative correlation between available phosphorus content and nitrogenase and nitric oxide synthase activity in soil. There is a significant negative correlation between soil water content and the activities of nitric oxide synthase and glutamine synthetase in soil. The activity of leucine aminopeptidase in soil was positively correlated with the activities of glutamine synthetase and acid phosphatase. There is a significant positive correlation between nitrogenase activity and nitric oxide synthase acid phosphatase in soil. The activity of nitric oxide synthase was positively correlated with the activities of glutamine synthetase and acid phosphatase. Glutamine synthetase activity was positively correlated with acid phosphatase activity.
Table 3. Correlation coefficients of crop rhizosphere soil index with yield (n= 20)

|       | OC   | NH4_N | NO3_N | AP   | pH   | WC   | NAG  | LAP  | Nitro | NOS  | GS   | ACP |
|-------|------|-------|-------|------|------|------|------|------|-------|------|------|-----|
| Yield | **   | *     | **    | **   | **   | **   | **   | **   | **    | **   | **   | **  |
| OC    |     |       |       | **   | **   | *    | **   | **   | *     | **   | **   | **  |
| NH4_N | *    |       |       |     | **   | **   |     |     | **    |     |     | **  |
| NO3_N | *    |       |       |     |     |     |     | **   |       |     |     | **  |
| AP    |     |       |       | **   | **   | **   | **   | **   | **    |     |     |     |
| pH    |     |       |       |     |     |     |     |     |       |     |     |     |
| WC    |     |       |       |     |     |     |     |     |       |     |     |     |
| NAG   |     |       |       |     |     |     |     |     |       |     |     |     |
| LAP   |     |       |       |     |     |     |     |     |       |     |     |     |
| Nitrogenase |     |       |       |     |     |     |     |     |       |     |     |     |
| NOS   |     |       |       |     |     |     |     |     |       |     |     |     |
| GS    |     |       |       |     |     |     |     |     |       |     |     |     |

Note: * * is significantly correlated at 0.01 level and * is significantly correlated at 0.05 level.

4. Conclusions
We found that the content of available N in plant rhizosphere soil increased in crop/soybean intercropping system, which was consistent with the findings of Chai Qiang et al. [16,17]. They found that the corresponding growth and development of gramineous plants competed with the N element in the soil, which triggered the inhibition mechanism of "nitrogen repression" of leguminous crops [16, 17], and prompted symbiotic rhizobia of leguminous plants to fix more N2 for the intercropping system. When the compensation effect [28] is greater than the need of plant growth, the content of available N in soil increases.

The increase of available P content in Sophora davidii rhizosphere soil may be due to nitrogen repression and inhibition caused by grass/family crops in cereal/bean intercropping system, which promotes legume crops to form more nodules [8-10], improves the ability of associated microorganisms in cereal/bean intercropping system to convert organic phosphorus to produce inorganic phosphorus, and makes the available P content in Sophora davidii rhizosphere increase. The decrease of available P content in silage corn rhizosphere may be caused by the competition of Sophora davidii and its associated microorganisms for phosphorus in intercropping system and the consumption of silage corn in specific growth period. According to the research of Zhang Enhe et al. [32], the reflection of
phosphorus in rhizosphere may be due to the intercropping of different crop roots. The dense root zone of compound population increases the consumption of soil nutrients, but the specific mechanism plays a leading role needs further study.

In a word, compared with monoculture, intercropping silage corn between Sophora davidii rows can effectively improve the land use efficiency of Sophora davidii plantation. Compared with monoculture system of Sophora davidii, the benefit of intercropping system is obvious. Intercropping Sophora davidii with silage corn can significantly promote the ability of soil water and moisture conservation. Intercropping did not significantly change the contents of organic carbon, ammonium nitrogen, nitrate nitrogen, available phosphorus, activities of carbon acquisitive enzyme, nitrogenase and phosphorus acquisitive enzyme in corn rhizosphere soil, but significantly decreased the contents of carbon and nitrogen in water and microbial biomass, and significantly increased the activities of nitrogen acquisitive enzyme (NAG, LAP) and nitric oxide synthase. Intercropping did not significantly change the activities of ammonium nitrogen, nitrate nitrogen, microbial biomass carbon and all five nitrogen-related enzymes in Sophora davidii rhizosphere soil, but significantly increased the contents of organic carbon, available phosphorus, water, microbial biomass nitrogen and the activities of carbon acquisition enzymes in Sophora davidii rhizosphere soil. Intercropping can improve the available P and N contents of Sophora davidii rhizosphere soil and the available N contents of silage corn rhizosphere soil, which has higher benefits of improving soil fertility and improving the sustainability of cultivated land planting.

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