



Impact of Maize-soybean Intercropping on Nitrogen Dynamics, Yield, and Nitrogen Fixation

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Article Info

Keywords:

Intercropping, Nitrogen fixation, Maize-Soybean, Legumes, Yield

Date:

Received: 06 April 2023

Accepted: 14 November 2023

Published: 12 December 2023

Volume: 2(1), 2023



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Abstract

Soybean (Glycine max L.) is an important legume as well as oilseed crop, having considerable potential to fix atmospheric nitrogen. Maize (Zea mays) is the world's third leading cereal crop. Both are important food, fodder and industrial crops of the world. Specific objectives of this study are: i) Evaluation of nitrogen use efficiency of maize (cereal) and nitrogen fixation of soybean (legume) as influenced by maize /soybean intercropping. ii) Examine the intercropping effects on yield and yield components of soybean and maize. The experiment was carried out at University Research Farm, Koont in split plot design with three replications. The main plot treatments were: i) Soybean ii) maize iii) soybean-maize intercropping. Sub plot treatments were: i) Fodder cutting ii) Grain cutting. Soil samples were collected after harvesting of soybean and maize crops to measure nitrate-nitrogen, available phosphorous, extractable potassium and organic matter. Soybean plant sap samples were collected to quantify nitrogen fixation. Plant samples of both crops were taken to quantify yields and nitrogen uptake. Soil nitrogen content was significantly greater in sole soybean and soybean-maize intercropping under grain harvest. Total organic carbon (TOC) was highest in sole soybean under fodder harvest. Soil potassium concentration was significantly elevated in sole maize, while phosphorus content was highest in soybean-maize intercropping under grain harvest. The highest nitrogen fixation was determined by sole soybean where soybean crop was grown as fodder.

1.Introduction

Soybean (*Glycine max* L. Merrill) is a versatile crop cultivated for human consumption, oil production, industrial applications, and livestock feed (Popovic et al., 2013). Its seeds contain approximately 37–42% high-quality protein, 6% ash, 29% carbohydrates, and 17–24% oil, of which 85% are polyunsaturated fatty acids, including two essential fatty

acids—linoleic and linolenic acids—that cannot be synthesized by the human body. It is a good source of vitamins and natural resources such as Fe, Zn, P, Ca, Cu, K, and Mg (Xavery, Kalyebara, Kasambala, & Ngulu, 2006). Soybean crop is mainly produced annually. Few countries including Pakistan have opportunity of harvesting two (spring and autumn) crops in a year. Soybean is commonly rotated with other crops such as wheat, maize, rice, cotton

sorghum and sugarcane. It is nitrogen fixing crop, hence need less chemical fertilizer.

Maize is the third important cereal crop in Pakistan after wheat and rice. Maize is categorized as a main cereal crop of Potohar in Pakistan. Mainly its grain is a source of starch, protein, oil, fiber and nutrition (Hokmalipour et al., 2010; Mahesh, Wali, Gowda, Motagi, & Uppinal, 2014). It contributes 2.6 percent to value addition in agriculture and 0.5 percent to GDP. During 2018-19, maize was cultivated on 1,318 thousand hectares and witnessed an increase of 5.4 percent over last year's 1,251 thousand hectares. Its production increased by 5.1 percent to 6.309 million tones compared to target 6.0 million tones and 6.9 percent to the last year's production 5.902 million tones.

Intercropping is a widely adopted cropping system globally, primarily for food and fiber production. In this system, at least two crops are grown on the same land for a defined period, often in alternating strips that partially overlap during their growth stages (Gou, van Ittersum, & van der Werf, 2016). Combining cereals with legumes is a common intercropping strategy, with maize-soybean intercropping being a prominent cereal-legume pattern in China (Yang et al., 2015). While both crops compete for nutrients, particularly nitrogen, this competition encourages legumes to fix atmospheric nitrogen, thereby enhancing soil fertility (Giller et al., 2011). Legume crops are capable to enhance soil fertility and promote nitrogen availability up to 70-340 kg in a hectare (Mobasser, Vazirimehr, & Rigi, 2014). Biological nitrogen fixation takes place in legumes through Rhizobium bacteria in the symbiotic relationship; the accessible nitrogen used throughout agronomic system is 64% (Lodwig et al., 2003).

Previous studies have shown that intercropping systems enhance biodiversity, improve soil quality and carbon sequestration, increase land-use efficiency, and reduce pathogen incidence compared with continuous monoculture systems (Zhu et al., 2000; Li et al., 2007a; Cong et al., 2015). However, there is a need to investigate the effect of intercropping on nitrogen fixation of legume crop. Hence the main objective of this study was to evaluate the nitrogen fixation of soybean and nitrogen use efficiency of maize under soybean-maize intercropping.

2. Materials and Methods

2.1 Experimental Site, Design, and Treatments

An experiment was performed on soybean and maize during summer 2018 at the University Research Farm, Koont to see the effect of intercropping (maize- soybean) on nitrogen fixation of

soybean plus nitrogen use efficiency of maize crop. Experiment was carried out in split plot design with 3 replications and six treatments. The main plot treatments were: i) Soybean ii) maize iii) maize-soybean Intercropping. Sub plot treatments were: i) Fodder cutting ii) Grain cutting.

2.2 Soil Sampling and Determination of Soil N, P, K, and TOC

After harvesting of soybean and maize at the depth of 0-15 cm soil samples were collected with auger. Then samples were prepared for analysis of nitrate-nitrogen, available phosphorous, extractable potassium, total organic carbon and nitrogen fixation by xylem solute technique. Nitrate-Nitrogen in soil was determined by Salicylic acid method using Spectrophotometer (Vendrell & Zupancic, 1990). Phosphorous was determined by Olsen method using spectrophotometer (Soltanpour, 1991). Potassium was determined by extraction method using flame photometer (Helmke & Sparks, 1996) and TOC was determined by titration method (Nelson & Sommers, 1996).

2.3 Plant Sampling and Determination of TN and N₂-Fixation

At the harvesting plant, samples of both crops were taken. Plant samples were prepared for analysis of TN and N₂-fixation. Xylem sap was collected in the Vacutainer at the pod-fill stage by the Vacuum Extraction Method and stored in the freezer (Peoples, Faizah, Rerkasem, & Herridge, 1989). TN was determined through Kjeldahl digestion method (Bremner & Mulvaney, 1996), and N₂-fixation was determined by the xylem solute technique.

2.4 Statistical Analysis

Data recorded for different parameters were analyzed by using statistical software. The Least Significant Difference (LSD) values were computed at the probability level of > 0.05 (Steel, Torrie & Dickey, 1997).

3. Results

3.1 Soil Nitrate-Nitrogen

Soil nitrogen content was significantly higher in sole soybean and soybean- maize intercropping as compared to sole maize. Among combination of treatments, the highest nitrate-nitrogen was determined in plots where soybean-maize intercrop 3.84 mg kg⁻¹ was grown for grain closely followed by plots where sole soybean 3.52 mg kg⁻¹ grown as grain while the lowest nitrate-nitrogen was recorded in plots where sole maize 2.68 mg kg⁻¹ was grown as fodder (Table 1).

3.2 Available Phosphorus

Table 1 showed that soil phosphorus content was significantly higher in soybean-maize intercropping 1.98 mg kg⁻¹ as compared to sole soybean and sole maize under grain. Phosphorous was higher in soil where crops were grown as grain as compared to the soil where crops were grown as fodder. while the lowest phosphorous was determined in plots where sole soybean was grown 1.52 mg kg⁻¹ as fodder closely followed by the plots where sole maize crop was grown as fodder (Table 1). Phosphorous is lower in sole soybean as compared to sole maize.

intercrop as fodder 127.47 mg kg⁻¹. There is no significant change in potassium of soybean and maize under fodder and grain but the little difference is due to fertilizer application.

3.4 Total Organic Carbon

TOC of soil was significantly higher in sole soybean as compared to sole maize and soybean-maize intercropping. Among treatments, the highest TOC was determined in plots where sole soybean was grown as fodder 5.13 g kg⁻¹ closely followed by sole maize crop was grown as fodder 4.78 g kg⁻¹ while the lowest TOC was recorded in soil where soybean-maize intercrop was grown 3.53 g kg⁻¹ as grain (Table 1). TOC of soils under fodder and grain is little different due to the fact that over short period of time TOC does not change extensively.

3.5 RUN%

Run% of soybean and soybean-maize intercrop has been shown in Figure 1. It is apparent from figure that Run % was higher in sole soybean as compared to soybean-maize intercrop. Among combination of treatments, the highest Run% values of sole soybean were determined where

Table 1: Effect of soybean-maize intercropping on NO₃-N, P, K and TOC

Treatment	Nitrate-N mg kg ⁻¹	Available Phosphorous mg kg ⁻¹	Extractable Potassium mg kg ⁻¹	TOC g kg ⁻¹
Sole Soybean (fodder)	3.42	1.52 A	132.93 BC	5.13 A
Sole Soybean (grain)	3.52	1.70 A	140.53 AB	4.43 AB
Sole Maize (fodder)	2.68	1.52 A	130.4 BC	4.78 A
Sole Maize (grain)	3.40	1.78 A	146.53 A	4.43 AB
Soybean-Maize (fodder)	3.58	1.92 A	127.47 C	4.09 BC
Soybean-Maize (grain)	3.84	1.98 A	136.27 BC	3.53 C

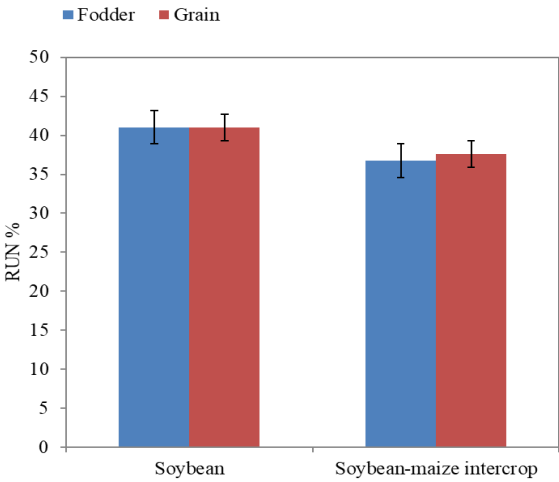
3.3 Extractable Potassium

In sole maize soil potassium concentration was significantly higher as compared to sole soybean and soybean-maize intercropping has been shown in (Table 1). Among combination of treatments, the highest potassium was determined in plots where sole maize was grown 146.53 mg kg⁻¹ as grain closely followed by plots where sole soybean was grown as grain 140.53 mg kg⁻¹ while the lowest potassium was recorded in plots where soybean-maize

soybean crop was grown as grain while the lowest Run% was recorded in soybean-maize intercrop grown as fodder. Run % is the relative abundance of ureide with respect to other nitrogenous compounds. It is apparent from the figure that ureide ratio is more than other nitrogenous compounds specially under grain purpose. As discussed earlier the crop

near to maturity leads to accumulation of ureide in plant. That ultimately results in feedback mechanism.

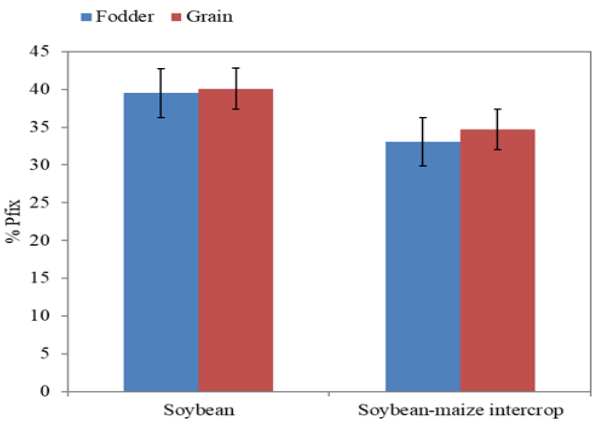
Figure 1: RUN % of soybean and soybean-maize intercrop under fodder and grain.



3.6 Percent (%) P_{fix}

The % P_{fix} of soybean and soybean-maize intercrop has been shown in figure 4.7. It is apparent from figure that % P_{fix} was higher in sole soybean as compared to soybean-maize intercropping. Among combination of treatments, the highest % P_{fix} was determined in sole soybean where soybean crop was grown as grain while the lowest P_{fix} was recorded in soybean-maize intercropping grown as fodder (Figure 2).

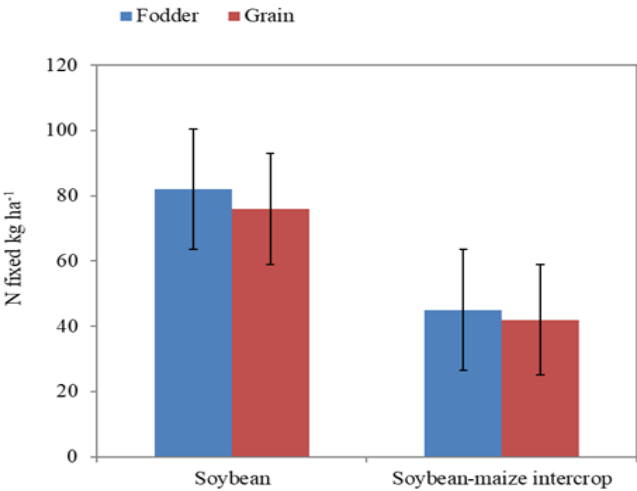
Figure 2: Percent (%) P_{fix} of soybean and soybean-maize intercrop under fodder and grain.



3.7 N_2 Fixed

Nitrogen fixation by soybean and soybean/maize intercrop has been shown in Figure 3. It is apparent from figure that nitrogen fixation was higher in sole soybean as compared to soybean-maize intercropping. Among combination of treatments, the highest nitrogen was determined by sole soybean where soybean crop was grown as fodder while the lowest nitrogen was recorded by soybean-maize intercrop grown as grain.

Figure 3: Nitrogen Fixation by soybean and soybean-maize intercrop under fodder and grain.



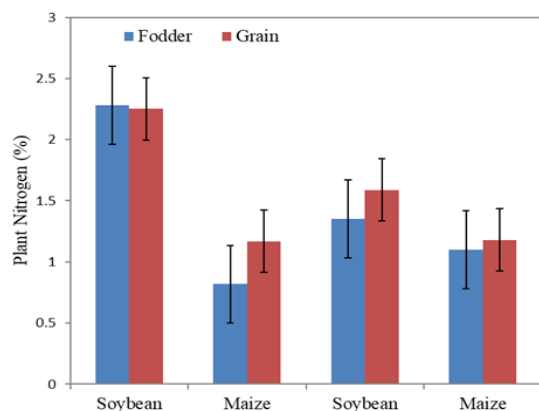
3.8 Plant Nitrogen

As compared to soybean intercrop, maize intercrop and sole maize nitrogen was significantly higher in sole soybean. Among combination of treatments, the highest nitrogen was determined in plots where sole soybean crop was grown as fodder closely followed by soybean intercrop where soybean crop was grown as grain while the lowest nitrogen was recorded in plots where sole maize was grown as fodder closely followed by maize intercrop where crop was grown for fodder (Figure 4).

4. Discussion

Soil nitrogen is not significantly different in sole soybean and maize/soybean intercropping. Soybean do not compete with maize for nitrogen due to the fact that intercropped legumes fixed nitrogen from the atmosphere (Adu-Gyamfi et al., 2007). The grain yield was increased from soybean-maize intercropping due to higher number of nodules having good nitrogen fixing capacity. A latest study initiates that soybean

Figure Error! No text of specified style in document.: Plant Nitrogen of soybean, maize, soybean intercrop and maize intercrop under fodder and grain.



(legume) biological nitrogen fixation (BNF) in maize/soybean intercropping increases through nodule formation (Li et al., 2016).

Soybean phosphorous content is not increased. Reason for difference in phosphorous is the addition of previous crop residues and chemical fertilizer to soybean plots (Khan, Shaikat, Rao, & Ahmed, 2014). The crop residue act as a source of energy for microorganisms and can help for releasing phosphorous. The results are supported by (Gangwar, Singh, Sharma, & Tomar, 2006). Although more soil nutrient reduction mainly for phosphorous by the intercropping to the cereal crops due to higher removal through crop harvesting and more proficient use of soil nutrients (Mucheru-Muna et al., 2010).

The results are supported by (Khan, Shaikat, Rao, & Ahmed, 2014) who concluded that potassium concentration in legume based cropping with maize is higher in grain. Soybean increase soil fertility and enhances soil quality without taking any costly procedures.

Total organic carbon under soybean enhanced by mutual effect of last two seasons of soybean crop in the same field and the residue addition from previous crop. The results are supported by (Qiao, Miao, Yue, Wu, & Han, 2016) who proved that total organic carbon increase in soybean plot by continuing soybean cultivation in the same field. Organic carbon input to soil was increased upto 9.27 t ha⁻¹ in soybean plot after 23 years crop cultivation.

The results were within the range observed by (Peoples & Herridge, 1990). They indicated that the proportion of soybean N derived from N₂ fixation ranged between 4 and 96%. As % P_{fix} represent the ratio of biologically fixed nitrogen to the total, so nearly half of the plant nitrogen content was gained by biological nitrogen fixation. Nitrogen supplied by symbiotic fixation to soybean can range from 25% to 85% of the total nitrogen in the plant depending on the conditions (Varco, 1999). Furthermore, it has been reported that soybean typically exports over 80% of nitrogen compounds from the nodules as ureides, which are transported via the xylem to the shoots for catabolism (Winkler et al., 1987).

Similar views were also expressed that the amount of N₂ fixed was substantially greater in soybean than soybean-maize intercropping, results were also observed in comparison between soybean and soybean-maize intercrop (Ahmad, Hafeez, Mahmood, & Malik, 2001) that soybean have maximum capacity to fix nitrogen hence significant difference was observed in nitrogen fixed. Previous studies have reported that soybean growth is negatively affected by shading, as the close planting of maize in maize-soybean relay-strip intercropping systems blocks much of the available light (Wu, Wang, & Yang, 2008).

Plant nitrogen is significantly higher in sole soybean under fodder because soybean has nodules formation to fix maximum nitrogen. The results are supported by (Zhang et al., 2015) who proved that sole soybean significantly add more total N than the sole maize. Legumes enhance N stability of cropping system (Bender, Haegle, Ruffo, & Below, 2013).

Conclusion

Intercropping enhances the provision of ecosystem services within rural farming systems. Compared with monocultures, intercropping can improve soil fertility, increase resource-use efficiency, reduce disease pressure, and enhance both crop and fodder productivity and quality. Understanding these ecosystem services helps land users make informed decisions about suitable crop combinations. Moreover, on-farm trials in smallholder farming areas are essential to build farmers' awareness, interest, and confidence, ultimately encouraging the adoption and integration of intercropping technologies into their production systems.

Authorship contribution statement

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Ayesha Nisar: Conceptualization, Investigation, Formal analysis, Writing original draft. Data curation, Methodology; Hira Aslam and Sheraz Fatima: Formal analysis and data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We acknowledge the Institute of Soil and Environmental Sciences, PMAS-Arid Agriculture University Rawalpindi, Punjab, Pakistan, for their support in conducting this research and providing all other facilities.

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