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Assessment of Nitrogen Fixation, Uptake, and Leaching in Maize/Soybean Intercropping System at Varied Soil Depths and under Phosphorus Application in Chinese Agricultural Settings

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Abstract

The study of Nitrogen fixation, uptake, and leaching at different soil depths in the co-cultivation of maize and soybean under phosphorus fertilization is important for sustainable agriculture. This study was conducted in Quzhou, Hebei Province, China, with MC812 maize and Jidou12 soybean varieties. Soil samples were taken from each plot to create a composite sample. The results show that nitrogen concentration varies at different depths and is higher in all treatments between 40 and 100 cm. Incorporating intercropping of maize and soybeans into farming practices can lead to more sustainable and environmentally friendly agriculture in China.

Keywords

Nitrogen, Maize/Soybean, Fertilization, Intercropping, Soil, Fixation

1. Introduction

The assessment of nitrogen concentration and leaching dynamics at different soil depths in Maize/soybean intercropping systems with phosphorus fertilization is essential for sustainable agriculture [1] [2]. This knowledge requires a comprehensive understanding of the factors affecting nitrogen availability and movement, including nutrient dynamics, soil-water interactions, and agronomic practices [3]. According to Long *et al.* (2021), [4] leaching is the process by which nutrients, including nitrogen, move down through the soil profile, potentially out of the root zone. By assessing nitrogen leaching at different soil depths, this study evaluates nutrient losses and their impact on crop productivity and environmental sustainability [5]. Investigating nitrogen dynamics at different soil depths is crucial as nutrient availability varies greatly within the soil profile [6]. Deeper soil layers have different nutrient concentrations and physical properties that influence the processes of nitrogen fixation, uptake, and leaching [7]. This study also focused on applying phosphorus fertilizer to the intercropping system, as phosphorus is vital for plant growth and plays a role in nitrogen metabolism. The analysis of nitrogen dynamics in phosphorus fertilization helps to understand the interactions between the different nutrients and their effects on plant performance [8].

Closed cropping systems that make little or no use of chemical fertilizers rely heavily on the microorganisms and fauna in the soil to promote nutrient cycling and plant growth [9] [10]. The microbial process of mineralization plays a crucial role in the release of nitrogen (N) and phosphorus (P) from decomposing plant material [11], however, atmospheric N deposition can cause soil and water body acidification, as well as leaching of N into surface and ground waters resulting in eutrophication and water quality degradation [12], whereby bacteria and fungi are also very crucial in the initial stages of mineralization, with the bacteria decomposing the crop residues in the soil and the fungi acting on the residues on the soil surface [13]. Mineralization is a process in which microorganisms convert nitrogen (N) into nitrate (NO_3^-) and ammonium (NH_4^+) [14]. In this process, active ammonia (NH_3) reacts with protons to form stable ammonium (NH_4^+) which can then be oxidized to nitrate (NO_3^-), a process known as nitrification. In soils with sufficient oxygen, nitrate (NO_3^-) is the primary form of nitrogen available to plants. Nitrate (NO_3^-) is more abundant in dry areas and its movement in the soil profile is influenced by water infiltration [15]. Studies in maize-soybean intercropping systems have shown a decrease in nitrate leaching [8] [16] [17]. However, previous studies suggest that conventional soil sampling methods may not accurately capture the spatial variability and vertical distribution of nitrate concentration in these intercrops [18].

The aim of this study was therefore 1) to determine the nitrate content at different soil depths (0 - 20 cm, 20 - 40 cm, 40 - 60 cm, 60 - 80 cm, and 80 - 100 cm) and 2) to evaluate nitrogen fixation, uptake, and leaching. Nitrogen fixation is the process by which soybean plants, through symbiotic relationships with nitrogen-fixing bacteria in root nodules, convert atmospheric nitrogen into a form that can be utilized by both soybeans and maize, thereby reducing the need for external nitrogen inputs. In addition, the study aimed 3) to determine how efficiently the maize and soybean plants in intercropping take nitrogen from the soil. Understanding nitrogen uptake patterns is important to gain insights into nutrient availability and utilization by plants.

2. Literature Review

The study of intercropping systems, particularly those involving maize (*Zea mays L.*) and soybean (*Glycine max L.*), is of interest because of their potential to enhance nitrogen (N) fixation and uptake, reduce N leaching, improve crop yield and nutrient use efficiency [19]-[21]. The complex interaction between soil depth, phosphorus (P) applications, and their effects on N dynamics within maize/soybean intercropping systems warrants detailed examination. Research indicates that intercropping maize with legumes, such as soybean, can significantly reduce nitrate leaching, especially at soil depths of 100 - 200 cm, compared to maize monoculture [22]. This reduction in nitrate leaching was attributed to the complementary use of soil nutrients between intercropped species. Additionally, the application of P fertilizers at varying depths has been shown to influence crop biomass, grain yield, and P uptake, with deeper applications (15 cm) resulting in higher biomass and yield [23]. This suggests that appropriate P application strategies can enhance nutrient use efficiency and potentially affect N dynamics by influencing root growth and microbial interactions. Furthermore, the presence of soybeans in intercropping systems has been associated with increased N fixation, which can contribute to the N pool and reduce the need for synthetic N fertilizers [24]. The reduction in N fertilization rates in intercropping systems has been linked to a decrease in greenhouse gas emissions and an increase in the diversity and function of soil microbial communities, including symbiotic arbuscular mycorrhizal fungi (AMF) [25]. These microbial communities play crucial roles in nutrient cycling and plant health, further influencing N uptake and leaching.

Maize/soybean intercropping systems have shown the ability to enhance nitrogen fixation and uptake while decreasing nitrogen leaching, particularly when combined with strategic phosphorus applications and managed at suitable soil depths. The interaction between phosphorus fertilization, soil depth, and intercropping practices plays a crucial role in optimizing nutrient use and promoting environmental sustainability in agriculture. Additional research is required to uncover the underlying mechanisms and to develop guidelines for implementing these systems in various agroecological zones.

3. Materials and Methods

3.1. Plant Material

The vegetative materials used were one of the varieties of quality maize (MC812) and one variety of soybean (Jidou12). The two materials were chosen for their yield potential availability of seeds to users and breeders and also food preferences.

3.2. Experimental Site and Method

The field trial was conducted from June to October 2023 at the Quzhou experimental station of China Agricultural University, which is situated in the North China Plain of Hebei Province at a latitude of, 1150E, 36.50N, and an elevation of 37 m above sea level. The trial was carried out during the short growing season from 15 June to 03 October 2023. The dimensions of each plot were 8m x 9m in size for both monoculture and intercropped maize/soybean. Maize and soybeans were harvested after 110 days of planting. Soil samples were taken with a soil auger at three locations within each plot in the monoculture and intercropping plots. Originally, the soil samples were taken using a zigzag method, but systematic sampling was used to determine nitrate leaching. A composite sample was then formed from the collected soil samples.

3.3. Design of the Experiment

The study was conducted using a randomized block split-plot design in which the main plot was divided into three cropping systems: MM/SS and M/S. There were two variants of phosphorus treatments, one without P application (P0) and the other with a P application of 80 kg P_20_5 ha^{-1}. The treatments included four different arrangements: 1) maize/soybean intercropping, where two legume rows were planted between two maize rows and two legume rows, respectively 2) Maize alone, 3) soybean alone, and 4) soybean/maize intercropping. All plant species were planted within two days. The study was conducted in plots 9 m long and 4 m wide (9 m × 4 m) with an area of 36 m^2. The plots were divided into two phases, with some plots being fertilized and others not.

3.4. Density

In a monoculture of maize, the row spacing is 50 cm, the plant spacing is 25 cm and the sowing density is 40,000 plants per hectare. In a soybean monoculture, the row spacing is also 50 cm, the plant spacing is 10 cm and the planting density is 100,000 plants per hectare. In the intercropping system, the row and plant spacing for maize and soybeans is the same as in their respective monocultures. The intercrop plot consists of 4 intercrop strips, each with 2 rows of maize and 2 rows of soybeans, resulting in a total of 8 rows of maize and 8 rows of soybeans. The row ratio for the intercropping system is 2:2, *i.e.* there are twice as many rows of maize as rows of soybeans.

3.5. Fertilizer treatment

The basic nitrogen fertilizer for maize and soybeans was applied at 90 kg N ha^{-1} using urea with an N content of 46. Potassium fertilizer was applied at a rate of 54 kg with 20 content of potassium chloride ha^{-1}. Phosphate fertilizer was either not applied or applied at a rate of 80 kg P_20_5 ha^{-1} depending on the experimental treatment. After the fertilizers were applied to the respective plot, they were applied to the tile floor in the jointing phase. For maize, 90 kg N ha^{-1} was applied, while no nitrogen was applied to soybeans [24].

3.6. Sowing Management

The basic nitrogen fertilizer for maize and soybeans was applied at 90 kg N ha^{-1} using urea with an N content of 46. Potassium fertilizer was applied at a rate of

54 kg K20 ha^{-1} using potassium chloride with a K20 content of 52. Phosphate fertilizer was either not applied or applied at a rate of 80 kg P_20_5 ha^{-1} depending on the experimental treatment. After the fertilizers were applied to the respective plot, they were applied to the tile floor in the jointing phase. For maize, 90 kg N ha^{-1} was applied, while no nitrogen was applied to soybeans.

3.7. Data Collection

The soil samples were taken at depths of 0 - 20 cm, 20 - 40 cm, 40 - 60 cm, 60 - 80 cm, and 80 - 100 cm [26]. At each depth, soil from three different locations was mixed and then separated before being placed in a small plastic container and refrigerated until preparation. The collected soil was then sieved using a 1 mm mesh sieve to remove any debris and the resulting soil samples were also refrigerated until the day of preparation. The soil samples were packed in plastic bags and sent to the soil laboratory in Quzhou, where they were sieved with a 1 mm sieve and prepared for laboratory analysis. The inorganic nitrogen content was analyzed using standard analytical methods [27]. At the beginning of the test, 6 g of soil was weighed into a plastic bottle using a tare balance, and 1.11 g of $CaCl_2$ was added to the bottle together with 1 L of distilled water, which was then shaken well. Then 50 ml of the $CaCl_2$ solution was added to the 6 g of soil in a plastic container and the solution was placed in a shaking machine at 180 rpm for 60 minutes. After 60 minutes, the solution was removed from the shaker and filtered into 10 ml centrifuge tubes. Finally, the solution was placed in a freezer at 20 degrees Celsius for the final test.

3.8. Statistical Analyses

All the measured variables were analyzed using the Analysis of Variance (ANOVA), Statistical Package for the Social Sciences (SPSS), and sigma plot version 2020, while Microsoft Excel was utilized to organize the data in the form of tables. Origin Pro 2022b was employed for graphical representation.

4. Results

4.1. Nitrate and Ammonium Concentrations Leaching at Different Soil Depths of Maize/Soybean Intercrop under Phosphorus Fertilization

Nitrate concentration at 60 cm depth in intercropped soybeans during the VT stage in non-fertilized treatments was relatively low (**Figure 1(a)**). However, the results showed that the nitrate concentration at a depth of 60 cm was higher in all fertilized treatments, although there was no significant difference ($P < 0.05$) from the other treatments (**Figure 1(b)**). The ammonium concentration was constant at the R1 growth stage at a depth of 40 - 60 cm in the non-fertilized treatments (**Figure 1(e)**), and this was also true for the fertilized treatments. In addition, ammonium concentration remained constant from a depth of 60 cm to 100 cm at all growth stages, although there was no significant difference ($P < 0.05$) in ammonium concentration at all growth stages.

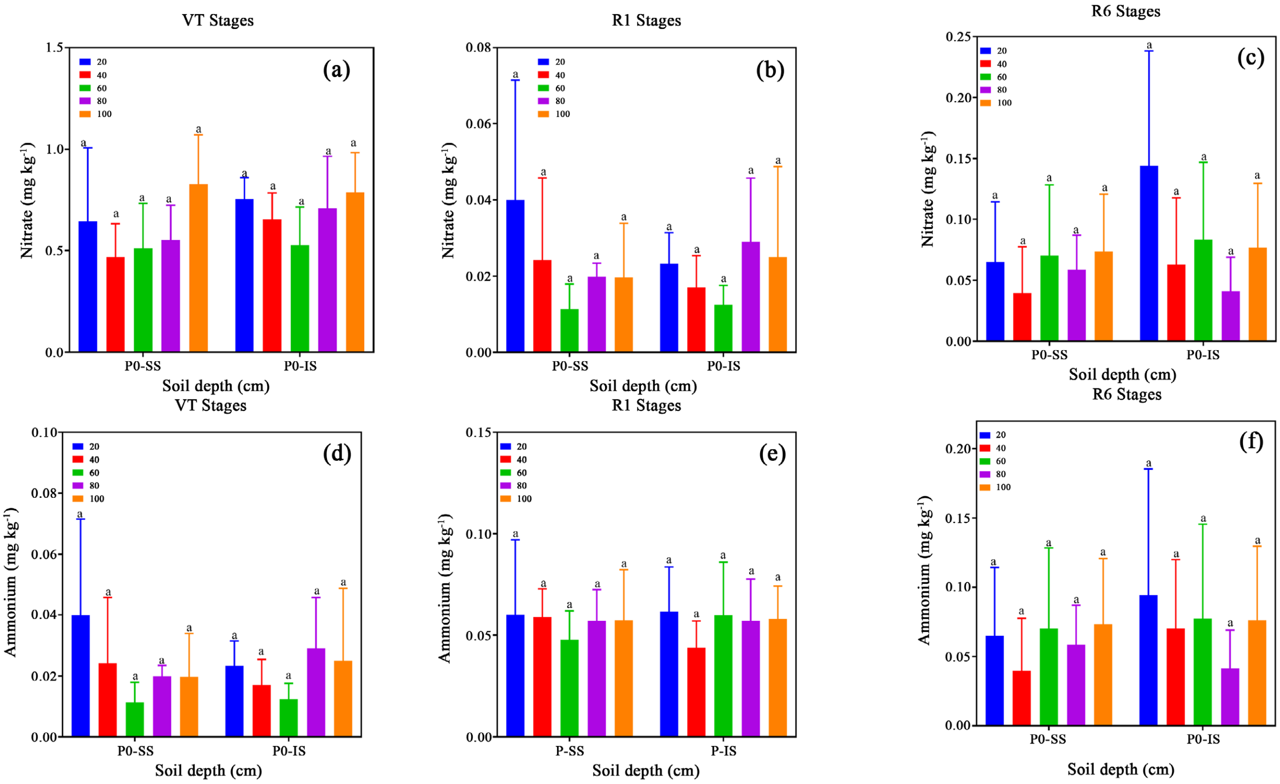

Figure 1. The nitrate and ammonium concentrations, as well as leaching at various soil depths, were assessed for intercropped maize/soybean (P-IS), and monoculture soybean (P-SS) without phosphorus fertilizer (P-IS and P0-IS, respectively), the vertical bars represent the values for each category; error bars indicate 95% confidence intervals.

At the VT growth stage and a depth of 60 cm, the nitrate concentration was higher in all fertilized treatments, as shown in Figures 2(a) and Figures 2(e). However, at the R1 and R6 growth stages, there was a noticeable decrease in nitrate concentration at a soil depth of 60 cm in both intercropped maize treatments, as shown in Figures 2(b) and Figures 2(c). Interestingly, the intercropped maize showed an increase in nitrate concentration at a depth of 100 cm at all growth stages in both the fertilized and non-fertilized treatments. It is worth noting that there was no significant difference ($P < 0.05$) between the non-fertilized treatment and the other treatments.

The ammonium concentration was low in all stages VT, R1, and R6 in the non-fertilized treatments at a depth of 60 cm, as shown in Figures 2(d)-(f). In the fertilized treatments, however, the ammonium concentration was relatively low in growth stage R6 at a depth of 80 cm and differed significantly from the other stages.

4.2. Nitrate and Ammonium Concentrations Leaching at Different Soil Depths of Sole Maize under Phosphorus Frtilization

The nitrate concentration at growth stages VT, R1, and R6 at a soil depth of 40 cm was relatively low in the non-fertilized maize intercrop treatments, as shown in Figures 3(a)-(c). Nevertheless, there was an increase in nitrate concentration at 60 cm to 100 cm soil depth at growth stage R6 in the non-fertilized treatments

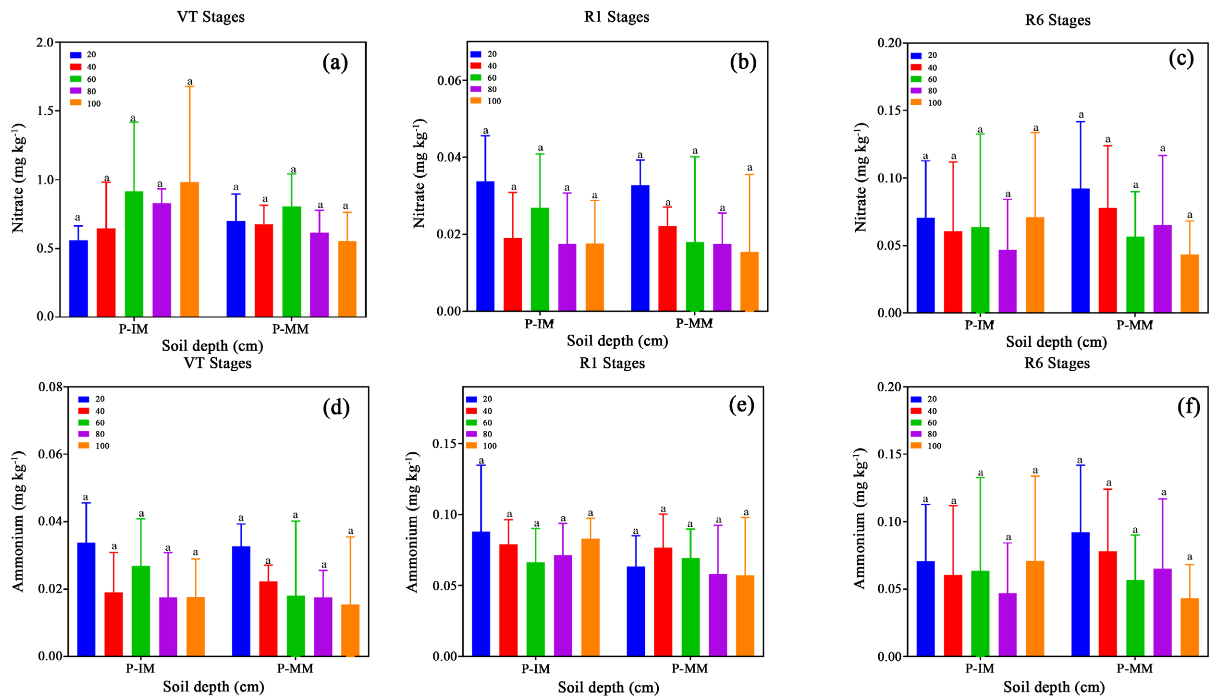

Figure 2. Nitrate and Ammonium concentration and leaching at different soil depths of intercropped Maize/soybean intercrop (P-IM), and monoculture maize (P-MM) under phosphorus Fertilizer; P-IM (intercropped maize), P-with fertilizer, the vertical bars represent mean values for each category; error bars indicate 95% confidence intervals.

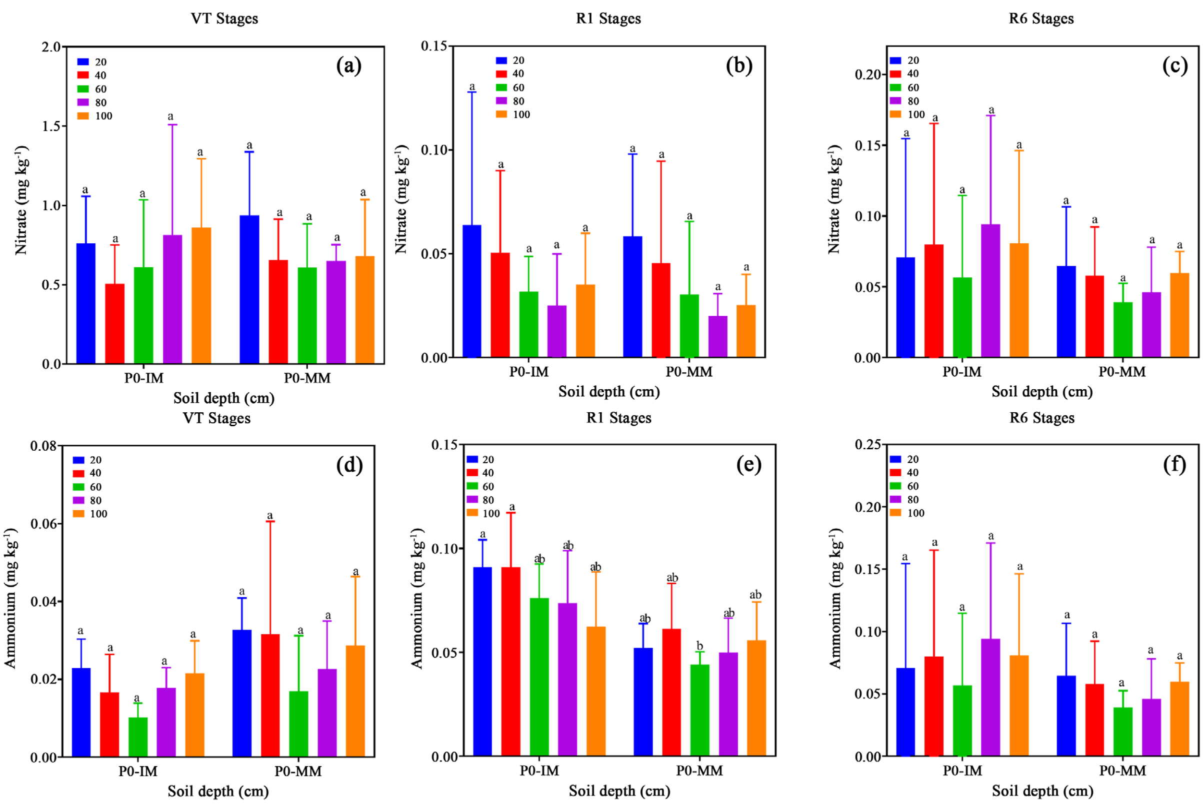

Figure 3. Nitrate and Ammonium concentration and leaching at different soil depths of monoculture(P-MM) and intercropped maize/soybean (P-IM) under phosphorus fertilizer; P-MM (Sole maize), P-with fertilizer, the vertical bars represent mean values for each category; error bars indicate 95% confidence intervals.

(Figure 3(c)). Leaching was high at this stage, resulting in lower nitrate uptake by the plants. Although there was no significant difference between the VT and R1 stages (Figure 3(a) and Figure 3(b)), there was a significant difference at the R6 stage. The ammonium concentration at 60 cm soil depth at the VT stage was relatively low in the non-fertilized treatments (Figure 3(d)). However, in the fertilized treatments, the ammonium concentration was slightly low at all stages in all treatments, and there was no significant difference between all fertilized treatments with maize alone.

4.3. Nitrate and Ammonium Concentrations Leaching at Different Soil Depths of Sole Soybean under Phosphorus Fertilization

The levels of Nitrate were noticeably greater at the VT growth stage, reaching 60 cm soil depth in the fertilized treatments shown in Figure 4(a). Additionally, the results showed higher levels of Nitrate in both the fertilized and non-fertilized treatments in Figure 4(b). and at 40 cm soil depth in Figure 4(c). However, there was

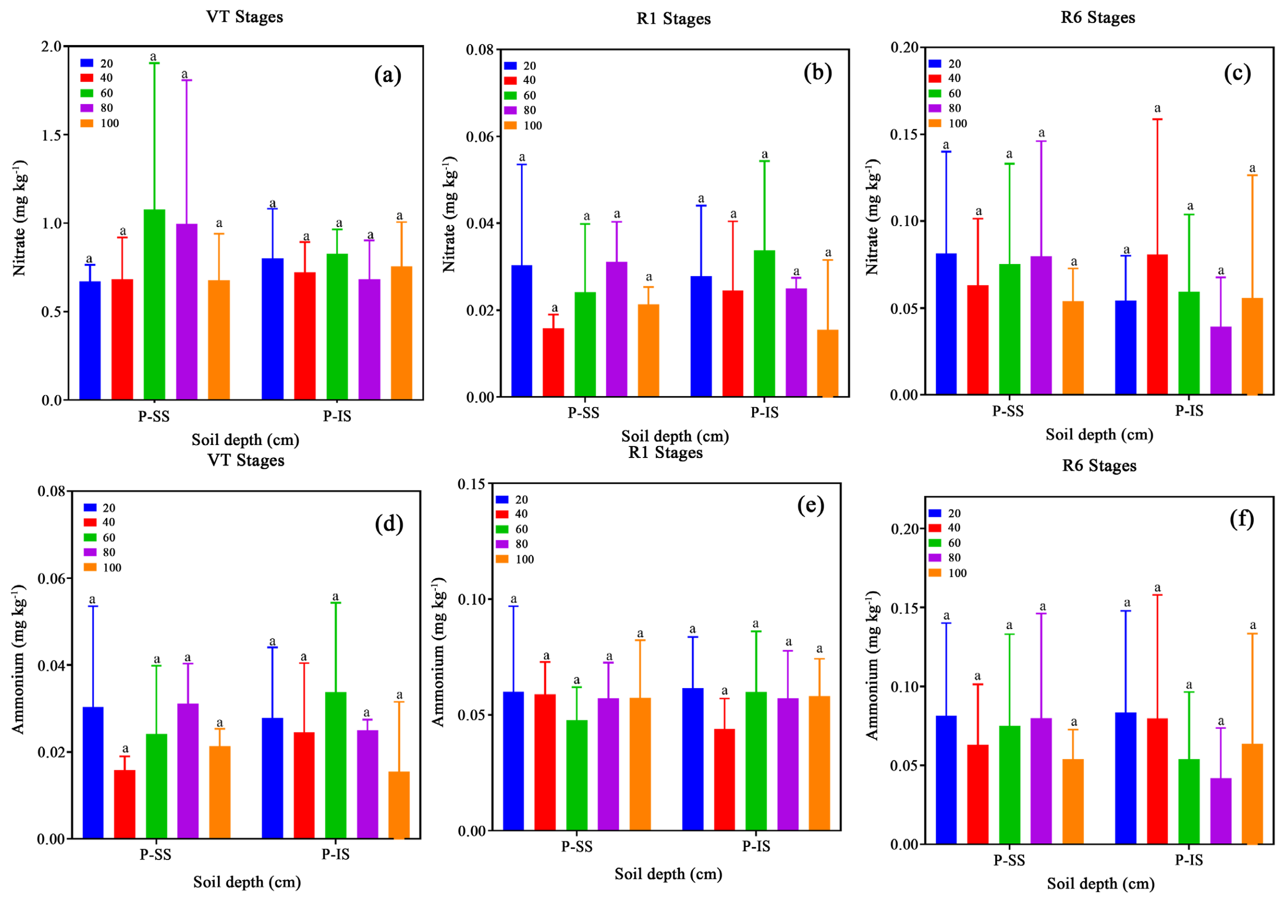

Figure 4. Nitrate and Ammonium concentration and leaching at a different soil depth of monoculture soybean (P-SS) and intercropped soybean (P-IS) under phosphorus fertilization; P-SS (monoculture Soybean), P-IS (intercropped soybean), P-with fertilizer, the vertical bars represent mean values for each category; error bars indicate 95% confidence intervals.

no significant difference between the fertilized and non-fertilized treatments in the R6 growth stage. The results also indicated that the levels of Ammonium were low at 40 cm soil depth in the R6 stage in both the fertilized and non-fertilized treatments in **Figure 4(f)**. Furthermore, the levels of Ammonium were significantly higher at 60 cm soil depth in the same stage. Although there were graphical differences, there was no significant difference (P = 0.05) between the fertilized and non-fertilized treatments in all the stages, as indicated by the statistical analysis.

4.4. Discussion

4.4.1. Effect of Phosphorus in Nitrogen Uptake, Leaching

The results show that nitrogen loss through leaching was very high in fertilized treatments where additional nitrogen fertilizers were added to the soil in intercropping maize/soybean compared to monoculture treatments, however, the nitrate concentration was also higher in treatments where phosphorus fertilizers were absent than compared to those treatments where fertilizers were present, suggesting that the leached nitrate and ammonium nitrogen were in excess and not needed by the plant. However, this study did not conclusively determine nitrate losses and plant requirements. The same results showed that the ammonium concentration at the R1 growth stage was constant between 40-60 cm soil depths in the non-fertilized treatments, indicating that this form of nutrient remained locked for plant use [23]. In a soybean intercrop system, the movement of nitrogen in the form of nitrate and ammonium through the soil profile and possibly out of the root zone is referred to as leaching [20].

Nitrogen is an essential nutrient for plants as it is an important component of amino acids, proteins, chlorophyll, and nucleic acids [28]. It is crucial for the growth, development, and general health of plants. Nitrogen is mainly taken up by plants in the form of nitrate (NO_3^-) and ammonium (NH_4^+) [29]. Therefore, the high accumulation of nitrate and ammonium outside the root zone indicates that these nutrient forms have been lost. Maize is a shallow-rooted plant, and the extraction of nitrogen from the soil, where nitrate and ammonium were concentrated at 80 - 100 cm, hence not very useful to the plant. The results are consistent with previous research that the availability of nitrogen is limited in many agricultural systems due to leaching and other inefficiencies [30]. In this study, nitrate concentrations were found to be significantly higher beyond the root zone when the corn plant reached maturity. Although the plant did not consume the nitrate at this stage, this suggests that important plant minerals were lost when they were most needed.

In crop production systems, nitrogen is often added to the soil in the form of fertilizers to improve plant growth and yield . It is important to understand the degree of leaching of nitrate and ammonium to assess nutrient losses, environmental impact, and nutrient management efficiency in the intercrop system. Nitrate (NO_3^-) is a mobile form of nitrogen in the soil that can easily leach into deeper soil layers and potentially into groundwater, leading to nutrient deficiencies in the root zone, groundwater contamination, and environmental problems such as eutrophication in water bodies if leached excessively [31]. Monitoring nitrate leaching in a soybean intercrop helps assess the risk of nutrient loss [32].

and allows fertilization practices to be adjusted to minimize environmental impact, however, enhanced nitrification increases soil NO_3-N, which may then leach and potentially elevate groundwater NO_3-N concentrations (Mailapalli & Thompso 2012).

Ammonium (NH_4^+) is another form of nitrogen that is leached through the soil profile, although it is less mobile than nitrate. Ammonium can be converted to nitrate by nitrification processes in the soil, which increases the risk of nitrate leaching [33] [34]. However, monitoring the extent of ammonium leaching in a soybean crop provides insight into nitrogen conversion, nutrient availability, and potential losses of this form of nitrogen from the system [23]. Several factors can influence leaching, such as soil properties, including texture, structure, drainage, and organic matter content, which can affect the movement of nitrate and ammonium in the soil profile. In addition, the timing, amount, and replacement of nitrogen fertilizers in the intercrop system can affect the availability of nitrate and ammonium in the soil and their susceptibility to leaching [35]. Cultivation practices, including intercropping and irrigation, can also affect nitrogen dynamics and leaching levels in soybean crops.

4.4.2. Effect of Phosphorus Mechanisms of Nitrogen Fixation, Uptake, and Leaching

Phosphorus plays a crucial role in the mechanisms of nitrogen fixation, uptake, and leaching in the soil-plant system [36]. These include Nitrogen fixation, the process by which nitrogen gas from the atmosphere is converted into a form that plants can utilize, primarily through the action of nitrogen-fixing bacteria [11]. Phosphorus is important for the enzyme nitrogenase, which is responsible for the conversion of atmospheric nitrogen into ammonia during the fixation process [35]. Phosphorus is an important component of the energy molecule ATP (adenosine triphosphate), which is crucial for the activity of the nitrogenase enzymes. Without an adequate supply of phosphorus, the energy required for nitrogen fixation is limited, resulting in reduced nitrogen fixation rates [37]. The availability of phosphorus in the soil has a direct impact on the efficiency of nitrogen fixation in legumes and other nitrogen-fixing bacteria.

Phosphorus is also important for maintaining the health and function of plant roots, which are essential for efficient nitrogen fixation [38]. Phosphorus is involved in the formation of root hairs and the development of the root surface, which are important for nutrient uptake. Adequate levels of phosphorus in the soil promote efficient nutrient uptake mechanisms in plants, including nitrogen uptake. Plants take up nitrogen in the form of nitrate (NO_3^-) or ammonium (NH_4^+), the two primary forms of nitrogen available to plants in the soil. Phosphorus plays a crucial role in the uptake of nitrogen by plants [39].

Nitrogen leaching occurs when nitrogen in the form of nitrate is washed through the soil profile by excess water and eventually enters groundwater or surface waters [40]. Phosphorus can influence the leaching of nitrogen by affecting plant growth and root development. If plants have sufficient phosphorus, they can absorb nitrogen better, which reduces the amount of nitrate available for leaching [41]. Phosphorus can also influence soil structure and aggregation, which in turn can affect water movement and nitrogen leaching rates [12] [33] [42]. Imbalances in phosphorus levels can lead to poor root growth, reducing plant uptake of nitrogen and increasing the potential for nitrogen leaching.

5. Conclusion and Prospects

Nitrogen, available in both nitrate and ammonium forms, plays a critical role in plant growth. The concentration of these nutrients varies with soil depth, ranging from 0 - 20 cm to 80 - 100 cm in 20 - 40 cm, 40 - 60 cm, and 60 - 80 cm increments. In general, nitrate concentrations are higher in all treatments at depths of 40 to 100 cm. However, the concentrations of nitrate and ammonium can differ due to factors such as fertilization in each treatment. The results indicate that intercropping systems have the potential to enhance nutrient utilization, reduce dependence on fertilizers, and contribute to sustainable farming practices compared to monoculture. Researchers have gained insight into the mechanisms that contribute to increased nitrogen uptake in the maize/soybean intercropping system by examining the interactions between root systems, nutrient complementarity, and increased microbial activity in the rhizosphere. Adopting intercropping systems, particularly maize/soybean, enables farmers in China to improve nitrogen uptake efficiency and reduce their reliance on synthetic fertilizers, leading to more sustainable and environmentally friendly farming practices.

This study highlights the significance of intercropping as a sustainable method for improving nitrogen uptake and reducing dependence on external nitrogen inputs in agricultural systems. By incorporating maize/soybean intercropping into farming practices, China can promote more sustainable and eco-friendly agricultural practices. This research contributes to the growing body of evidence supporting intercropping as a viable strategy for enhancing nutrient uptake and reducing fertilizer use in agricultural systems.

Maize and soybean intercropping exhibits the potential to enhance nitrogen fixation and uptake while reducing nitrogen leaching in Chinese agricultural settings. Moreover, the application of phosphorus can further optimize nitrogen utilization efficiency in the intercropping system. Nevertheless, additional research is required to comprehensively understand the underlying mechanisms and optimize the benefits of this system. Prospects for this research include investigating the impacts of different soil depths and levels of phosphorus application on nitrogen dynamics in the intercropping system, as well as devising management strategies to maximize nitrogen use efficiency and sustainability in agricultural practices. This study offers valuable insights into improving nitrogen management and fostering environmental sustainability in Chinese agriculture.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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