NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 41, 2024
www.incda-fundulea.ro; doi.org/10.59665/rar4114 First Online: December, 2023. DII 2067-5720 RAR 2024-49

Nitrogen Fate of Chemical Fertilizer and Green Manure-Wheat Rotation Using ^{15}N Tracer Technique

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ABSTRACT

To investigate the accumulation, distribution, and translocation of nitrogen (N) from chemical fertilizers and green manure in wheat plants, we conducted an experiment using leguminous green manure crop hairy vetch and wheat as test materials. During the anthesis and maturity stages of wheat, the ^{15}N enrichment and Nitrogen content of wheat plant samples were determined in different organs, and ^{15}N enrichment and nitrogen content of soil samples were measured at maturity. The accumulation and distribution characteristics of nitrogen from different sources during the wheat growth stages were investigated to explore the impacts of green manure and chemical fertilizers on wheat growth and nitrogen uptake. The results showed that nitrogen from chemical fertilizers in the soil-plant system exhibited the following pattern: plant absorption > soil residue > loss, while nitrogen from green manure showed: soil residue > loss > plant absorption. Wheat had higher absorption of nitrogen from chemical fertilizers compared to green manure. The absorption of nitrogen from chemical fertilizers by wheat ranged from 46.00% to 54.08%, while the absorption of nitrogen from green manure ranged from 20.71% to 28.21%. The residual nitrogen from green manure in the soil (38.47% to 58.22%) was higher than that from chemical fertilizers (31.44% to 40.69%), and the nitrogen loss from green manure (21.07% to 33.31%) was higher than that from chemical fertilizers (5.22% to 22.56%). Supply of nitrogen from the two sources could coordinate the distribution of nitrogen in various organs and increase the proportion of nitrogen allocation in grains. This was beneficial for facilitating nutrient transfer to reproductive organs.

Keywords: chemical fertilizer, green manure, ^{15}N tracer technique, N translocation, spring wheat.

INTRODUCTION

Nitrogen (N) is a critical nutritional element for crops and is considered as one of the main limiting factors that substantially affects crop yields (Piotrowska-Długosz and Wilczewski, 2020). In China, N fertilizer application has increased the grain yield of crops from 83.4 to 474.2 Mt from 1961 to 2009 (Fan et al., 2012), thus making it possible to feed one-fifth of the world population from <9% of the world arable land (Li et al., 2022). However, this has also caused a series of serious environmental pollution (e.g., eutrophication and air pollution) at regional and global scale (Chen et al., 2021). Measuring and monitoring the fate of fertilizer N is required to guide improved N management strategies and decrease its impact on the environment (water and air phases), which impact establishment and development of source-sink relationship between various organs (Monowar and Yoshida, 2012). Uneven water distribution and inefficient fertilizer N uptake are the major problems in agricultural production of China, especially for the Northwest (Moriondo et al., 2011). Moreover, excessive application of N fertilizers is particularly prominent in agricultural activities, and nitrate leaching losses in farmland have threatened the balance of farmland ecosystems (Jiang et al., 2022). It has been reported that nitrate in groundwater mainly came from agricultural fertilizers, and the contribution rate could reach up to 52.3% (Kong et al., 2023). Therefore, studying the cycling and translocation processes and regulatory mechanisms of N is essential to solve agriculture-related ecological and environmental problems.

N fertilization management practices, including N application rate, timing, and methods, as well as irrigation rate and timing

Received 1 November 2023; accepted 8 December 2023.

can influence N fate in wheat production (Jia et al., 2011). Under different integrated nutrient management conditions, approximately 50% of the crop's absorbed N is derived from the soil (Tonitto et al., 2006). Specifically, N derived from green manure crops tends to have a longer residence time in the soil (Macholdt et al., 2020), thereby enabling the crop to access more organic N (Gao et al., 2023). Several studies have demonstrated that the application of green manure also enhances N fixation (Gondim et al., 2020). Thus, increasing the utilization of N from green manure crops is a crucial approach for maintaining the ecological balance of N-fixing microorganisms in the soil and reducing the reliance on synthetic fertilizers (Hu et al., 2023). Moreover, optimizing the recycling of plant and soil microbial nutrient pools can reduce the need for fertilizers in subsequent crop seasons (Mahmood et al., 2022). Combining green manure with chemical fertilizers has been proven effective in mitigating biomass reduction, improving crop growth, and enhancing soil fertility (Braos et al., 2023; Fan et al., 2023). Additionally, the practice of applying green manure through mulching or plowing down can contribute to an increased rate of N translocation and conversion (Liang et al., 2023).

As a non-radioactive and non-destructive tracer, the ^{15}N isotope is widely utilized in ecological cycle and atmospheric circulation research (Ma et al., 2023). Due to its non-disruptive nature, the use of ^{15}N ensures the safety of the current experiment and the accuracy of the results. Currently, the ^{15}N labeling technique has been gradually adopted in studies related to biological N fixation, plant N uptake and utilization, plant N transport, N losses, and N fate (Zhang et al., 2022; Lei et al., 2023). This technique has also been employed in green manure-related research. For instance, in studies investigating the N nutrition status in wheat, researchers have explored the fate of N in green manure, straw, and fertilizers (Wan et al., 2021). Previous research on N and the application of green manure and chemical fertilizers has primarily focused on aspects such as crop productivity and quality, as well as soil quality in farmland. However, little attention has been given to the effects of planting and incorporating hairy vetch on the composition of the soil N pool or the accumulation and distribution of N from different sources in wheat organs.

This study aimed to utilize the ^{15}N isotope tracer technique to examine the translocation of N and the associated transformation processes at the atomic level. Specifically, N derived from green manure and chemical fertilizer was labeled separately to serve as our study subjects. We simultaneously analyzed and quantified multiple translocation pathways to investigate the variations in ^{15}N content in different plant organs and soil at different growth stages. The main objective was to explore the acquisition and distribution of N from different sources in wheat, as well as to understand the patterns of N translocation and transformation. Additionally, we aimed to elucidate the relationship between the planting and plowing down of hairy vetch, the soil N pool, and N uptake and utilization by crops. Furthermore, we assessed the impact of hairy vetch on soil fertility and fertilizer efficiency.

MATERIAL AND METHODS

1. Experimental site

The experiment was conducted at Mojiaquan Bay in Ershilipu Town, located in the northern suburb of Xining, Qinghai Province, China (101°45′E, 36°42′N). This area falls within the highland continental semi-arid climate zone, characterized by an altitude of 2230 m and a cold climate. The annual average temperature is recorded at 5.9°C, showcasing a notable diurnal temperature range. The crop growth period spans 220 days. Additionally, the mean annual precipitation measures 367.5 mm, while the mean annual evaporation stands at 1729.8 mm. The water source for the experimental plots is the Beichuan canal. The soil type within the plots is classified as chestnut soil. Before the experiment, soil properties of the top 0-30 cm soil layer were 1.07 g/kg total N, 76.0 mg/kg available N, 21.95 g/kg organic matter, 9.40 mg/kg mineral

N, 8.22 pH, and 23.70 mg/kg available phosphorus.

2. Experimental design

This potted experiment utilized ceramic pots with a diameter of 10 cm and a height of 30 cm. Each pot contained 10 kg of air-dried soil. The experiment consisted of five treatment groups, namely ^{15}G, ^{15}U, $^{15}G+U$, $G+^{15}U$, and a control group (CK) with no application of nitrogen (N) fertilizer (Table 1). The experimental treatments, where were replicated four times, were arranged in a single-factor experiment design. In the ^{15}G treatment, 134 g of hairy vetch (fresh weight) labeled with ^{15}N was crushed and pressed into the pot during the anthesis stage. Similarly, in the G treatment, 134 g of unlabeled hairy vetch (fresh weight) was added to the pot. For the ^{15}U treatment, each pot received 2.14 g of ^{15}N-labeled N (equivalent to 4.66 g of urea), while in the U treatment, the same amount of unlabeled N was applied. Additionally, each fertilization treatment received 1 g of P_2O_5. The P fertilizer used in this study was calcium superphosphate (0-12-0 of N-P_2O_5-K_2O) and N fertilizer was urea (46-0-0 of N-P_2O_5-K_2O).

On 8th April 2020, two plots with an area of 6 m^2 each were designated for the cultivation of hairy vetch. One plot was allocated for the ^{15}N-labeled hairy vetch, while the other for the common unlabeled hairy vetch. The application rate of N to the hairy vetch was 75 $kg \cdot ha^{-1}$, with a 1:1 distribution as a basal fertilizer during sowing and as a top-dressing at the budding stage. The basal fertilizer was formulated by incorporating 22.5 g of labeled or regular urea into fine soil for furrow application. The top-dressing solution was prepared by dissolving 22.5 g of urea in 6 L of water, which was evenly sprayed onto the roots of the hairy vetch. At the full-blooming stage on 28th June 2020, the labeled and unlabeled green manure was harvested. The labeled green manure exhibited a N content of 3.77%, whereas the ordinary green manure showcased a N content of 4.05%. Subsequently, appropriate quantities of labeled and unlabeled fresh hairy vetch samples, along with soil samples, were collected and subjected to drying for the determination of their ^{15}N abundance and N content.

On June 28, 2020, wheat (*cv. Qingchun 38*) was sown. After incorporating the green manure into the soil, wheat seeds were sown with four seeds per hole and five holes per pot. Two wheat plants at the three-leaf stage were planted in each pot.

Table 1. Detailed fertilization information

Treatment	Treatments	Fertilizer dosage
Control group without nitrogen application	CK	1 g P_2O_5
Labeled green manure were plowed into soil	^{15}G	134 g ^{15}N hairy vetch + 1 g P_2O_5
Labeled urea were applied in customary dosage only	^{15}U	2.14 g ^{15}N urea + 1 g P_2O_5
Labeled green manure were applied with regular urea	$^{15}G + U$	134 g ^{15}N hairy vetch + 2.14 g N urea + 1 g P_2O_5
Unlabeled green urea were applied with labeled urea	$G + ^{15}U$	134 g N hairy vetch + 2.14 g ^{15}N urea + 1 g P_2O_5

Abbreviations used in the table: ^{15}G - labeled green manure were plowed into soil; ^{15}U - labeled urea were applied in customary dosage only; $^{15}G + U$ - labeled green manure were applied with regular urea; $G + ^{15}U$ - unlabeled green urea were applied with labeled urea.

3. Sampling and measurement

3.1. Sampling

Five wheat plants were collected from each treatment during the anthesis stage and the maturity period. After sampling, all aboveground samples were oven-dried at 105°C for 30 min and then oven-dried at 80°C to a constant mass to determine the aboveground dry matter and N content. Soil samples were taken from 0-30 cm depth at the maturity stage.

During the anthesis stage and the maturity period, a total of five wheat plants were collected from each pot. Subsequently, all aboveground samples were subjected to oven-drying at 105°C for 30 min, followed by further drying at 80 °C until a constant mass was achieved, enabling the determination of aboveground dry matter and nitrogen (N) content. At the maturity stage, soil samples were obtained from a depth of 0-30 cm.

The dry plant and soil samples were first passed through a 100-mesh sieve, and then subjected to digestion using boiling concentrated sulfuric acid-hydrogen peroxide solution. The determination of total N concentration was performed using the semi-micro Kjeldahl method, while the ^{15}N abundance ratio was measured using a mass spectrophotometer, following the method described by Wang et al. (2020).

3.2. Measurement

^{15}N relative abundance

The relative abundance of sample ^{15}N ($\delta^{15}{}_{N}$) was obtained as the relative abundance of ^{15}N in the sample minus the background value of ^{15}N abundance in the control：

$$\delta_{^{15}N}‰ = \left[\frac{Sample_{atom\%^{15}N} - Control_{atom\%^{15}N}}{Control_{atom\%^{15}N}}\right] \times 1000\#(1)$$

where Sample $_{atom\%}{}^{15}{}_{N}$ represents the ^{15}N atomic abundance of the sample, Control $_{atom\%}{}^{15}{}_{N}$ represents the ^{15}N atomic abundance in the atmosphere (0.3663%), which is used as the standard isotope abundance of ^{15}N.

N accumulation

The N accumulation for each sample was calculated using the following equation (Xu et al., 2023):

$$NA(\mathrm{g}) = DMw \times TN\#(2)$$

where NA represents the N accumulation of wheat, DMw represents the dry matter, TN represents the total N concentration.

Further, the amount of ^{15}N-derived N in different plant parts was calculated using the following equation:

$$N\ accumulation\ derived\ ^{15}N\ amount\ in\ plant(g) = Plant\ \delta_{^{15}N}‰ \times NA_p\#(3)$$

where NAp is the N accumulation of the roots, stems, leaves, spikes, glumes, grains and whole plant.

Nitrogen use efficiency

The N harvest index (NHI) was calculated as follows (Lü et al., 2012):

$$N\ harvest\ index(\%) = \frac{NA_g}{NA_{aboveground}}\#(4)$$

where NA_g is the N accumulation of grain and $NA_{aboveground}$ is the N accumulation of the aboveground parts.

The residual rate of green manure N (or chemical fertilizer N) was calculated according to the following equation:

$$CR_F(\%) = \frac{\delta_{^{15}N}‰}{\delta_{^{15}N}‰_F}\#(5)$$

$$R_F(g) = \frac{TN_{soil}}{CR_F}\#(6)$$

$$RR_F(\%) = \frac{R_F}{I_F}\#(7)$$

where CR_F represents the contribution rate of green manure N or chemical fertilizer N, $\delta^{15}{}_{N}‰$ represents the relative abundance of sample ^{15}N, $\delta^{15}{}_{N}‰_F$ represents the relative abundance of green manure ^{15}N or chemical fertilizer, R_F represents the residue amount of green manure N or chemical fertilizer, TN_{soil} represents the total N in the soil during the wheat maturity stage, RR_F represents the residual rate of green manure N or chemical fertilizer N, and I_F represents the input amount of green manure N or chemical fertilizer N.

The ^{15}N loss rate was calculated as follows:

$$UR_{^{15}N}(\%) = \left(Plant\ \delta_{^{15}N}‰/TI^{15}N\right) \times 100\#(8)$$

$$LR_{^{15}N}(\%) = 100 - UR_{^{15}N} - RR\#(9)$$

where $UR^{15}{}_{N}$ is the utilization rate of ^{15}N, Plant $\delta^{15}{}_{N}‰$ is the plant uptake amount of

^{15}N, $TI^{15}{}_{N}$ is the total input amount of ^{15}N, and $LR^{15}{}_{N}$ is the loss rate of ^{15}N.

The absorption rate of ^{15}N sourced from green manure N or chemical fertilizer N by each organ was calculated as follows:

$$AR_{^{15}N}\,(\%) = \frac{U_{^{15}N\ Organs}}{U_{^{15}N\ Plant}}\,\#(10)$$

where $UR^{15}{}_{NOrgans}$ is the amount of ^{15}N uptake by the organ, and $UR^{15}{}_{NPlant}$ is the amount of ^{15}N uptake by the plant.

4. Statistical analysis

Data were analyzed using Statistical Analysis Software (SPSS software, 17.0, SPSS Institute Ltd, USA). The treatment effects were investigated using Duncan's multiple-range test, and significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Nitrogen content in various organs of wheat

The N content in different organs of wheat undergoes changes throughout its growth period, as shown in Figure 1. During the anthesis period, characterized by active growth and photosynthesis in the leaves, higher N content was observed in the leaves compared to other organs. The treatments involving green manure ^{15}U combined with chemical fertilizers, G + ^{15}U and ^{15}G + U, exhibited significantly higher N content in the leaves by 14.3% and 16.3%, 16.7%, and 18.8% compared to ^{15}G and, respectively. Conversely, the CK had significantly lower N content in the leaves at 1.93%. This suggests that the combination of green manure and chemical fertilizers effectively enhances N content in wheat leaves.

At the maturity stage, the N content in the various organs followed the order of grains > leaves > glumes > roots > stems. The grains treated with G + ^{15}U showed 24.5%, 17.4%, and 35.7% higher N content compared to ^{15}G + U, ^{15}G, and CK, respectively, with no significant difference observed compared to ^{15}U. This indicates that the increase in leaf N content during the anthesis period due to G + ^{15}U treatment is subsequently transferred to the wheat grains during maturity, leading to an overall improvement in grain N content.

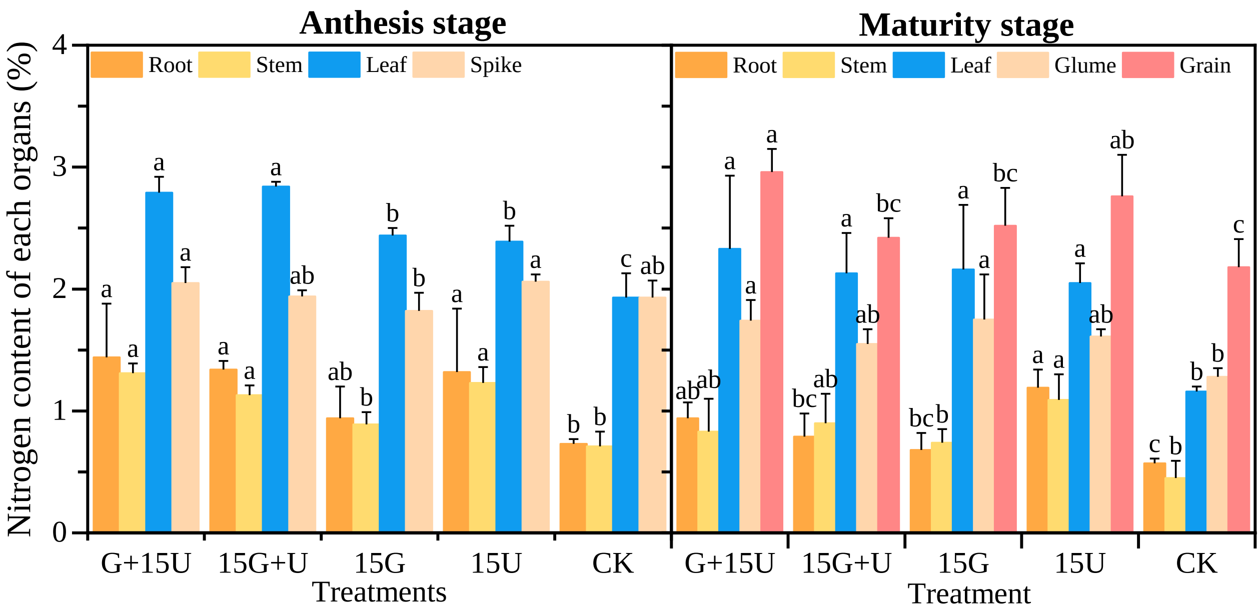

Figure 1. Nitrogen content (%) in various organs of wheat at the anthesis and maturity stages

N accumulation in various organs of wheat

As illustrated in Figure 2, the N accumulation in various organs continues to increase throughout the growth period. During the anthesis stage, the order of N accumulation in different organs is as follows: leaves > spikes > stems > roots. Among the different treatments, the G + ^{15}U treatment notably increased N accumulation by 86.5% and 227.8% compared to ^{15}G and CK, respectively, with no significant difference compared to ^{15}G + U and ^{15}U.

During the maturity stage, the N stored in various nutrient organs is transferred to the grains, with the order of accumulation being grains > leaves > stems > glumes > roots. The G + ^{15}U treatment exhibited the highest N accumulation in the grains, with a significant increase of 75.5% and 148.5% compared to ^{15}G and CK, respectively, while showing no significant difference compared to ^{15}G + U and ^{15}U. This highlights the effectiveness of the G + ^{15}U treatment in boosting N accumulation in wheat during both stages, resulting in a total N accumulation of 640.4 mg/pot, higher than that achieved by other treatments.

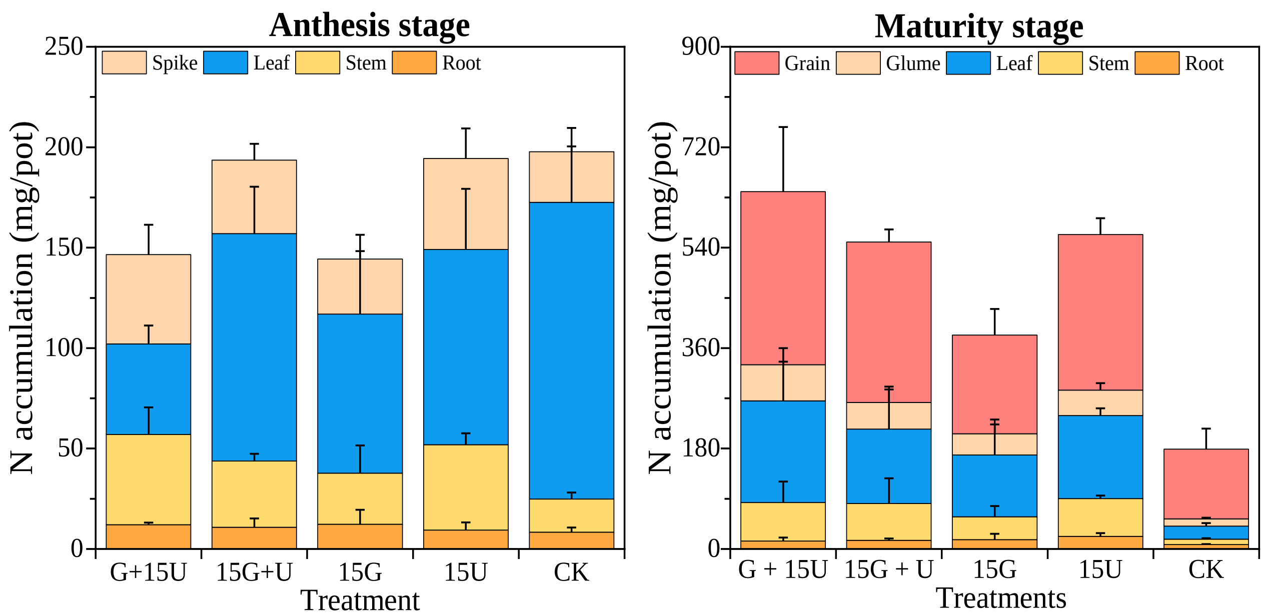

Figure 2. The nitrogen accumulation in various organs of wheat at the anthesis stage and maturity stage

Dynamic of N from chemical fertilizer and green manure in the soil-plant system

The N dynamics in the soil-plant system from chemical fertilizer and green manure is presented in Table 2. The observed trend reveals that the order of N utilization for fertilizer is plant absorption and utilization, soil residue, and loss, whereas for green manure, it is soil residue, loss, and plant absorption and utilization. Wheat plants exhibited significant differences in N absorption from different sources under varied treatments. N uptake from fertilizer ranged from 46.0% to 54.0%, while that from green manure ranged from 20.7% to 28.2%.

The residual rates of fertilizer and green manure N in the soil also exhibited significant differences among treatments. The ^{15}G treatment demonstrated notably higher residual rates compared to ^{15}G + U and G + ^{15}U, while the residual rate of ^{15}U treatment was significantly lower than other treatments. N loss rates also varied significantly among treatments, with the ^{15}G + U treatment showing a considerably higher loss rate compared to other treatments.

Furthermore, analysis of the G + ^{15}U, ^{15}U, and CK treatments revealed that the application of green manure, in conjunction with chemical fertilizer, improved N absorption and utilization by wheat plants. Similarly, comparing the ^{15}G + U, ^{15}G, and CK treatments, it was observed that the application of chemical fertilizer enhanced wheat's N uptake from green manure sources, thereby reducing N residue and loss. Moreover, a comparison between the ^{15}G + U and G + ^{15}U treatments indicated that when both fertilizer N and green manure N were supplied simultaneously, wheat theoretically absorbed 66.7-82.2% of N from both sources.

Table 2. Analysis of dynamic flow direction variance of N from chemical fertilizer and green manure in the soil-plant system

Treatment	Biomass(g/pot)	^{15}N absorption rate by plant (%)	^{15}N residual rate in the soil (%)	^{15}N loss (%)
G + ^{15}U	14.30 ± 2.41a	54.08 ± 3.63a	40.69 ± 1.25b	5.22 ± 4.04c
^{15}G + U	13.86 ± 4.24ab	28.21 ± 1.69c	38.47 ± 4.14b	33.31 ± 2.52a
^{15}G	9.61 ± 3.25bc	20.71 ± 4.18d	58.22 ± 4.64a	21.07 ± 0.53b
^{15}U	12.96 ± 1.36ab	46.00 ± 2.65b	31.44 ± 3.17c	22.56 ± 4.46b
CK	5.72 ± 1.69c	-	-	-

Note: Mean ± standard deviation, the significance level of the mean difference was 0.05.

Absorption of ^{15}N from chemical fertilizer or green manure by various organs of wheat

The combination of chemical fertilizer and green manure can effectively enhance the distribution ratio of N from both sources in wheat grains. During the anthesis period, N distribution in the stem and leaf organs is relatively high, while it is comparatively low in the root and spike (Table 3). The ^{15}G + U treatment shows the highest N distribution proportion in the roots, indicating that wheat roots primarily absorb N from green manure during the anthesis stage. The ^{15}G treatment results in the highest N distribution proportion in the stem, significantly higher than other treatments. There is no significant difference in N distribution in leaves among the different treatments. The G + ^{15}U and ^{15}U treatments exhibit the highest N distribution proportion in the panicle, suggesting greater absorption of fertilizer N by the panicle compared to green manure N.

At maturity stage, the N distribution ratio in roots remains consistent with the anthesis stage. Under the ^{15}U treatment, the proportion of N in the stem is significantly higher than in other treatments. The ^{15}G and ^{15}U treatments lead to significantly higher N distribution proportions in leaves compared to other treatments. There is no significant difference in N distribution in glumes between the two sources. The G + ^{15}U and ^{15}G + U treatments result in higher N distribution proportions in grains, significantly higher than other treatments. The ^{15}G + U treatment exhibits the highest N distribution proportion in grains, surpassing the ^{15}U and ^{15}G treatments by 15.46% and 22.09%, respectively. Overall, the combination of chemical fertilizer and green manure improves N absorption and increases the proportion of N distribution from both sources in wheat grains.

Table 3. Absorption of ^{15}N source from chemical fertilizer or green manure by various organs of wheat at the anthesis and maturity stages

Treatment	Anthesis stage (%)				Maturity stage (%)				
	Root	Stem	Leaf	Spike	Root	Stem	Leaf	Glume	Grain
G + ^{15}U	18.90 ± 0.68c	23.61 ± 0.76b	22.57 ± 0.51a	23.20 ± 0.49a	14.72 ± 0.70b	18.11 ± 1.06b	19.26 ± 0.78b	20.27 ± 0.73a	23.86 ± 0.67ab
^{15}G + U	32.22 ± 3.77a	20.18 ± 1.43c	21.47 ± 3.07a	16.15 ± 1.90c	16.45 ± 1.62b	17.24 ± 1.28bc	17.26 ± 1.35b	22.49 ± 0.84a	25.94 ± 0.37a
^{15}G	25.52 ± 3.94b	27.30 ± 1.87a	21.06 ± 1.75a	18.73 ± 1.89b	22.59 ± 3.28a	15.52 ± 1.14c	23.37 ± 3.87a	20.52 ± 3.51a	20.21 ± 2.30c
^{15}U	21.51 ± 0.63bc	24.02 ± 0.51b	24.24 ± 0.54a	23.38 ± 0.12a	16.68 ± 3.72b	20.10 ± 0.39a	21.13 ± 0.20ab	21.68 ± 0.55a	21.93 ± 0.26bc
CK	-	-	-	-	-	-	-	-	-

Note: Mean ± standard deviation, the significance level of the mean difference was 0.05, the nitrogen in plants includes two portions: the N sourced from chemical fertilizer and green manure.

Nitrogen accumulation and distribution from different sources in various organs of wheat

Nitrogen (N) regulation is essential for maintaining regular material circulation and energy metabolism in plants. N fertilizers serve various functions in the agro-ecosystem (Savasli et al., 2023), but their excessive use can disrupt the ecological balance due to N surplus (Ahmed et al., 2020). To mitigate these negative impacts, the combined application of chemical fertilizer with organic fertilizer has been explored (Zhang al., 2023). This study aimed to investigate the accumulation, distribution, and translocation of N in different organs of wheat at different growth stages following the incorporation of hairy vetch as green manure, with a focus on exploring the potential for utilization. We quantitatively analyzed the uptake and utilization of N from both chemical fertilizer and hairy vetch in the wheat plant system. The accumulation and translocation of N in wheat plants are influenced by dry matter accumulation in vegetative organs, including roots, stems, and leaves, before the anthesis stage, as well as the translocation of photosynthetic products from leaves to grains after the anthesis stage, which ultimately determine wheat yield (Fan et al., 2023).The coordinated distribution of N in different organs of wheat plants, including stems, leaves, glumes, and grains, is crucial for their normal development and growth. In this study, we investigated the N content and accumulation in wheat plants, with leaves showing the highest N content at the anthesis stage and grains having the highest N content at maturity. The ^{15}N content in the aboveground reproductive organs was significantly higher than that in the underground parts, and the N content in stems and leaves exhibited a declining trend. At the anthesis stage, leaves exhibited the highest N content among all organs. Similarly, at the maturity stage, the accumulation of N from chemical fertilizer and green manure in different wheat organs followed the order of grain, leaf, stem, glume, and root. The combined application of chemical fertilizer and green manure resulted in significantly higher N accumulation in various wheat organs compared to individual applications of the two fertilizers or no N fertilizer application. A previous study on green manure incorporation in spring wheat demonstrated that N fertilization combined with green manure incorporation increased the conversion rate of N from vegetative organs to grains, with a 38.8% to 49.5% increase in N translocation from leaves to the spike and a 49.1% to 64.7% increase in N translocation from stems to the spike (Guo and Yin, 2023).

In the present study, the application of green manure significantly increased the uptake of N by wheat plants. This led to the release of soil nutrients and improved retention of fertilizer nutrients (Ward et al., 2022). Organic fertilizers, such as green manure, play a significant role in enhancing plant nutrient levels while balancing the interaction between soil N supply and plant demand. This helps to regulate hormone metabolic processes, promote plant growth and development, and ultimately affect the translocation and distribution of N in plants (Qiu et al., 2022). Before the anthesis stage, leaves and stems serve as nutrient reservoirs, with continuous accumulation of nutrients in these organs. From the anthesis stage to the maturity stage, leaves and stems act as nutrient distribution centers, where nutrients and dry matter are translocated to reproductive organs, particularly grains, which become the primary nutrient accumulation centers. The study demonstrated that the combined application of chemical fertilizer and green manure can regulate the distribution of N in different wheat organs and facilitate the translocation of accumulated nutrients to reproductive organs.

The proportion of accumulated N of wheat from different sources

In this experiment, the fate of N applied to the soil was mainly categorized into three aspects: plant uptake and utilization, residues in the soil, and various forms of losses (Li et al., 2023). The performance of chemical fertilizer N was observed to prioritize plant uptake and utilization, followed by residues in the soil and losses. On the other hand, the performance of green manure N was ranked as residues in the soil, losses, and plant uptake and utilization. N utilization by plants involves two key processes: uptake and translocation. To improve N utilization efficiency in crops, it is important to understand the functions and regulatory mechanisms of these critical components. When there is an adequate supply of N, the efficiency of plant N use primarily relies on N uptake efficiency. However, under N deficiency, the efficiency of plant N use is primarily limited by N translocation (Gao et al., 2023). In the present experiment, the N supply was sufficient. The wheat plants exhibited an N uptake efficiency range of 46.0% to 54.1% for N sourced from chemical fertilizer, while the uptake efficiency for green manure N ranged from 20.71% to 28.21%.

Using the ^{15}N tracer technique, researchers have found that when organic and chemical fertilizers are applied together, plants tend to initially absorb the N from chemical fertilizers. N is taken up by the roots, transported to the aboveground parts of the plant, and then redistributed back to the roots (Shao et al., 2020). In the present study, the analysis of N accumulation and distribution in various wheat organs revealed a consistent pattern of N being transported from the bottom to the top of the plant, with continuous translocation to reproductive organs. Studies by Liu et al. (2020) using isotopes found that intercropping wheat with green manure increased total N accumulation in plants, with 11.4% to 31.6% of the absorbed N coming from green manure. In a study, Zhang et al. (2022) observed that plants absorbed 25.46% to 40.99% of the total fertilizer N. Similarly, Somers et al. (2019) discovered that soil was the primary provider of N and the largest source of N supply for plants. Their results showed that 18.1% to 33.7% of the N uptake by plants was from chemical fertilizer, while 66.4% to 81.9% was derived from soil N. In a study (Azeem et al., 2023) found that the uptake of fertilizer N and utilization efficiency of the plant was in the range of 45.0–60.0%. In China, the uptake of N fertilizer and utilization efficiency of wheat and other food crops is usually in the range of 40-50% (Yang et al., 2012). The fertilizer uptake efficiency obtained under the treatment conditions in the present experiment ranged from 20.21 to 54.08%, which was higher than that reported by Zhang et al. (2021). N uptake and utilization by wheat may be influenced by genetics and various anthropological and environmental factors (Guo et al., 2022). The regulatory effects and mechanisms of nutrient uptake, utilization, and translocation capacity of wheat vary among different ecotype regions. In this study, the wheat harvest index was in the range of 48-69%, which was much higher than the national average wheat harvest index of 0.440 (Xu et al., 2015).

In this study, the loss rate of N from green manure was found to be between 21.07% and 33.31%, while the loss rate of chemical fertilizer N ranged from 5.22% to 22.56%. However, when the two sources of N were applied together, the loss rate dropped to 5.22%, which was lower than the results of other studies (Zhang et al., 2023). The reduced loss rate in this study can be attributed to the pot experiment, which minimized losses associated with surface runoff and leaching. Overall, when green manure and chemical fertilizer were combined, the loss rate of N from both sources was reduced. The residual rate of N from green manure in the soil ranged from 38.47% to 58.22%, while for chemical fertilizer N, it ranged from 31.44% to 40.69%. N from green manure is released slowly into the soil, contributing to a significant aftereffect (El-Shamy et al., 2022). Consequently, the combined application of chemical fertilizer and green manure promotes nutrient uptake and utilization by the current crop season and provides a continuous supply of nutrients to subsequent crops. This helps retain nutrients within the soil system, thereby improving the soil environment and fertility.

CONCLUSIONS

In terms of performance, for nitrogen from chemical fertilizer, it was ranked as plant uptake and utilization, residues in the soil, and losses. On the other hand, for nitrogen from green manure, the performance was ranked as residues in the soil, losses, and plant uptake and utilization. By applying the two sources of nitrogen together, the distribution of nitrogen into different plant organs can be coordinated, and the distribution ratio of nitrogen in grains can be increased. This approach also promotes the source-sink relationship between the soil and plants, facilitating the translocation of nutrients to the reproductive organs. The uptake of nitrogen by wheat from chemical fertilizer ranged from 46.0% to 54.1%, which was higher than the uptake from green manure (ranging from 20.7% to 28.2%). Interactions between the nitrogen from the two sources were observed. This study demonstrated that applying chemical fertilizer with green manure can enhance the uptake and utilization of nutrients in wheat plants.

ACKNOWLEDGEMENTS

This work was supported by the Fund Project of Qinghai Academy of Agricultural Sciences (2019-NKY-06) and the China Agriculture Research System of MOF and MARA (Green manure) [grant number CARS-22]. The authors thanks Mrs. Mei Han, Ke Xu, Mingdan Song and Mr. Zhengpeng Li , Qingbiao Yan for guiding the papers, and other students for assistance the trial. They also appreciate the teachers and staff at Xining Comprehensive Experimental Station of National Green Manure Industry System in managing field plots.

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