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Dynamics of Post-Flowering Nitrogen Uptake and Nitrogen Recovery Efficiency Using 15N Isotope Labeling in Corn

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Abstract

In corn (*Zea mays* L.), breeding and selection for grain yield over time has been accompanied by a simultaneous increase in plant nitrogen (N) uptake. The understanding of plant N dynamics has attracted attention due to the environmental concerns related to N losses coming from fertilization. This research study was implemented to 1) describe N uptake and allocation dynamics, and 2) quantify fertilizer recovery efficiency across late-N strategies. Two field experiments (one under irrigation and one rainfed) were conducted at the Ashland Bottoms Research Farm, KS, during 2017. Three hybrids with different year of release and three N scenarios were tested. Isotope ^{15}N was utilized as tracer to determine ^{15}N recovery and N fate within plant organs when both timings of late-N were evaluated. As ^{15}N fertilizer was applied later in the season, lower recovery of the fertilizer was achieved and proportionally more N was allocated to the developing grains. These findings can motivate future investigations using ^{15}N labelling technique to evaluate fertilizer recovery efficiency in corn.

Keywords

maize, nitrogen uptake, post-flowering, fertilizer recovery efficiency, corn

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Summary

In corn (*Zea mays* L.), breeding and selection for grain yield over time has been accompanied by a simultaneous increase in plant nitrogen (N) uptake. The understanding of plant N dynamics has attracted attention due to the environmental concerns related to N losses coming from fertilization. This research study was implemented to 1) describe N uptake and allocation dynamics, and 2) quantify fertilizer recovery efficiency across late-N strategies. Two field experiments (one under irrigation and one rainfed) were conducted at the Ashland Bottoms Research Farm, KS, during 2017. Three hybrids with different year of release and three N scenarios were tested. Isotope ^{15}N was utilized as tracer to determine ^{15}N recovery and N fate within plant organs when both timings of late-N were evaluated. As ^{15}N fertilizer was applied later in the season, lower recovery of the fertilizer was achieved and proportionally more N was allocated to the developing grains. These findings can motivate future investigations using ^{15}N labelling technique to evaluate fertilizer recovery efficiency in corn.

Introduction

Over time, breeding and selection for grain yield in corn has been accompanied by a simultaneous increase in N uptake (Ciampitti and Vyn, 2012; Haegele et al., 2013). The understanding of plant N dynamics has attracted attention due to the environmental concerns related to N losses coming from fertilization. Although late-season N-fertilization could be used as an alternative to synchronize N supply and demand, benefits of delayed N application strategies have not been consistently reported.
A recent meta-analysis provided evidence for a lack of a repeatable effect of late N application on yield (Fernandez et al., 2020), but specifically quantified when late N might improve yield. Therefore, studies on post-flowering N uptake are necessary in order to warrant an efficient utilization of N. Integration of physiological indicators within the plant can help us identify productive opportunities to realize suitable fertilization strategies. The objectives of this study are to 1) describe N uptake and allocation dynamics, and 2) quantify fertilizer recovery efficiency across late-N strategies.

Procedures

Field Experiments

Two field experiments (one under irrigation and one rainfed) were conducted at the Ashland Bottoms Research Farm, Manhattan, KS, 2017 (39°08' N, 96°37' W). Soil

analyses were conducted at pre-planting to characterize initial conditions. Overall, the area presented pH of 5.9, soil organic matter (SOM) 1.34%, 50 ppm of phosphorus (P) (Mehlich), and 158 ppm of potassium (K) at 6-inch soil depth. Table 1 presents climatic data for the growing season.

The experimental design consisted of a split plot design with two factors evaluated: genotype with three levels in the main plot, and fertilizer N rate with three levels in the sub-plot. Three hybrids with different year of release (3394, 1991; P1151, 2005; and P1197, 2014) and three N scenarios (zero N, N0; fertilized with N at R1 - flowering, NL1; and fertilized with N two weeks after R1, NL2) were tested in both studies. The studies were planted on May 5, 2017, in plots of 4 rows, 30 in. apart, and size of 10-ft wide × 70-ft long. For the two fertilized treatments, an initial 50 lb/a was added at planting, and a second application was added at V6 growth stage (50 lb/a and 100 lb/a for dryland and irrigated, respectively). Depending on the treatment, the last application (22 lb/a and 44 lb/a for dryland and irrigated, respectively) was performed at flowering (R1; Ritchie et al., 1997) or two weeks after R1. Total fertilizer N rate applied for the treatments receiving N was 122 lb/a for the rainfed and 194 lb/a for the irrigated condition. The experimental area was kept free of weeds, pests, and diseases during the growing season.

Isotopic Labeled Fertilizer Application and Calculation of ^{15}N Abundance

Isotope ^{15}N was utilized as tracer to determine ^{15}N recovery and N fate within plant organs when both timings of late-N were evaluated. For this evaluation, two 5-plant microplots, one for each ^{15}N-timing, were established within each experimental unit in order to trace the fate of N at R1 and two weeks after R1. Labeled fertilizer $Ca(NO_3)_2$ (10.15% ^{15}N) at 1 g per plant was applied with plastic syringes on both sides of the plants after diluting in 30 mL of water. Fertilizer was injected using the methodology employed in de Oliveira Silva et al. (2017), and the three center plants from each microplot were harvested five days after the ^{15}N application. Additionally, non-enriched plants were sampled to determine the background ^{15}N abundance in the fertilized and unfertilized soils, in order to account for possible small variations in the standard values of natural ^{15}N abundance (Cabrera and Kissel, 1989; Högberg, 1997). Plants were separated into leaves (leaf blades), stem (stems + leaf sheaths + tassels), ear (husks + cobs), and grain fractions; after that, samples were dried at 150°F until constant weight, and then ground through a 0.10 mm sieve for laboratory analyses. Nitrogen content and ^{15}N abundance were determined using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS) at the Kansas State University Stable Isotope Mass Spectrometry Laboratory.

For each plant fraction, the atom percentage excess [At% (^{15}N)Excess] was calculated using the following equation:

$$\text{At\% } (^{15}N)\text{Excess} = \text{At\% } (^{15}N)_{\text{sample}} - \text{At\% } (^{15}N)_{\text{control}}$$

, where At% $(^{15}N)_{\text{sample}}$ represents the percentage of ^{15}N abundance in the ^{15}N labeled samples, and At% $(^{15}N)_{\text{control}}$ corresponds to the percentage of ^{15}N abundance in non-labeled control plants.

Total ^{15}N uptake expressed in lb/a was estimated by the following equation:

$$^{15}N \text{ uptake} = N \text{ uptake} \times \left(\frac{\text{At\% } (^{15}N) \text{ Excess}}{100} \right)$$

, where N uptake per plant fraction is expressed in lb/a.

^{15}N uptake rate expressed in lb/a/°C day was obtained as follows:

$$^{15}N \text{ uptake rate} = \frac{^{15}N \text{ uptake}}{\text{Thermal time between fertilizer application and sampling}}$$

Lastly, ^{15}N recovery lb/lb was calculated according to the following equation:

$$^{15}N \text{ recovery} = \frac{^{15}N \text{ uptake}}{^{15}N \text{ applied}}$$

, where ^{15}N applied denotes the amount of N applied (lb/a) multiplied by At% (^{15}N) $\text{Excess}_{\text{fertilizer}}$.

Statistical Analyses

Data were subjected to an analysis of variance (ANOVA) for each studied trait at the 2017 sites. Mixed effects models were fitted to the data using R program (version 3.6.1) in RStudio interface (RStudio Team, 2016). We combined the data from both studies (irrigated and dryland) and accounted for the study difference by including a site-level random effect. Adjustments on the distributional assumption of the residuals were taken into consideration for model fitting. Homogeneity of error variances was verified by plotting the residuals and fitted values. Significance of the factors were tested via ANOVA Type 3 tests using the *car* (Fox and Weisberg, 2019) package. Differences between the mean values of each treatment were determined by the LSD Fisher test (alpha = 0.05) with *emmeans* (Lenth, 2019) package.

Results

Non-significant interactions between factors for yield and numerical components allowed for inferences at a marginal mean level. Figure 1 summarizes the average yield for N fertilization levels (N) and corn hybrids (H) evaluated in the 2017 experiment. Differences in yield were significant between N and H treatments ($P < 0.05$). Fertilized treatments differed from the zero N treatment. However, the two weeks delay of the last N application did not cause significant yield variations across these hybrids. Comparing genotypes, grain yield increased with year of introduction of each hybrid and, accordingly, the modern material (P1197, 206 bu/a) outyielded the older genotype (3394, 177 bu/a).

Figure 2 summarizes estimates for ^{15}N uptake rate (A) and (B), and for ^{15}N fertilizer recovery (C) and (D) across fertilizer N rate levels in the experiment at R1 and two weeks after R1. No evidence for interactions between sampling time, N, and genotypes were detected for ^{15}N uptake rate, allowing for inferences at a marginal mean level

for each of the factors. In this way, differences in uptake rate were significant across sampling time ($P < 0.001$), but not for N treatments (Figure 2A and B).

Regarding ^{15}N recovery (at physiological maturity), a significant 2-way interaction between sampling time and N treatment was observed, so pairwise comparisons were performed across N levels within each sampling time. At R1, greater recovery efficiency was identified for both fertilized treatments when compared to the control without applied N (Figure 2C). In parallel, a significant increase over the zero N control was observed at mid-grain filling only when N was delayed two weeks after R1 (Figure 2D). Respecting hybrids, no evidence for differences was detected in fertilizer recovery efficiency ($\alpha = 0.05$). However, it is important to consider that for this experiment reduced statistical power for H factor (whole plot) might be restraining inferences at this level.

Plant dynamics of N absorbed from R1 to maturity were quantified with ^{15}N fertilizer to investigate whether different N fertilization strategies have altered the fate and efficiency of N absorbed during the reproductive period. The proportion of ^{15}N uptake partitioned into leaves, stem, ear (cob + husk), and grains for each N treatment are represented in Figure 3. Nitrogen partitioning at physiological maturity allow us to conclude that plants differed in the allocation of post-flowering N depending on the supply of N. However, no changes were identified between late-season fertilization treatments. When N was applied, hybrids were proportionally more efficient in the allocation of post-flowering N towards the grains (Figure 3D). This difference was principally related to a reduced conservation of N in cob and husks (Figure 3C). In addition, a significant percentage of N from post-flowering uptake was present in stem tissue at maturity (from 0.12 to 0.13 lb/lb N absorbed, Figure 3B). Ning et al. (2017) concluded that this N retention in stems could constrain N utilization efficiency, especially under high N supply.

The current study proposes the utilization of isotope ^{15}N as tracer to describe N dynamics among hybrids under a late-N fertilization strategy. Results showed that as N fertilizer was applied in later reproductive stages, lower recovery of the fertilizer was achieved. These results acquire relevance considering that both yield and total N uptake were not affected. Under these conditions, we can expect a greater proportion of N demand to be covered by the soil N pool, instead of N coming from fertilizer. In addition, proportionally more N was allocated to the grain as fertilization was applied in the crop, in detriment to its distribution to cob and husk organs. Overall, these outcomes can motivate future investigations using isotope ^{15}N technique to span a wider range of historical hybrids and environmental conditions in order to improve the utilization of N in corn and the N environmental footprint in agricultural systems.

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Table 1. Monthly values for daily solar radiation, temperature, and total precipitation for the 2017 growing season

	May	June	July	August	September
Solar radiation (MJ m^{-2} day^{-1})	25.2	27.3	26.5	23.0	18.5
Mean temperature (°F)	65.8	75.4	80.4	72.1	72.0
Precipitation (inches)	3.74	2.82	1.33	6.09	0.81

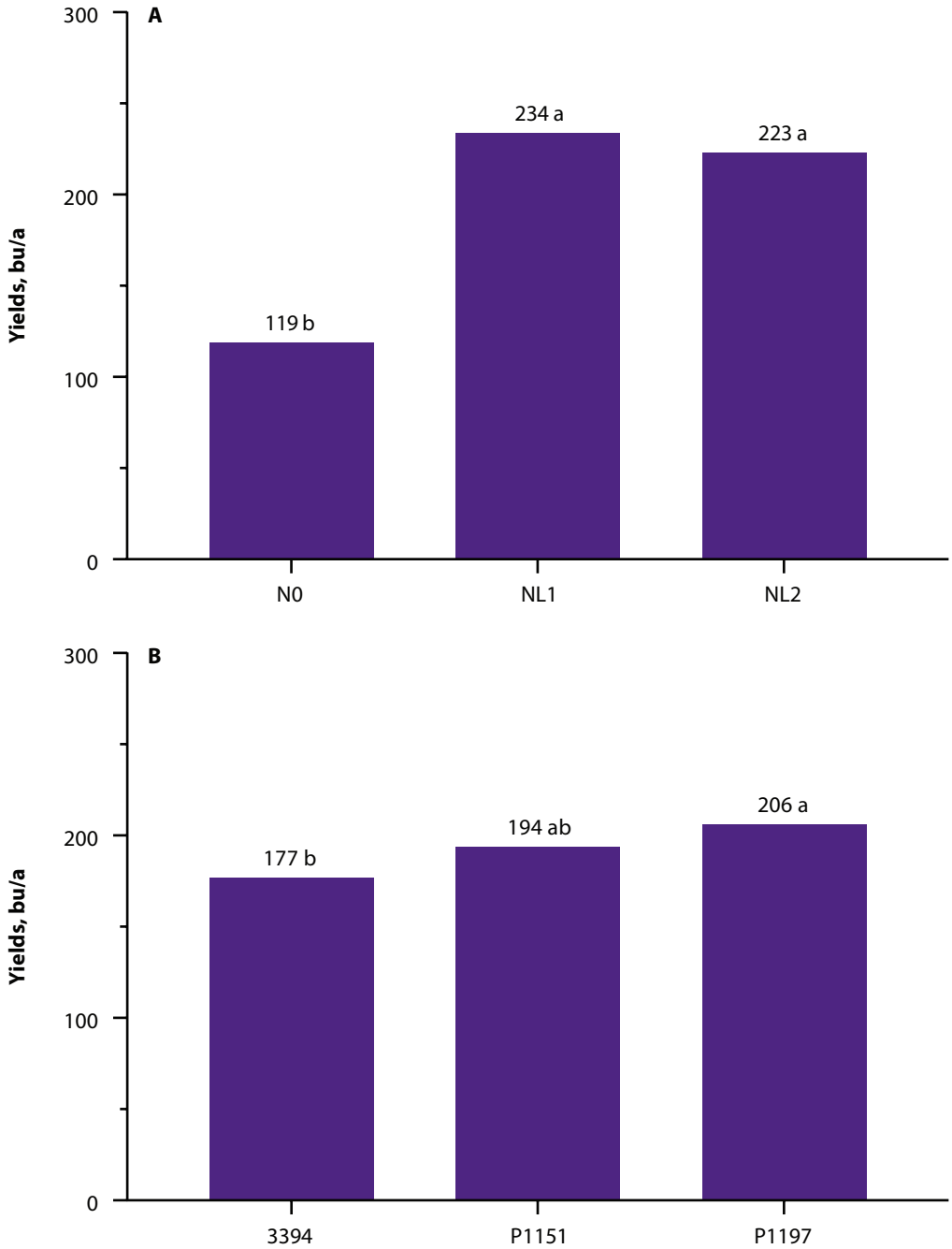

Figure 1. Analysis of variance and means for yield (15.5% moisture) for three nitrogen (N) treatments (A) and three hybrids (B). Different letters indicate significant differences at $P \leq 0.05$.

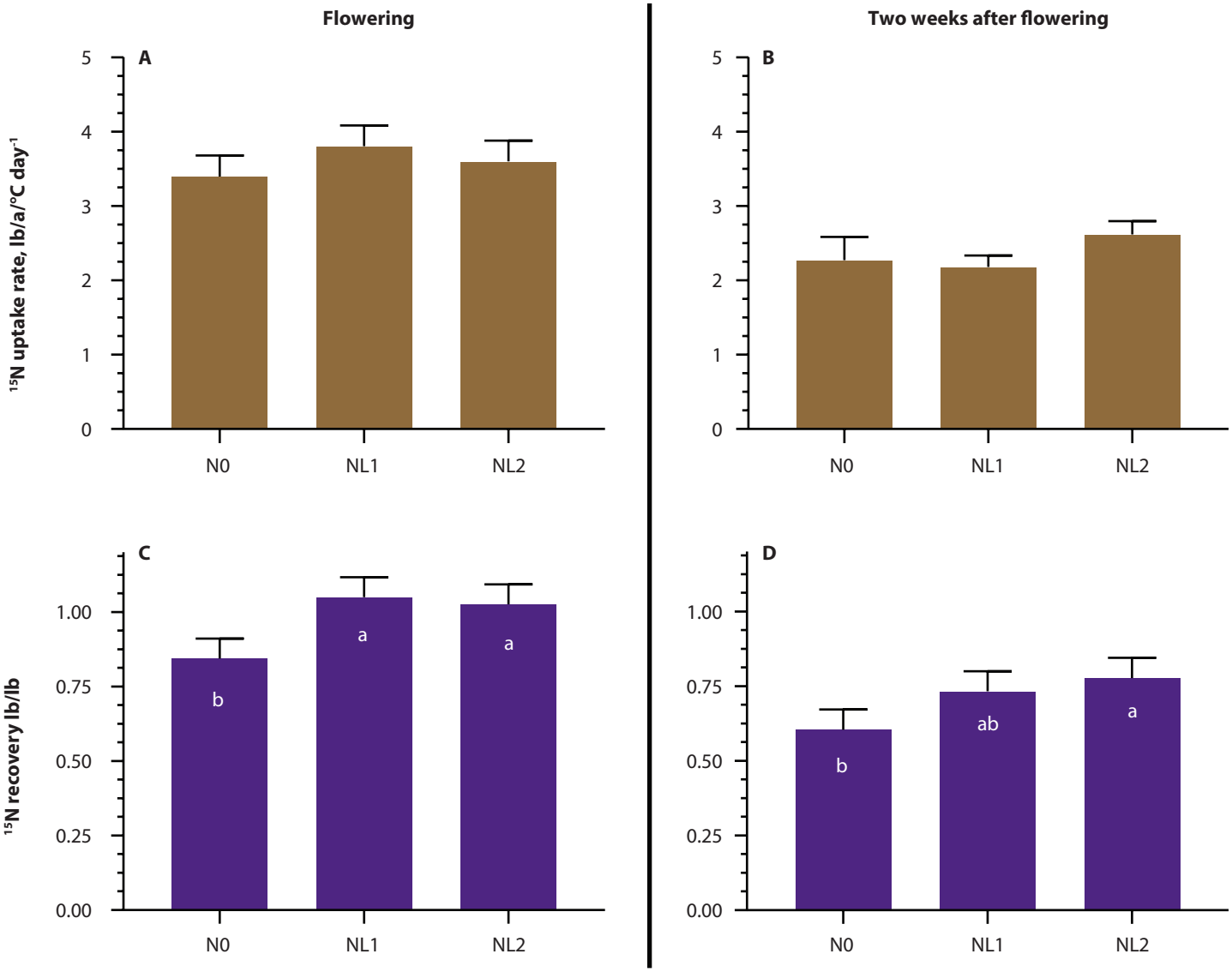

Figure 2. Least-squares estimates for ^{15}N uptake rate (A and B), and for ^{15}N recovery (C and D) for three N treatments. Variables were equally measured at flowering (left section) and two weeks after flowering (right section). Isotope ^{15}N recovery was measured at physiological maturity (R6). Different letters indicate significant differences at $P \leq 0.05$.

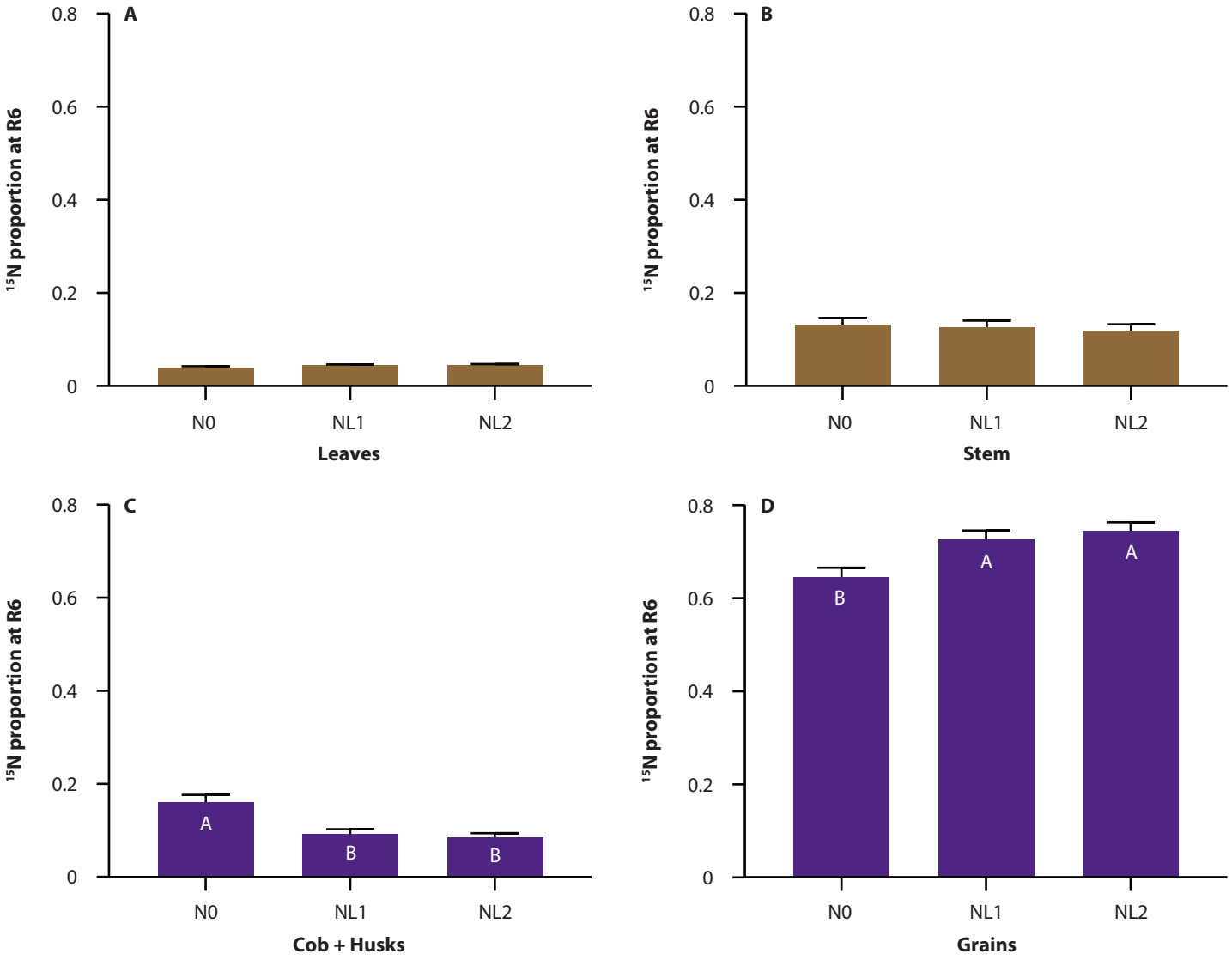

Figure 3. Nitrogen allocation of ^{15}N absorbed at flowering stage (R1) across three N fertilization treatments in 2017. Relative proportion at maturity of ^{15}N applied at R1 stage to leaves (A), stem (B), cob + husks (C), and grains (D). Bars represent estimates averaged across genotypes, and whiskers the standard errors (SE) of the mean. Different letters indicate significant differences at $P < 0.05$.