1 **Dynamic change of mineral nutrient content in different plant organs**
2 **during the grain filling stage in maize grown under contrasting**
3 **nitrogen supply**

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ABSTRACT

The introduction of new hybrids and integrated crop-soil management have been causing maize grain yield to increase. However, less attention has been paid on the nutrient concentration of the grain; this aspect is of great importance to supplying calories and nutrients in the diets of both humans and animals worldwide. Increasing the retranslocation of nutrients from vegetative organs to grain can effectively increase the nutrient concentration of grain and general nutrient use efficiency. The present study involved monitoring the dynamic change of macro- and micronutrients in different organs of maize during the grain filling stage. In addition, the mobility of different elements and their contribution to grain nutrient content were evaluated in a 2-year experiment under low (LN, no N supplied) and high N (HN, 180 kg N ha⁻¹) supply. Under HN supply, the net remobilization efficiency (*RE*) of the vegetative organs as a whole (calculated as nutrient remobilization amount divided by nutrient content at silking) of N, P, K, Mn, and Zn were 44%, 60%, 13%, 15%, and 25%, respectively. The other nutrients (Mg, Ca, Fe, Cu, and B) showed a net accumulation in the vegetative organs as a whole during the grain filling stage. Among the different organs, N, P, and Zn were remobilized more from the leaves (*RE* of 44%, 51% and 43%, respectively) and the stalks (including leaf sheaths and tassels) (*RE* of 48%, 71% and 43%, respectively). K was mainly remobilized from the leaves with *RE* of 51%. Mg, Ca, Fe, Mn, and Cu were mostly remobilized from the stalks with the *RE* of 23%, 9%, 10%, 42%, and 28%, respectively. However, most of the remobilized Mg, Ca, Fe, Mn, Cu, and Zn were translocated to the husk and cob, which seemingly served as the buffer sink for these nutrients. The *REs* of all the nutrients except for P, K, and Zn were vulnerable to variations in conditions annually and were reduced when the grain yield and harvest index were lower in 2014 compared with 2013. Under

LN stress, the *RE* was reduced in P and Zn in 2013, increased in Cu and unchanged in other nutrients. The concentration of these nutrients in the grain was either unchanged (P, K, Ca, Zn, and B) or decreased (N, Mg, Fe, Mn, and Cu). It is concluded that grain N, P, K, Mn, and Zn, but not Mg, Ca, Fe, Cu, and B concentration, can be improved by increasing their remobilization from vegetative organs. However, enhancing the senescence of maize plant via LN stress seems unable to increase grain mineral nutrient concentration. Genetic improvement aiming to increase nutrient remobilization should take into account the organ-specific remobilization pattern of the target nutrient.

Keywords: Grain nutrient concentration; Low nitrogen; Maize; Mineral nutrient; Remobilization; Vegetative organs

1. Introduction

As a valuable staple food worldwide, maize provides proteins, carbohydrates, and minerals for human beings and animals. During the past several decades, maize breeding has resulted in dramatically increased yield while grain protein and mineral nutrient concentrations have decreased (Duvick and Cassman, 1999; Anandan et al., 2011; Chen et al., 2013). The nutritional value of food is of great importance to human and animal health (Grusak and DellaPenna, 1999). For example, Fe and Zn deficiencies can cause impairments of the immune system and increases in anemia (Cakmak et al., 2004). In addition, the concentration of the nutrients in maize seed greatly affects the ability of a seedling to tolerate various biotic (such as diseases) and abiotic stresses (such as mineral nutrient deficiency) (Masclaux-Daubresse et al., 2010). Thus, the primary target for genetic improvement is increasing the mineral nutrient concentration of maize grain.

Nutrients in maize grain are derived from post-silking uptake and the remobilization of nutrients accumulated in vegetative organs during the pre-silking stage (Hirel et al., 2007). While post-silking nutrient uptake depends on soil nutrient availability and may require additional nutrient input, increasing nutrient remobilization from vegetative organs can make the best use of nutrients already in the plant to increase grain nutrient content, and thus increase nutrient use efficiency (Masclaux-Daubresse et al., 2008, 2010). In addition, the capacity of maize to redirect nutrients from vegetative organs to grain would be even more important when nutrient uptake is severely restricted during the grain filling stage because of biotic and/or abiotic stresses (Marschner, 1993). In maize, 25–82% of grain N is derived from the remobilization of vegetative N accumulated before silking (Ta and Weiland, 1992; Ma et al., 1998; Mi et al., 2003; Lemaire

and Gastal, 2009; Chen et al., 2014). The contributions of vegetative P and K to the grain are 1–75% and 45–447%, respectively (calculated from the results of Ning et al., 2013). In contrast, the contribution to grain from the remobilization of most micronutrients such as Fe, Mn, Cu, and B seems very low (Karlen et al., 1988; Brown and Shelp, 1997; Bender et al., 2013). The remobilization efficiency (*RE*) may be different among different vegetative organs. For example, Chen et al. (2014) found that the *REs* of N in the stalks and leaves were 49–55% and 65–70%, respectively. P and K were also remobilized more intensely from the leaves than from the stalks (Shi, 2010; Ning et al., 2013). Mg and Ca were remobilized only from old leaves, while Fe, Mn, and Zn were only remobilized from stalks under high N supply (Grzebisz et al., 2008; Shi, 2010; Bender et al., 2013). Less information is available about whether the remobilized nutrients from various organs are directly translocated into the grain or have been redistributed into other organs.

Nitrogen nutrition plays an essential role in plant senescence, and therefore may greatly affect the remobilization of various nutrients from vegetative organs. Low-N stress may increase, or does not have an effect on the remobilization of N from vegetative organs (Ta and Weiland, 1992; Mi et al., 2003; Chen et al., 2014), but reduces the redistribution of P from vegetative parts into the grain (Venekamp et al., 1986). The transport of many metal ions (Fe, Mn, Cu, and Zn) depends greatly on chelates such as organic acids, nicotianamine, and mugineic acids etc. (Marschner, 1995; Curie et al., 2009; Sperotto et al., 2012). Nitrogen deficiency may possibly affect the remobilization of these elements by regulating the synthesis of the nitrogenous chelates.

Few studies have investigated the remobilization characteristics of all mineral nutrients simultaneously. Further, the analysis of nutrient remobilization has been largely focused on the net change of the nutrients in vegetative organs as a whole between silking and maturity. However,

the net change of the content of a nutrient in the vegetative organs as a whole may mask the behavior of that nutrient in different organs. A nutrient remobilized from an organ may not necessarily be redistributed into the grain. Knowing the dynamic changes of various nutrients content in different vegetative organs (leaves, stalks, and husk + cob) is essential for understanding the key organs and timing of the remobilization of different nutrients, as well as their contribution to the nutrient content of grain, which is important for genetic improvement related to increasing grain mineral nutrition. Additionally, the relationship between N nutrient-mediated leaf senescence and the remobilization of various nutrients needs to be elucidated, because of the central role of N management on maize growth and yield formation. In the present study, the dynamic changes in the content of N, P, K, Mg, Ca, Fe, Mn, Cu, Zn, and B in the leaves, stalk, husk + cob and grain were monitored in field-grown maize under low and high N input levels. The aim of this work was to understand the spatial and temporal behaviors of these nutrient elements within maize plants during the grain filling stage in relation to grain nutrient concentrations under contrasting N supplies.

2. Materials and methods

2.1. Experimental site

A field experiment was conducted during the 2013 and 2014 growing seasons at Shangzhuang Experimental Station of China Agricultural University, Beijing (116°11'N, 40°8'E). The typical Ustochrept soil had a silty loam texture with soil chemical properties of the 0–30cm soil layer at the beginning of the experiment as follows: organic matter 9.09 g kg⁻¹, CaCl₂-extracted mineral N (N_{min}) 3.0 mg kg⁻¹, Olsen-P 22.53 mg kg⁻¹, NH₄OAc extracted K

108.17 mg kg⁻¹, and pH (H₂O) 8.2. Fig. 1S shows weekly precipitation, mean daily temperature and sunshine. Total precipitation during the maize growing season was 495.2 mm in 2013 and 484.5 mm in 2014. In 2014, less rain fell around silking stage (during the 11–13th weeks after sowing) than in 2013; therefore, irrigation was conducted on 11 July to alleviate drought stress. Plots were not irrigated in 2013.

2.2. Experimental design

The experiment applied a randomized block design with four replicates. Each 9 m × 15 m plot had 25 rows, each 9 m long, that were spaced 60 cm apart. Plots were fertilized with 135 kg ha⁻¹ P₂O₅ (as superphosphate) and 100 kg ha⁻¹ K₂O (as potassium chloride) before sowing as base fertilizer. Two N fertilization rates were used, using urea as N fertilizer: (1) no N application (LN); (2) 180 kg N ha⁻¹ (HN), half applied before sowing as base fertilizer and the other half applied at the V6 (six expanded leaves) stage as top dressing. The experimental field had been previously used to grow maize continuously. The N treatments were continued in the plots for both years of the current experiment.

The plots were over-seeded with hand planters and subsequently thinned during the seedling stage to achieve a final plant density of approximately 60,000 plants ha⁻¹ and plant spacing of 28 cm within the rows. The field was irrigated and plowed prior to sowing. The base fertilizer was sprinkled on the ground surface before plowing. The N top dressing was hand applied in a diffuse band between the rows at the V6 stage (the 6th leaf expanded) before a rain event. Seeds were sown on the 28 April 2013 and 29 April 2014 and harvested on the 7 September 2013 and 3 September 2014. The plots were kept free of weeds, insects, diseases during the maize growing

season.

2.3. Plant sampling and tissue nutrient analysis

At silking, plants with the same silking date were tagged in each plot for further sampling. Three representative plants were sampled at each of following growth stages: during the silking stage, 10 days after silking (DAS), and 20, 30, 40, and 50 DAS (R6). The silking stage was determined when at least 50% of the plants within a plot exhibited exposed silk. The stage of physiological maturity was determined when at least 50% of the plants exhibited a visible black layer at the base of the kernels. Plant samples were separated into four components: 1) stalk (including stem, leaf sheaths and the tassel), 2) leaves, 3) husk + cob, and 4) grain. All samples were heat-treated at 105°C for 30 min, dried at 70°C to a constant weight, weighed to obtain the dry weight (DW), and then ground into fine powder (passed through a 2-mm mesh screen). Appropriate amounts of ground plant dry matter (DM) were used to determine the N, P, K, Mg, Ca, Fe, Mn, Cu, Zn, and B concentrations. Nitrogen was analyzed by a micro-Kjeldahl procedure after digestion with H₂SO₄-H₂O₂ (Nelson and Sommers, 1973). Other nutrients were analyzed using a two-part process of HNO₃-H₂O₂ microwave digestion (CEM, Matthews, NC, USA) followed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, OPTIMA 3300 DV, Perkin-Elmer, USA) (Zarcinas et al., 1987). For yield and component measurements, 15 intact rows of each plot were hand harvested and the number of harvested plants was counted. Grain yields were expressed as grams per plant with no moisture content included. In addition, 100-grain weights and grain number per ear were also determined.

2.4. Calculations

Based on the measured plant dry weight (DW) and nutrient content, the following parameters were calculated (Chen et al., 2013; Chen et al., 2014). The equations of (1) to (6) are suitable for both one specific vegetative organ and the total vegetative organs (leaves, stalks, and husk + cob) for DM or each nutrient.

$$\text{Dry matter (DM) remobilization amount} = \text{DW at silking} - \text{DW at maturity}; \quad (1)$$

$$\text{DM remobilization efficiency (\%)} = (\text{DW at silking} - \text{DW at maturity}) / \text{DW at silking} \times 100; \quad (2)$$

$$\begin{aligned} \text{Apparent contribution to grain DM by DM remobilization (\%)} &= (\text{DW at silking} - \text{DW at maturity}) \\ &/ \text{grain DW at maturity} \times 100; \end{aligned} \quad (3)$$

$$\text{Nutrient remobilization amount} = \text{nutrient content at silking} - \text{nutrient content at maturity}; \quad (4)$$

$$\begin{aligned} \text{Nutrient remobilization efficiency (\%)} &= (\text{nutrient content at silking} - \text{nutrient content at maturity}) \\ &/ \text{nutrient content at silking} \times 100; \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Apparent contribution to grain nutrient by nutrient remobilization (\%)} &= (\text{nutrient content at} \\ &\text{silking} - \text{nutrient content at maturity}) / \text{grain nutrient content at maturity} \times 100; \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Nutrient accumulation amount after silking} &= \text{total nutrient content at maturity} - \text{total nutrient} \\ &\text{content at silking}; \end{aligned} \quad (7)$$

$$\begin{aligned} \text{Contribution to grain nutrient by nutrient accumulation after silking (\%)} &= (\text{total nutrient content} \\ &\text{at maturity} - \text{total nutrient content at silking}) / \text{grain nutrient content at maturity} \times 100; \end{aligned} \quad (8)$$

$$\text{DM harvest index (HI; \%)} = \text{grain DW at maturity} / \text{total DW at maturity} \times 100; \quad (9)$$

$$\begin{aligned} \text{Nutrient harvest index (\%)} &= \text{grain nutrient content at maturity} / \text{total nutrient content at maturity} \times \\ &100. \end{aligned} \quad (10)$$

For DM or nutrient remobilization (equation 1, 2, 4, and 5), a negative value indicates net

accumulation of DM or the nutrient in a certain organ. For the contribution to the grain (equation 3, 6, and 8), higher values than 100 indicate that nutrient remobilization amount exceeded grain demand, while negative values indicate that nutrient remobilization has no contribution to the grain.

2.5. Statistical analysis

Statistical analysis was accomplished using SPSS Statistics 17.0 (SPSS, Inc., Chicago, IL, USA). All treatment factors (N and year) were considered as fixed factors. After verifying the homogeneity of error variances, the experimental data were analyzed by a two factor variance analysis program and the differences were compared using the least significant difference (LSD) test at a 0.05 level of probability. Significant differences of the remobilization and post-silking accumulation (Tables 4 and 5) between the two N treatments in 2013 and 2014 were determined using Student *t* test and indicated by *** ($P < 0.001$), ** ($P < 0.01$), and * ($P < 0.05$). The analysis was mainly focused on the effects of N application. Unless necessary, the year effect was not analyzed.

3. Results

3.1. Grain yield and grain nutrient composition

Significant differences in grain yield (GY), grain number, and 100-grain weight were found between N treatments (Table 1). Of the two treatments, LN stress reduced GY, grain number, and 100-grain weight by 24%, 25%, and 14%, respectively. No difference in harvest index (HI) was found between the two N treatments. However, the GY and HI in 2014 were lower than in 2013.

The N $\times$ year interaction was not significant for all these parameters.

The concentrations of N, Mg, Fe, Mn, and Cu in the grain decreased by 21%, 14%, 23%, 24%, and 21%, respectively, in the LN compared with the HN treatment (Table 2). The concentration of other nutrients in the grain was not affected by N. The N $\times$ year interactions for the grain concentration were significant for P, K, Mg, and B. The grain concentration of P and Mg decreased by 13% and 21%, respectively, in the LN treatment in 2014, while no significant difference was observed in 2013. In contrast, grain B concentration decreased by 27% and K increased by 11% in LN in 2013 and there was no significant difference in 2014.

3.2. Remobilization of DM and the nutrients from vegetative organs as a whole

Nitrogen did not affect the total remobilization amount of DM, K, Mg, Fe, and Mn, but significantly affected that of N, P, Ca, Cu, Zn, and B from vegetative organs (Table 3). Across two years, the value of the average remobilization amount under HN was positive for N, P, K, and Zn; however, it was negative for DM and Ca (Table 4), indicating a net increase of DM and Ca in the vegetative organs during the grain filling stage. For Mg, Fe, Mn, Cu, and B, their remobilization from the total vegetative organs was positive in 2013, but negative in 2014.

Across the two years, LN reduced total remobilization amount of N, P, and Zn by 49%, 50%, and 126%, respectively; however, that of Ca, Cu, and B increased by 77%, 117%, and 97%, respectively (Table 4). The N $\times$ year interaction was also significant for the total remobilization amount of all nutrients but not DM, K, and Cu (Table 3). The lower GY and HI in 2014 might have had an overall effect on nutrient remobilization. In 2014, the remobilization of Mg, Fe, Mn, Cu, and B was completely inhibited and more Ca was accumulated in vegetative organs during the

grain filling stage in the HN treatment.

When averaged over 2 years, the average remobilization efficiency (*RE*) under HN was −15%, 44%, 60%, 13%, −5%, −35%, −20%, 15%, −29%, 25%, and −25% for DM, N, P, K, Mg, Ca, Fe, Mn, Cu, Zn, and B, respectively (Table 4). Only the *REs* of P, Ca, Cu, Zn, and B were significantly affected by the LN treatment (Table 3). When averaged over 2 years, the *RE* of Ca, Cu, and B from the total vegetative organs increased by 61%, 118%, and 86%, respectively, whereas that of P and Zn decreased by 37% and 137%, respectively in LN compared with in HN (Table 4). Interaction between N × year had a similar effect on *RE* as on the total remobilization amount of the nutrients.

When averaged over 2 years under HN, the potential contribution of the remobilization of N, P, K, Mn, and Zn to their content in the grain was 44%, 41%, 40%, 151%, and 21%, respectively (Table 4). The remaining amount of these nutrients in the grain was contributed by the post-silking uptake (Fig. 1). For Mg, Ca, Fe, Cu, and B, the post-silking accumulation exceeded their content in the grain by 18%, 2085%, 9%, 72%, and 94%, respectively (Table 5; Fig. 1). When averaged over 2 years, LN stress reduced the post-silking accumulation amount of N, Mg, Ca, Cu, and B by an average of 33%, 50%, 74%, 76%, and 61%, respectively (Table 5). The contribution of post-silking accumulated P and Zn to the grain increased by 21% and 35%, respectively, while that of Ca, Cu, and B decreased by 73%, 76%, and 48%, respectively, in the LN treatment. The N × year interaction effect was significant on the post-silking accumulation of the nutrients other than N, K, and Cu (Table 3). Under LN, the harvest index of P, K, Fe, Zn, and B decreased by 14%, 16%, 26%, 23%, and 9%, respectively; meanwhile, that of N, Mg, and Cu increased by 13%, 12%, and 62%, respectively (Table 5). The N × year interaction had an effect on the harvest index of N,

P, Fe, Mn, Zn, and B (Table 3).

3.3. Remobilization of DM and the nutrients from different vegetative organs

When averaged over 2 years under HN conditions, the remobilization of N and P occurred more intensely from the leaves ($RE = 44\%$ and 51% for N and P, respectively) and the stalks ($RE = 48\%$ and 71% for N and P, respectively), and less so from the husk + cob ($RE = 25\%$ and 57% for N and P, respectively; Table 4; Fig. 1). K was mainly remobilized from leaves ($RE = 51\%$) and less so from the stalks ($RE = 5\%$). Mn was mainly remobilized from the stalks ($RE = 42\%$), and less so from the leaves ($RE = 0.04\%$). The RE of Zn in leaves and stalks were as high as 43% . In the husk + cob, the content of K, Mn, and Zn increased by 76% , 113% , and 80% as shown by the negative RE value, respectively. Therefore, the K, Mn, and Zn remobilized from the leaves and the stalks may have been possibly stored in the husk + cob.

Although the net remobilization of Mg, Ca, Fe, and Cu from the vegetative organs as a whole was negative, these nutrients were remobilized from the stalks with the RE of 23% , 9% , 10% , and 28% , respectively (Table 4). The content of these nutrients in the husk + cob increased, indicating that the remobilized Mg, Ca, Fe, and Cu from the stalk was accumulated in the husk + cob rather than translocated to the grain. B was the only element which showed net accumulation in all the vegetative organs during post-silking stage.

When averaged over 2 years, LN stress increased the RE by 28% and 42% for N and K, respectively, in the leaves but not in other organs (Tables 3 and 4). For P, the RE decreased by 54% and 24% in the stalk and leaves, respectively, in the LN treatment, and the RE of P in the husk + cob was not affected by N. N treatment had no significant effect on the RE of other nutrients in the

stalk. In the leaves, the LN treatment resulted in an increase in the *RE* of Mg, Ca, Fe, Cu, and B by 67%, 68%, 119%, 54%, and 84%, respectively, while LN treatment had no effects on the *RE* of Mn and Zn. In the husk + cob, the *RE* increased by 51% for Cu and decreased by 234% for Zn in the LN treatment, with the *RE* of Mg, Ca, Fe, Mn, and B were not affected by the addition of N.

3.4. Dynamic change of nutrients in different organs during the grain filling stage

DM and the content of the investigated nutrients in each organ changed greatly during the grain filling stage (Figs. 2–12). Only DW and the dynamic change of N, P, K, Mn, and Zn content in different organs were analyzed further because the remobilization of these nutrients had a contribution to their content in the grain (Table 4, Fig. 1).

Under the HN treatment, the DW of the stalk increased from the silking stage, reached a maximum at 20 DAS, and then decreased gradually until maturity (Fig. 2). Leaf DW increased slightly from silking to 30 DAS, and then decreased slightly. The DW of the husk + cob showed a great increase from silking to 20 DAS, remained stable for the next 10 days, and then decreased slightly. A net increase was observed in the DW of the husk + cob. As expected, the DW of the grain started to increase from 10 DAS and continued to increase until maturity. In the LN treatment, the DW of stalk and the leaves remained almost unchanged until 40 DAS and then decreased. The DW of the husk + cob showed a dramatic increase between 0 and 10 DAS, and then remained stable until maturity (Fig. 2).

Depending on N supplied and years, N remobilization in the stalk and leaves started from 0–20 DAS from silking (Fig. 3). The N content in the husk + cob increased between 0 and 10 DAS, and then decreased gradually until maturity. In 2014, the remobilization of N in all of the

vegetative organs was delayed. The concentration of N in all the vegetative organs decreased from silking until maturity (Table 1S). Grain N content increased continuously from silking to maturity (Fig. 3). However, grain N concentration was highest on 10 DAS, and then decreased by 50% on 20 DAS, and then remained stable until maturity (Table 1S). The LN treatment did not change the pattern of N remobilization in each organ and N accumulation in the grain (Fig. 3).

Under HN, the patterns of P remobilization from different vegetative organs and P accumulation in the grain were quite similar as that of N (Fig. 4). At the silking stage, LN greatly reduced the accumulation of P in the leaves but not in the stalk, and husk + cob (Fig. 4). LN also reduced the amount of P remobilized from the stalk and leaves (Fig. 4; Table 4). As a result, the concentration of P in the stalk was much higher under LN than under HN during the entire grain filling stage (Table 1S).

The K content in the leaves continued declining during the grain filling stage (Fig. 5). In the stalk, the K content fluctuated considerably during the grain filling stage, but remained balanced between silking and maturity. The K content in the husk + cob increased during the early grain filling stage (0 to 20 DAS), remained stable, and then decreased from 30 DAS to maturity. Notably, the grain K content increased from 10 to 30 DAS when the K content in all the vegetative organs remained largely unchanged (Fig. 4). Furthermore, the increase in the K content in the grain ceased on 30 DAS, whereas the K content in the leaves and husk + cob continued to decrease from 30 DAS to maturity. At the silking stage, low N resulted in increased K content in the stalk without an effect on that in the leaves and husk + cob (Fig. 4). Low N did not affect the pattern of K remobilization in the vegetative organs. Under LN, the grain K content continued to increase until maturity; however, the grain K concentration was always lower under LN when

compared with that under HN (Table 1S).

During the grain filling stage under HN, Mn was mainly remobilized from the stalk (Fig. 6). Remobilization was found in the leaves only in the HN treatment in 2013. In 2013, the amount of remobilized Mn from either the stalk or the leaves was more than that accumulated in the grain at maturity (Table 4). In 2014, however, the amount of Mn remobilized from the stalk was only 57% of that in the grain. Meanwhile, the Mn content in the leaves showed a net accumulation from silking to maturity (Table 4). LN reduced the content of Mn in the vegetative organs (especially from the leaves) at silking and that in the grain at maturity (Fig. 6). The amount of Mn remobilized from the stalk was still more than that accumulated in the grain (Table 4). The changing pattern of Mn in different organs was similar in the two N treatments. Regardless of N treatment, the Mn content in the husk and cob increased from silking to 10 DAS and then remained quite stable.

The content of Zn in the stalk decreased starting from silking (Fig. 7). The Zn content in the leaves started to decrease from 20 DAS. Zn content in the husk + cob increased for a short time from silking to 10 DAS, and then decreased. As found in P, LN reduced the Zn content in the leaves without much effect on those in the stalk and husk + cob at the silking stage. As a result, the Zn concentrations in the stalk and husk + cob were much higher under LN compared with under HN (Table 1S).

Year effect strongly affected the remobilization pattern of Fe from vegetative organs (Fig. 8). In 2013, Fe content decreased in the stalk and leaves, and the amount of remobilized Fe in the stalk was more than that accumulated in the grain (Table 4). The remobilization of Fe in the stalk started at silking and in the leaves at 30 DAS (Fig. 8). In 2014, Fe content in the stalk and leaves

increased temporally and then decreased at the late grain filling stage. At maturity, a net accumulation of Fe was observed in the stalk and leaves under the HN treatment (Table 4). LN did not have an effect on the changing pattern of Fe content in different organs during the grain filling stage. Regardless of N treatment, Fe content in the husk + cob increased from silking to 10 DAS, and then remained unchanged until maturity.

In both years and under both N treatments, the content of Mg, Ca, Cu, and B in the leaves and the husk + cob showed an increasing tendency from silking to maturity (Figs. 9–12, Table 4). Depending on different N treatment/year combinations, Mg content in the stalk decreased at different stages and showed a net reduction to a certain extent from silking to maturity (Fig. 9, Table 4). In 2013, but not in 2014, the content of Ca, Cu, and B in the stalk showed a decrease from silking to maturity in both the HN and LN treatments (Figs. 10–12). The reduction of Ca and Cu content in the stalk was more than that accumulated in the grain (Table 4). Again, the content of Mg, Ca, Cu, and B in the husk + cob increased from silking to 10 DAS, and then decreased a little or remained unchanged until maturity (Figs. 9–12). LN did not have an effect on the changing pattern of the content of these elements in different organs during the grain filling stage.

4. Discussion

4.1. Mobility of the nutrients during the grain filling stage in maize

Based on whether a nutrient element can be re-translocated from an organ to other organs via phloem transport, and with consideration of the data related to the composition of phloem sap, mineral nutrients can be roughly classified into those that are highly mobile (such as N, P, K, and Mg), intermediately mobile (such as Fe, Cu, Zn, and B) and those having low mobility (Ca and

Mn; Marschner, 1995). However, this classification is only a first approximation because certain factors are ignored, such as genotypical differences or the nutritional status of plants. In the present study, the content of N, P, K, Mg, Fe, Mn, Cu, and Zn showed a net decrease in at least one vegetative organ, suggesting they are mobile, to various extents, during the grain filling stage of maize. Although some exceptional data were observed, the content of Ca and B in the vegetative organs generally increased or remained unchanged during the grain filling stage, suggesting Ca and B were immobile. Previously, Karlen et al. (1988) and Bender et al. (2013) reported that N, P and Zn are highly plant mobile in maize and begin translocation to grain at the R2 (blister) stage, while most micronutrients such as Fe, Mn, Cu, and B exhibit limited or nonexistent remobilization characteristics. The present study indicates that Mn, Fe, and Cu could be remobilized in some growth conditions (Table 4).

Among the mobile nutrient elements, N and P were remobilized from all the vegetative organs (Figs. 3 and 4, Table 4). The *RE* of N and P of the vegetative organs as a whole was also very high (44–51% for N, and 38–60% for P under two N levels across 2 years; Table 4). The *RE* of N in the stalk, leaves, and the husk + cob averaged 49%, 50%, and 29%, respectively, while that of P averaged 52%, 45%, and 55%, respectively. Chen et al. (2014) provided comparable data, suggesting that the *RE* of N in the stalk and leaves could be 49–55% and 65–70%, respectively. Ta and Weiland (1992) reported that the *RE* of N in the stalk (75–85%) was much higher than that in the leaves (60–73%), and leaves and stalk each contributed 45% of total remobilized N. In wheat, 72% and 76% of the N in stem and leaves, respectively, and 76% and 86% of the P in stem and leaves, respectively, was apparently redistributed to developing grain (Hocking, 1994). In general, the remobilization of N and P in the stalk happened earlier than in the leaves (Figs. 3 and 4).

Because the leaf is the main photosynthetic organ, the late remobilization of N or P from the leaves should help the plants to sustain photosynthetic productions as long as possible. This prior remobilization of N in the stalk was similar to that reported previously (Ta and Weiland, 1992), even though the remobilization from stalk was initiated before anthesis in their study.

Much like N and P, Zn was readily remobilized from the leaves and stalk. Averaged over the 2 years and two N levels, the *REs* of Zn in stalks and leaves were 35% and 41%, respectively. Bender et al. (2013) however, found only the stalk tissue served as a temporal but major source for Zn remobilization (*RE* = 60%). In wheat, Hocking (1994) found the *RE* of Zn in the stem (45%) was also much lower than that in the leaves (*RE* = 51%). However, some of the remobilized Zn might have been distributed into the husk + cob, as shown by the net increase in Zn content in the later (Fig. 7). As a result, the calculated *RE* of Zn in vegetative organs as a whole was only 8%, much lower than that of N and P (Table 4).

Nutrient remobilization could be organ-specific. K was mainly remobilized from the leaves (Fig. 5, Table 4). In contrast, the remobilization of Mg, Mn, and Cu was barely observable in the leaves under either HN or LN in both years (Figs. 6 and 9–10, Table 4). Previous research also found this phenomenon in the seasonal uptake patterns of Mg, Mn, and Cu in maize (Karlen et al., 1988; Bender et al., 2013), in which there was no decrease of the nutrients content and even an increase in the leaves during the grain filling stage. One possible explanation might be that Mg, Mn, and Cu were involved in photosynthesis. For example, Mg could modulate RuBP carboxylase in the stroma of chloroplasts (Pierce, 1986), 50% of Cu in plants was found in chloroplasts, bound to plastocyanin and took part in photosynthetic reactions (Hansch and Mendel, 2009), and Mn participated in photosynthetic O₂ evolution in PS II (Husted et al., 2009; Gong et al., 2010). The

remobilization of Mg, Mn, and Cu reserves from the leaf will affect photosynthesis, and thus there is a conflict between sustaining photosynthesis and Mg, Mn, and Cu remobilization from leaves during the grain filling stage. Taken all, the above data suggest that genetic improvement aiming to increase nutrient remobilization should take into account the organ-specific remobilization pattern of the target nutrient.

The *RE* of various nutrients seemed to be greatly affected by weather conditions (Tables 3 and 4). The drought during silking and the early grain filling stage in 2014 had an irreversibly influence on plant pollination, and resulted in a lower grain number per ear, GY and HI (Table 1). These may reduce the demand for the remobilization of some nutrients into grain. Thus, the *RE* of all the nutrients, except for N, P, and K in the stalk and/or leaves was reduced (Table 4). Ning et al. (2013) also reported that the percentage of post-silking remobilization of N and P was much lower when DM accumulation in the grain decreased. Clarke et al. (1990) found that a moist environment was beneficial for more post-anthesis N and P uptake than a dry environment. In the present study, water supply via precipitation and irrigation was much greater during the grain filling stage in 2014 compared with 2013 (Fig. 1S), which may explain the increased post-silking nutrient uptake in 2014 (Table 5).

In the husk + cob, the content of all analyzed nutrients increased from silking to 10 DAS (Figs. 3–12). Thereafter, the content of these nutrients either increased, remained unchanged or reduced at various times. At maturity, the content of all these nutrients except for N and P showed a net increase compared with that at silking (Figs. 3–12). The increase of the nutrients in the husk + cob during early grain filling stage may be contributed by both post-silking uptake and their remobilization from the stalk and/or the leaves, depending on year, nutrients, and/or N treatments.

Take N as an example; when N remobilization was low in 2014, the temporal increase of N in the husk + cob seemed to be contributed by post-silking N uptake. In 2013, however, the amount of the N increment in the husk + cob was almost compatible to the combined decrease of N in the stalk and leaves, suggesting that, in this case, the N was mainly contributed by N remobilized from the stalk and leaves. Therefore, the husk + cob seemed to serve as a buffer sink for storing nutrients from either remobilization or post-silking uptake. The nutrients stored in the husk + cob were further transported to the grain as needed. Previously, Crawford et al. (1982) and Cliquet et al. (1990) demonstrated by ¹⁵N label experiments that cob, husk, and shank acted first as N sinks and then as N sources during ear development. Furthermore, the accumulation of N, P, and K in the grain was low on 10 DAS (Figs. 3–5); the rapid reduction of the content of these nutrients in the leaves and/or the stalk might indicate that the remobilization of these nutrients seemed to be triggered by some “signals” from grain development rather than the balance between the need and supply of them in the grain.

4.2. Apparent contribution of the remobilization of the nutrients to their content in the grain

Although many nutrients could be remobilized in different organs and/or in different growth conditions, it appeared that only the remobilized N, P, K, Mn, and Zn contributed to their content in the grains. Across 2 years, the apparent contribution of nutrient remobilization to grain nutrient content was 44% for N, 41% for P, 40% for K, 151% for Mn, and 21% for Zn under HN supply conditions (Fig. 1, Table 4). Improvement of the remobilization of these nutrients may contribute to increase their content in the grains. Previous research, primarily discussing N, P, and K, has reported the contribution of nutrient remobilization to grain nutrient content (Mi et al., 2003;

Masoni et al., 2007; Bender et al., 2013; Chen et al., 2014). In wheat, the redistribution from stems and leaves could have provided 72%, 68%, 108%, 46%, 44%, and 33% of the N, P, K, Mg, Cu, and Zn accumulated by grain, respectively, while the redistribution of Ca, Fe, and Mn was negligible (Hocking, 1994). Also in wheat, 73–82% of grain N and 56–63% of grain P originated from the remobilization of N and P accumulated prior to anthesis with differences based on soil type (Masoni et al., 2007). Using ¹⁵N tracing techniques, Ta and Weiland (1992) found that 40–70% of the ear (kernels + other reproductive tissues) N was obtained from vegetative remobilization in maize, very similar to findings published by Mi et al. (2003). The much higher contribution (69–80%) reported by Chen et al. (2014) may have occurred because of the longer growing period of spring maize in Northeast China. In summer maize, 18–23% of the grain P and 44–205% of the grain K was obtained from the remobilization from stalk and leaves, while the remobilization of Mn and Zn from total vegetative organs was only found under LN condition (remobilized Mn and Zn were contributed to grain at 59% and 14%, respectively) and there was no remobilization for Mg, Ca, Fe, and Cu (calculated from the results of Shi, 2010).

Note that the change of nutrient content in different organs might reflect a balance of nutrient remobilization and distribution of the newly absorbed nutrients (Figs. 3–12). For example, an ¹⁵N tracing experiment suggested that some of the N absorbed during the grain filling stage was deposited in the vegetative organs, and then was further hydrolyzed to provide N in the grain (Gallais et al., 2006, 2007). Therefore, the above data only indicated an apparent contribution of the nutrients to the grain. Studies using radioactive element tracing techniques are necessary to gain a true picture of the contribution of the nutrients from different organs to the grain.

Notably, grain K content increased from 10 to 30 DAS when the net reduction of the K

content in the vegetative organs as a whole was very low (Fig. 5). From 30 DAS to maturity, grain K content remained largely unchanged. However, the content of K in the leaves and the husk + cob continued to decrease. This indicated that K that had been remobilized from the leaves was not translocated to the grain. Because the K content of the stalk remained relatively stable from 30 DAS to maturity, the net loss of K from the leaves and the husk + cob must have been leached out of the plant. A net loss of K from maize plants has been frequently reported during the grain filling stage (Peng et al., 2012; Ning et al., 2013). Therefore, if the contribution of K remobilization to grain K was calculated based on the vegetative-K content at the maturity, then the contribution of remobilized K to the grain would be overestimated. This may explain the higher value of the contribution of K remobilization to grain K in Hocking (1994).

4.3. Nutrient remobilization in relation to grain nutrient concentration

The concentration of nutrients in the grain may come from either post-silking nutrient uptake or the remobilization of the nutrients accumulated before silking. Therefore, researchers have suggested that high nutritional quality of the grain was obtained through a combination of post-silking uptake and remobilization from vegetative organs (Masclaux-Daubresse et al., 2008; Bender et al., 2013; Chen et al., 2014). However, knowing whether the limiting factor for nutrient concentration in the grain is nutrient supply or nutrient accumulation in the grain remains unclear. In the present study, the concentration of N, P, Mg, Ca, Fe, Mn, Cu, and Zn in the grain remained stable after 20 DAS, when the grain filling process accelerated rapidly (Table 1S). That means the accumulation of these elements matched the accumulation of the DM in the grain. Therefore, one might conclude that under normal nutrient supply conditions, the concentrations of these nutrients

in the grain were primarily determined by physiological processes occurring within the grain itself. The remobilization of these nutrients might be regulated by the requirement for them in the grain. For example, Uribeharrea et al. (2007) reported that the selection for genotypes with high protein content resulted in an increase in N remobilization efficiency. This raises a question: is it practical to increase grain N concentration via genetic manipulation of N remobilization from the leaves (Gallais and Hirel, 2004; Martin et al., 2006). Grain N concentration might serve as a more direct target for genetic manipulation.

4.4. The effects of N supply on nutrient remobilization

As expected, the LN treatment resulted in a reduction in the content of all nutrients in the vegetative organs, in part because of the reduced DM (Figs. 2–12). Interestingly, the LN treatment resulted in greatly reduced accumulation of P and Zn in the leaves but not in the stalk. As a result, throughout the grain filling stage, the concentration of P and Zn in the stalk was much higher under LN than under HN (Table 1S). Furthermore, a constant P/Zn ratio was observed in the leaves and grain throughout the grain filling stage (data not shown). Similarly, Chen (2012) found a significant correlation between P and Zn concentration in maize grain released in different eras. These results suggest that a close linkage exists between the biological functions of P and Zn in these two organs. Other studies found that Zn has the similar function to that of P as it is related to the stability of the biological membrane (Chvapil, 1973; Webb and Loneragan, 1990).

Among the mobile nutrients, the *RE* of P and Zn in the vegetative organs as a whole was smaller in 2013 than in 2014 (Tables 3 and 4). Venekamp et al. (1986) reported that the redistribution of P from vegetative organs into seeds was restricted under a low N condition. Chen

et al. (2015) reported that the *RE* of N in the vegetative organs as a whole was not affected by the availability of N. However, Mi et al. (2003) reported that low N treatment increased N remobilization efficiency. Chen (2012) found the concentration of grain P, K, Mg, and Zn increased, while that of Fe, Mn, and Cu decreased under LN conditions. In the present study, however, the concentration of the nutrients in the grain was either unchanged (P, K, Ca, Zn, and B) or decreased (N, Mg, Fe, Mn, and Cu; Table 2), even though the grain number and 100-grain weight were reduced significantly under LN (Table 1). The decreased concentration of Fe, Mn, Cu, and Zn in the grain under the LN treatment had also been found in wheat and rice (Hao et al., 2007; Shi et al., 2010). Therefore, although low-N stress can increase the remobilization of some nutrients from various vegetative organs, the concentration of these nutrients in the grain may not be increased accordingly.

5. Conclusion

In the present study, the dynamic change of the macronutrients and micronutrients in different organs of maize was monitored during the grain filling stage, and the mobility of different elements and their contribution to grain nutrient content were evaluated. It is concluded that (1) the mobility of the nutrients is nutrient-specific, organ-specific and vulnerable to weather conditions; therefore, genetic improvement aiming to increase nutrient remobilization should take into account the organ-specific remobilization pattern of the target nutrient. (2) Only the remobilized N, P, K, Mn, and Zn contributed to their content in the grains, the concentration of these nutrients in the grains might be improved by increasing the remobilization of these nutrients from the vegetative organs. (3) In the grains, the accumulation of most nutrients matched the

accumulation of the DM. Therefore, the remobilization of the nutrients might be regulated by the requirement for them in the grain. Grain nutrient concentration might serve as a more direct target for genetic manipulation. (4) Although low-N stress can increase the remobilization of some nutrients from various vegetative organs, enhancing the senescence of maize plant via low-N stress seems unable to increase grain mineral nutrient concentration.

Acknowledgements

We gratefully acknowledge the National Basic Research Program (973 Program) of China (Grant No. 2015CB150402), the National Science Foundation of China (Grant Nos. 31272233 and 31121062) for financial support.

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1 **Figure legends**

2 **Fig. 1** Remobilization of mineral nutrients from different organs and the related post-silking
3 accumulation as well as nutrient contribution to grain nutrient content. RE: remobilization
4 efficiency; CG: apparent contribution to grain nutrient content. Data are the average of applied
5 nitrogen (HN) treatment in 2013 and 2014. Negative values indicate net accumulation of the
6 indicated nutrient in the indicated organ.

7 **Fig. 2** Dry matter (DM) accumulation after silking in four maize organs under two nitrogen levels
8 in 2013 and 2014; data are the average data for these 2 experimental years. Vertical bars indicate
9 standard error from the data of each nitrogen (N) treatment. Different letters indicate significant
10 differences among different stages ($P < 0.05$). LN, no N supplied; HN, 180 kg N ha⁻¹, half applied
11 before sowing as base fertilizer and the other half applied at the V6 (six expanded leaves) stage as
12 top dressing.

13 **Fig. 3** Post-silking changes of nitrogen (N) content in four maize organs under two nitrogen levels
14 in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N)
15 treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN
16 and HN are the same as in Fig. 2.

17 **Fig. 4** Post-silking changes of phosphorus (P) content in four maize organs under two nitrogen
18 levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N)
19 treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN
20 and HN are the same as in Fig. 2.

21 **Fig. 5** Post-silking changes of potassium (K) content in four maize organs under two nitrogen
22 levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N)

treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN and HN are the same as in Fig. 2.

Fig. 6 Post-silking changes of manganese (Mn) content in four maize organs under two nitrogen levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N) treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN and HN are the same as in Fig. 2.

Fig. 7 Post-silking changes of zinc (Zn) content in four maize organs under two nitrogen levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N) treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN and HN are the same as in Fig. 2.

Fig. 8 Post-silking changes of iron (Fe) content in four maize organs under two nitrogen levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N) treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN and HN are the same as in Fig. 2.

Fig. 9 Post-silking changes of magnesium (Mg) content in four maize organs under two nitrogen levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N) treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN and HN are the same as in Fig. 2.

Fig. 10 Post-silking changes of copper (Cu) content in four maize organs under two nitrogen levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N) treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN and HN are the same as in Fig. 2.

45 **Fig. 11** Post-silking changes of boron (B) content in four maize organs under two nitrogen levels
46 in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N)
47 treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN
48 and HN are the same as in Fig. 2.

49 **Fig. 12** Post-silking changes of calcium (Ca) content in four maize organs under two nitrogen
50 levels in 2013 and 2014. Vertical bars indicate standard error from the data of each nitrogen (N)
51 treatment. Different letters indicate significant differences among different stages ($P < 0.05$). LN
52 and HN are the same as in Fig. 2.

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Table 1 Yield, grain number, 100-grain weight, and harvest index under two nitrogen levels in 2013 and 2014.

Treatments	Yield (g plant ⁻¹)	Grain number (ear ⁻¹)	100 grain weight (g)	Harvest index (%)
Nitrogen				
HN	172.9	592	30.7	54.3
LN	131.6	444	26.5	53.7
Year				
2013	164.9	547	28.4	57.4
2014	139.6	490	28.9	50.6
Source of variation				
Nitrogen (N)	***	***	***	NS
Year (Y)	**	NS	NS	***
N × Y	NS	NS	NS	NS

LN, no N supplied; HN, 180 kg N ha⁻¹, half applied before sowing as base fertilizer and the other half applied at the V6 (six expanded leaves) stage as top dressing; NS, not significant ($P > 0.05$); *, **, ***, indicate significance at $P < 0.05$, 0.01, 0.001, respectively.

7 **Table 2** Grain nutrient concentration at maturity under two nitrogen levels in 2013 and 2014.

Treatments		Grain nutrient concentration									
		N	P	K	Mg	Ca	Fe	Mn	Cu	Zn	B
		-----g kg ⁻¹ -----					-----mg kg ⁻¹ -----				
Nitrogen											
	HN	13.8	3.1	3.8	1.2	0.06	22.5	4.8	1.5	21.7	1.6
	LN	11.0	2.9	3.9	1.1	0.07	17.3	3.6	1.2	20.3	1.5
Year											
	2013	11.6	2.9	3.9	1.0	0.06	19.8	3.7	0.92	19.7	1.5
	2014	13.2	3.2	3.8	1.2	0.07	20.0	4.7	1.7	22.3	1.6
Source of variation											
	Nitrogen (N)	***	NS	NS	***	NS	**	***	**	NS	NS
	Year (Y)	**	**	NS	***	NS	NS	***	***	NS	NS
	N × Y	NS	*	**	**	NS	NS	NS	NS	NS	*

8 LN, no N supplied; HN, 180 kg N ha⁻¹, half applied before sowing as base fertilizer and the other half applied at
9 the V6 (six expanded leaves) stage as top dressing; NS, not significant ($P > 0.05$); *, **, ***, indicate significance
10 at $P < 0.05$, 0.01, 0.001, respectively.

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Table 3 Variance analysis of the remobilization amount, remobilization efficiency, post-silking accumulation, the apparent contribution of remobilization and post-silking accumulation to the grain, and the harvest index of the dry matter (DM) and various nutrients.

DM and nutrient	Source of variation	Remobilization amount				Remobilization efficiency				Apparent contribution to grain				Post-silking accumulation		Harvest index
		Husk+				Husk+				Husk+				Contribution		
		Stalk	Leaf	cob	Total	Stalk	Leaf	cob	Total	Stalk	Leaf	cob	Total	Amount	to grain	
DM	Nitrogen (N)	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	NS	**	NS	NS
	Year (Y)	*	NS	NS	NS	*	NS	NS	NS	NS	NS	**	*	NS	*	***
	N × Y	*	NS	NS	NS	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
N	Nitrogen (N)	***	**	NS	**	NS	*	NS	NS	*	NS	NS	NS	*	NS	***
	Year (Y)	*	**	**	**	NS	NS	**	*	NS	*	**	*	NS	*	***
	N × Y	*	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	***
P	Nitrogen (N)	**	***	*	***	***	*	NS	***	NS	**	NS	*	NS	*	***
	Year (Y)	*	*	***	NS	**	NS	***	NS	*	NS	*	NS	NS	NS	NS
	N × Y	**	**	*	**	***	**	**	***	**	*	NS	**	*	**	***
K	Nitrogen (N)	NS	*	NS	NS	NS	***	NS	NS	NS	**	NS	NS	NS	NS	***
	Year (Y)	NS	NS	*	NS	NS	NS	**	NS	NS	NS	**	NS	NS	NS	***
	N × Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mg	Nitrogen (N)	NS	***	NS	NS	NS	*	NS	NS	NS	**	NS	NS	*	NS	*
	Year (Y)	*	**	***	**	**	*	***	**	NS	**	***	**	**	**	***
	N × Y	*	NS	*	*	*	NS	NS	NS	*	NS	NS	*	*	*	NS

NS, not significant ($P > 0.05$); *, **, ***, indicate significance at $P < 0.05, 0.01, 0.001$, respectively. Table 3 continues on the next page.

Nutrient	Source of variation	Remobilization amount				Remobilization efficiency				Apparent contribution to grain				Post-silking accumulation		Harvest index
		Husk+				Husk+				Husk+				Contribution		
		Stalk	Leaf	cob	Total	Stalk	Leaf	cob	Total	Stalk	Leaf	cob	Total	Amount	to grain	
Ca	Nitrogen (N)	NS	***	**	**	NS	**	NS	*	NS	***	NS	**	**	**	NS
	Year (Y)	*	**	**	**	*	NS	NS	*	*	NS	*	*	**	*	NS
	N × Y	*	**	**	**	*	*	NS	*	*	NS	NS	*	**	*	NS
Fe	Nitrogen (N)	*	**	NS	NS	NS	**	NS	NS	NS	**	*	NS	NS	NS	***
	Year (Y)	***	***	NS	***	***	***	*	***	***	***	*	***	***	***	***
	N × Y	**	***	NS	**	**	***	NS	***	NS	**	NS	*	**	*	**
Mn	Nitrogen (N)	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Year (Y)	**	**	*	**	**	**	*	**	**	**	NS	**	**	**	***
	N × Y	*	**	*	**	NS	*	NS	*	NS	*	*	*	**	*	*
Cu	Nitrogen (N)	NS	**	***	*	NS	*	*	*	NS	**	**	*	**	*	***
	Year (Y)	***	NS	***	**	***	NS	***	***	***	NS	***	**	***	**	NS
	N × Y	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Zn	Nitrogen (N)	NS	**	***	**	NS	NS	***	**	NS	NS	***	**	NS	**	***
	Year (Y)	NS	**	NS	*	NS	**	NS	NS	NS	**	NS	NS	NS	NS	NS
	N × Y	NS	NS	**	*	NS	NS	**	*	NS	NS	**	*	*	*	*
B	Nitrogen (N)	NS	**	NS	**	NS	*	NS	*	NS	**	NS	**	**	**	*
	Year (Y)	***	NS	**	**	***	NS	**	**	***	NS	**	**	**	**	***
	N × Y	**	**	NS	***	**	**	NS	**	NS	***	NS	**	**	**	***

16 **Table 4** Dry matter (DM) and nutrients remobilization amount, remobilization efficiency and the apparent contribution to the grain under two nitrogen levels in 2 yrs.

DM and nutrient	Year	Treatment	Remobilization amount (mg plant ⁻¹)				Remobilization efficiency (%)				Apparent contribution to grain (%)			
			Stalk	Leaf	Husk+cob	Total	Stalk	Leaf	Husk+cob	Total	Stalk	Leaf	Husk+cob	Total
DM	2013	HN	19217	6036	-25268	-15	23	14	-250	-0.89	10	3	-13	0.22
		LN	8448	7720	-21335	-5166	14	22	-210	-6	6	6	-15	-3
	2014	HN	-6332	-641 [*]	-28754	-35726 [*]	-10	-2 [*]	-318	-30	-4 [*]	-0.18 [*]	-18	-22
		LN	10023	5880	-22816	-6913	15	16	-268	-6	9	5	-19	-6
N	2013	HN	587	660	106	1353	60	52	43	54	24	27	4	56
		LN	145 [*]	356 ^{**}	74	575 ^{**}	48	59	39	53	10 [*]	26	5	41
	2014	HN	283	389	16	688	36	36 [*]	8 [*]	33 [*]	13	17	0.74 [*]	31
		LN	165	265	41	470	52	54	27	49	12	19	3	34
P	2013	HN	112	105	31	248	76	65	72	70	22	20	6	48
		LN	14 ^{**}	29 ^{**}	21 [*]	64 ^{***}	9 ^{***}	30 ^{**}	56 [*]	22 ^{***}	3 ^{**}	7 ^{**}	5	15 [*]
	2014	HN	97	58	16	171	66	38	41	50	19	11	3	33
		LN	91	37	16	144	56	48	50	53	26	11	4	41
K	2013	HN	118	373	-84	407	10	54 [*]	-37	21	19	55	-12	62
		LN	4	428	-91	341	-1	73	-43	18	3	74	-15	62
	2014	HN	6	308 ^{**}	-221	94 [*]	-0.68	48 ^{***}	-116	5 [*]	4	50 ^{***}	-35	19 [*]
		LN	211	467	-124	554	19	73	-72	29	51	104	-27	128
Mg	2013	HN	145	-55 [*]	-8	83	40	-23	-37	13	74	-28	-4	43
		LN	43	-5	-9	28	23	-6	-48	7	31	-2	-6	23
	2014	HN	25	-161 ^{**}	-44	-179 [*]	6	-49 [*]	-164	-23 [*]	13	-73	-20	-79 [*]
		LN	49	-35	-26	-12	18	-18	-121	-2	39	-29	-20	-10

17 Comparing N treatments, data followed by *, **, *** indicate significance at $P < 0.05$, 0.01, 0.001, respectively. Negative values indicate net accumulation of the indicated nutrient. For the
18 contribution to the grain, higher values than 100 indicate that nutrient remobilization amount exceeded grain demand, while negative values indicate that nutrient remobilization has no
19 contribution to the grain. Table 4 continues on the next page.

Nutrient	Year	Treatment	Remobilization amount ($\mu\text{g plant}^{-1}$)				Remobilization efficiency (%)				Apparent contribution to grain (%)			
			Stalk	Leaf	Husk+cob	Total	Stalk	Leaf	Husk+cob	Total	Stalk	Leaf	Husk+cob	Total
Ca	2013	HN	99498	-131276	-20735	-52514	31	-43	-207	-10	821	-1510	-215	-904
		LN	15457	-47976	-21217	-53736	10	-25	-228	-17	181	-492	-216	-527
	2014	HN	-35602*	-298332***	-44953**	-378887**	-12*	-89***	-394*	-60**	-282*	-2588***	-396	-3266***
		LN	21414	-43593	-23070	-45249	10	-17	-235	-10	303	-477	-293	-468
Fe	2013	HN	8694	1895	-1135	9454	70	23	-144	43	222	50	-27	245
		LN	2512*	601	-1450	1663*	31*	8	-219	8	103	28	-56*	74
	2014	HN	-2075	-5355***	-1997	-9426**	-50	-78***	-457	-82***	-58	-148**	-56	-262**
		LN	-703	166	-1461	-1998	-20	3	-307	-19	-29	9	-73	-93
Mn	2013	HN	1836	1078	-98	2816	64	33	-47	45	244	143	-11	376
		LN	730*	88*	-153	666*	48	5	-101	22	165	23*	-33*	156
	2014	HN	457	-847	-312	-702	20	-33	-179	-15	57	-94	-37	-73
		LN	508	-38	-147	323	31	-4	-124	10	108	-6	-30	73
Cu	2013	HN	340	-266*	-20*	53	62	-70	-37*	4	168	-130	-10*	28
		LN	194*	-31	6	169	71	-28	20	38	188	-28	6	166
	2014	HN	-16	-365**	-112*	-493*	-5	-98	-152	-62	-5	-127*	-39	-171*
		LN	20	-72	-43	-95	12	-50	-112	-27	13	-37	-23	-47
Zn	2013	HN	651	712	-73	1290	46	62	-21	43	18	20	-2	36
		LN	399	350*	-994***	-244**	23	50	-310***	-10**	14	13	-35***	-8**
	2014	HN	408	249	-507	150	39	25	-138	6	11	7	-13	6
		LN	396	143	-673	-135	31	27	-222	-8	16	6	-28*	-6
B	2013	HN	152	-51	-27	75	46	-15	-43	10	48	-14	-8	26
		LN	52	-56	-26	-30	23	-29	-52	-8	33	-29	-14	-10
	2014	HN	-164*	-274**	-75*	-513**	-68*	-48**	-162	-60**	-70	-112**	-31	-213**
		LN	-50	123	-55	18	-22	18	-132	0.70	-28	67	-29	11

21 **Table 5** Post-silking accumulation amount, its apparent contribution to the grain, and harvest index of the nutrients under two nitrogen levels in 2 yrs.

Nutrient	Year	Treatment	Post-silking accumulation		Harvest index (%)	Nutrient	Year	Treatment	Post-silking accumulation		Harvest index (%)
			Amount (mg plant ⁻¹)	Contribution to grain (%)					Amount (µg plant ⁻¹)	Contribution to grain (%)	
N	2013	HN	1109	44	69 ^{***}	Fe	2013	HN	-5279	-145	26
		LN	861	59	74			LN	830	26	17 ^{**}
	2014	HN	1611	69	63 ^{***}		2014	HN	13049	362	15
		LN	950 [*]	66	75			LN	4074 ^{**}	193 ^{**}	14
P	2013	HN	285	52 [*]	84	Mn	2013	HN	-2027 [*]	-276	19
		LN	349	85	65 ^{**}			LN	-212	-56	17 ^{**}
	2014	HN	364	67	76		2014	HN	1553	173	14
		LN	211 [*]	59	74			LN	174	27	15
K	2013	HN	297	38	34	Cu	2013	HN	150	72	18 ^{**}
		LN	249	38	29 [*]			LN	-63	-66	29
	2014	HN	529	81	30		2014	HN	781	271	18 ^{**}
		LN	-105 [*]	-28 [*]	25 ^{***}			LN	285 [*]	147 [*]	30
Mg	2013	HN	118	57	28	Zn	2013	HN	2352	64 ^{**}	70
		LN	116	77	31			LN	3063	108	49 ^{**}
	2014	HN	398	179	19		2014	HN	3631	94	63
		LN	144 ^{**}	110 [*]	21			LN	2612	106	54 [*]
Ca	2013	HN	63	1004	2	B	2013	HN	246	74	34
		LN	64	627	2			LN	208	110	26 ^{***}
	2014	HN	390	3366	1		2014	HN	757	313	15
		LN	54 ^{**}	568 ^{***}	2			LN	181 ^{**}	89 ^{**}	18

22 Comparing N treatments, data followed by *, **, *** indicate significance at $P < 0.05$, 0.01, 0.001, respectively. Negative values indicate net loss of the indicated nutrient between silking and

23 maturity. For the contribution to the grain, higher values than 100 indicate that nutrient accumulation exceeded grain demand.