Comparing localized application of different N fertilizer species on maize grain yield and agronomic N-use efficiency on a calcareous soil

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Running title: Localized application of NH_4^+–N plus P improves maize yield and agronomic N-use efficiency

With 18 text pages, 3 tables, 4 figures

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Abstract

Localized phosphorus (P) plus nitrogen (N) fertilization results in an ammonium-dependent enhancement of maize (*Zea mays* L.) growth and nutrient uptake at the seedling stage. However, how N forms affect maize growth, grain yield components, and agronomic N-use efficiency (AEN) in field conditions is not fully understood. A 2-year field experiment with maize growing on a calcareous soil was conducted with localized application of NH_4^+–N, P (superphosphate), P plus either NO_3^-–N, NH_4^+–N or NH_4:NO_3 (1:1) at sowing and jointing as well as no N and P as control (CK). The results showed that localized supply of NH_4^+–N+P as starter fertilizer and as side-dressing at jointing increased shoot dry weight and N and P accumulation at different stages when compared with CK, localized application of P or NO_3^-–N+P in 2012 and 2013, even when NH_4^+–N was applied with nitrate (1:1, meaning NH_4^+–N was reduced by 50%) or NH_4^+–N was supplied without P. Plant growth rate (PGR) and dry matter distribution in ear at grain filling (25 days after the onset of anthesis) were higher in localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P than CK, localized P and NO_3^-–N+P treatments. The grain yield and N concentration in grain increased by 10-48% and 4-27%, respectively, in localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments compared with CK, localized P and NO_3^-–N+P in two years. The AEN and kernel number per ear were enhanced by supply of NH_4^+–N, NH_4:NO_3 (1:1)+P or NH_4^+–N+P, but not with NO_3^-–N+P. There was a positive linear correlation between kernel number per ear and PGR. The NH_4^+–N supply in localized NH_4^+–N+P treatment accounted for more than 50% of variation in PGR, shoot dry matter and grain yield at maturity in 2012 and 2013. The results indicate that localized application of P with NH_4^+–N enhances maize grain yield, kernel number per ear and agronomic N-use efficiency on calcareous soil via improving post-anthesis growth, dry matter distribution and nutrient accumulation.

Keywords: Ammonium; Phosphate; Localized nutrient supply; Maize yield; Agronomic N-use efficiency; Calcareous soil

1. Introduction

Maize is an important cereal crop worldwide. However, there are two challenges: 1) improve the grain yield to satisfy increasing human requirements, and 2) increase nitrogen (N)-use efficiency for sustainable agriculture (Ladha et al., 2005; Tilman et al., 2002; Zhang et al., 2011). In China, maize yield has increased steadily from 1.14 t ha^{-1} in 1961 to 5.26 t ha^{-1} in 2009 (Fan et al., 2011). However, over the last 10 years there was a stagnant or even declining trend of maize production in most provinces in China, with a linear increase in fertilizer consumption (Fan et al., 2011). As a result, the average agronomic N-use efficiency (AEN, kg grain yield increase per kg N applied) of maize was 11.4 kg kg^{-1} (Xu et al., 2014). In comparison, the average AEN for cereals was 21 kg kg^{-1} worldwide, with AEN reaching an average value $>$25 kg kg^{-1} in well-managed systems (Dobermann, 2007). It has been recognized that grain yield and AEN can be improved by adopting N-efficient management strategies, including the choice of forms and placement of N fertilizers, as well as the combination of P and N fertilization (Hirel et al., 2007).

Ammonium N (NH_4^+–N) and nitrate N (NO_3^-–N) are the main inorganic forms of N in soils; both forms can be taken up by plants. The preferred N form varies in different plant species, and N uptake by plants is dependent on the applied N and soil conditions. For many species, including maize, the mixture of NH_4^+–N and NO_3^-–N supports better plant growth than either form supplied separately (Marschner, 2012). Furthermore, P supply combined with ammonium (as opposed to nitrate) could increase maize shoot biomass and shoot P content (Hoffmann et al., 1994; Leonce and Miller, 1966), with increased root proliferation in the nutrient-rich patches and enhanced N and P uptake efficiency per unit of root length/biomass (Hoffmann et al., 1994; Ma et al., 2013a). Addition of ammonium fertilizer (as ammonium sulfate) in soil could temporarily hamper nitrification through inhibiting growth of ammonium-oxidizing bacteria, probably due to ammonium toxicity and local acidification (Kozlovský et al., 2009; Tong and Xu, 2012). A localized enriched zone containing both ammonium and phosphate could attract roots and enhance lateral root proliferation (Drew, 1975), suggesting that ammonium can be taken up before being nitrified. Jing et al. (2012) reported a higher proportion of NH_4-N than NO_3 in the narrow row with localized application of NH_4^+–N+P in the field during the seedling stage. Both rhizosphere acidification and high concentration of ammonium at the local site could help maintain N mainly in the ammonium form for some time. Hence, localized supply of P and NH_4^+–N may be an effective way to improve crop growth and nutrient uptake via modifying root proliferation and nutrient availability in agricultural systems (Shen et al., 2013).

In maize, total kernel number at harvest is the main yield determinant (Andrade et al., 1999). This number is dependent on several processes (such as spikelet differentiation, pollination, kernel formation and abortion), with kernel formation after silking being the most important one (Uhart and Andrade, 1995). There was a close relationship between kernel number per ear and plant growth rate (PGR) during the critical kernel establishment period around anthesis (Andrade et al., 1999; Tollenaar et al., 1992). Similarly, it was found that kernel number per ear of two maize genotypes decreased linearly with decreasing plant growth rate during the 2-week period after anthesis (Paponov et al., 2005).

Lee and Tollenaar (2007) reported that increasing the partitioning of dry matter (DM) to developing ears during the critical period of kernel establishment can increase kernel number per ear. However, another study with maize showed that increased kernel number per ear due to N fertilization was not associated with an increase in plant biomass, but was related to a rise in dry matter distribution to ears during the critical silking-to-anthesis period (Paponov et al., 2005). Furthermore, it has been recognized that increased translocation of pre-anthesis N was beneficial to grain setting (Paponov et al., 2005). A recent study also showed that new maize varieties have higher post-silking net N and P accumulation than the old varieties, which has significantly contributed to the greater grain yield of new varieties (Ning et al., 2013).

Our previous studies showed that localized application of P plus ammonium (compared to nitrate) at sowing significantly improved maize growth and nutrient uptake at the seedling stage; ammonium played a critical role in stimulating root proliferation, but the positive effect disappeared at the 10-leaf stage (Jing et al., 2010, 2012). Further study demonstrated that maize growth and nutrient uptake at later stages can be improved with repeated (at the seedling and jointing stages) localized application of ammonium and P (Ma et al., 2013b). Therefore, we hypothesized that localized supply of NH_4^+–N+P as starter fertilizer and as side-dressing at jointing could impove plant growth, nutrient accumulation and dry matter distribution at a later stage, and increase grain yield and agronomic N-use efficiency compared to

NO_3^-–N +P.

In the present study, 2-year field experiment on a calcareous soil was conducted to test the above hypothesis with localized supply of P plus NH_4^+–N, NO_3^-–N or both, and the controls as only P, only NH_4^+–N, or no N/no P. The objectives of the present study were to investigate the effect of localized supply of different N forms plus P as starter fertilizer and side-dressing at the jointing stage on (i) maize growth up to maturity, concentration and accumulation of N and P in shoot, DM distribution and PGR during the critical kernel establishment period (from anthesis to 25 days after), and (ii) on kernel number per ear, AEN, harvest index and grain yield at maturity.

2. Materials and methods

2.1. Site characterization and experimental design

Field experiments were conducted in 2012 and 2013 at the research station of China Agricultural University in Shangzhuang, Beijing, China (40°N, 116°E). Maize is a usual crop in the intensive farming systems in the area. The soil at the study site is a typical calcareous soil with bulk density 1.45 g cm^{-3} in North China Plain. The chemical properties of the 0-30 cm soil layer (before the trial) were as follows: pH 8.2 (1:5 soil:water suspension), organic carbon 8.5 g kg^{-1}, total N 0.79 g kg^{-1}, Olsen-P 8.4 mg kg^{-1} and NH_4OAc-extractable K 92 mg kg^{-1}. The weather data during maize growing season were reported by Ma et al. (2014).

Maize seeds (*Zea mays* L. cv. DH661) were sown on May 1, 2012 and May 15, 2013. The plots were seeded with hand planters and then thinned at seedling stage to density of 75,000 plant ha^{-1}. Plants were grown in alternating 30- and 50-cm-wide rows referred to as narrow and wide rows, respectively (Ma et al., 2013b). There were six twin rows in each plot (6 m long and 5 m wide). The experiment was set up in a completely randomized block design with four replicates.

The experiment consisted of six treatments in each of the two years: (1) no N and P (CK), (2) localized ammonium nitrogen (as ammonium sulfate) as starter fertilizer and as side-dressing at jointing (NH_4^+–N), (3) localized superphosphate as starter fertilizer and as side-dressing at jointing (designated P hereafter), (4) localized nitrate nitrogen (as calcium nitrate) plus superphosphate as starter fertilizer and as side-dressing at jointing (NO_3^-–N+P), (5) localized ammonium nitrate plus superphosphate as starter fertilizer and as side-dressing at jointing [NH_4:NO_3 (1:1)+P], and (6) localized ammonium nitrogen (as ammonium sulfate) plus superphosphate as starter fertilizer and as side-dressing at jointing (NH_4^+–N+P). The application rates of localized nutrients were: 165 kg N ha^{-1} and 50 kg P ha^{-1}, of which 28% N and 65% P was used as starter at sowing, and the rest was applied as side-dressing at jointing 54 days after sowing (DAS). The starter nutrients were banded locally in a narrow row 5 cm to the side and 5 cm below seeds by using a hand-furrow opener at sowing. At jointing (54 DAS), the nutrients (72% of 165 kg N ha^{-1} and 35% of 50 kg P ha^{-1}) as side-dressing were banded in a wide row 10 cm to the side of plant and 15 cm below the soil surface in both years. A digging shovel was used by hand to open fertilizer trenches, and soil was replaced in trenches after fertilization.

Pre-sowing basal fertilizers [80 kg K_2O ha^{-1} (as potassium chloride) and 15 kg Zn ha^{-1} (as $ZnSO_4{\cdot}7H_2O$)] were broadcast. In addition, 43 mm irrigation was applied on April 20, 2012. In 2013, 43 mm and 69 mm were applied on April 20 and May 17, respectively.

2.2. Plant sampling and measurements of nutrient uptake

Plants were sampled at the following growth stages in 2012 (or 2013): seedling 31 (35), jointing 51 (53), anthesis 73 (75) (separated into leaves, stem and ear), grain filling 98 (100) (separated into leaves, stem and ear) and grain maturity 140 (145) (separated into leaves, stem, cob and grain) DAS. At each sampling time, five plants per plot were cut at the stem base to measure shoot dry weight and nutrient uptake. In this paper, the term “shoot” referred to all above-ground parts of maize plants including grain.

All plant samples were exposed to 105°C for 30 min, and then over dried at 60°C for 3-7 days to achieve constant weight. Dry samples were digested using a mixture of H_2SO_4 and H_2O_2 (5:8 volume ratio, 98% w/w H_2SO_4, 30% w/w H_2O_2), and digests were analyzed for N (Kjeldahl method) by titration of the ammonia liberated by distillation with alkali, and for P by molybdo-vanadophosphate method (Johnson and Ulrich, 1959).

2.3. Kernel number per ear, agronomic N-use efficiency, grain yield and harvest index at maturity

At maturity, three twin rows in each plot (8 m^2) were harvested by hand to determine the grain yield. Kernel number per ear was counted on six corn cobs per plot. Agronomic N-use efficiency was calculated as kg grain increase produced per kg N applied. Harvest index was calculated by dividing the amount of grain yield by the total dry matter of the above-ground part at grain maturity.

2.4. Statistics

One-way and two-way ANOVA were conducted using the SAS statistical software (SAS Inst., 1999). The statistical significance of differences among means was determined by LSD at the *$P \le 0.05$* probability level.

The effects of NH_4^+–N and P on maize growth in localized NH_4^+–N+P treatment were analyzed with F-tests using sums of squares (Schmid et al., 2002).

3. Results

3.1. Shoot dry weight at different stages, plant growth rate and biomass distribution at grain filling

Localized supply of NH_4^+–N, NH_4:NO_3 (1:1)+P or NH_4^+–N+P as starter fertilizer and as side-dressing at jointing had a significant influence on maize shoot dry weight at seedling and later stages, and plant growth rate and biomass distribution at grain filling (25 days after anthesis) (Tables 1 and 2).

Shoot dry weight was significantly or at least slightly higher in the treatments of localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P than that in CK, localized P and NO_3^-–N+P treatments at seedling (31 and 35 DAS in 2012 and 2013) and jointing (51 and 53 DAS), except the treatment of localized P at 31 DAS in 2012 (Table 1). Similarly, shoot dry weight in localized NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments was markedly greater at anthesis (73 and 75 DAS in 2012 and 2013) and grain filling (98 and 100 DAS) than that in CK, localized P and NO_3^-–N+P treatments, and was higher at grain maturity (140 and 145 DAS) than that in CK and localized P treatments in both years. Localized NH_4^+–N supply increased shoot dry weight at 75, 100 and 145 DAS in comparison with CK, localized P and NO_3^-–N+P treatments in 2013, and there was an increasing trend in 2012. No significant difference was found in shoot dry weight among localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments at 51 (53), 73 (75), 98 (100) and 140 (145) DAS in 2012 (2013) (except a decrease in localized NH_4^+–N at 73 DAS in 2012).

Compared with CK, localized P and NO_3^-–N+P treatments, plant growth rate at grain filling (25 days after the onset of anthesis) in localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments increased by 19-220% and 62-111%, respectively, in 2012 and 2013 (Table 2). The amount of dry matter distributed to ears was greater in the treatments with localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P than CK, localized P and NO_3^-–N+P in 2012 (11-89%) and 2013 (46-130%). The amount of dry matter in stem and leaves was lower in the CK treatment than other five treatments in 2012. In comparison with CK, localized P and NO_3^-–N+P treatments, the amount of dry matter in leaves was greater in localized NH_4^+–N, NH_4:NO_3 (1:1) +P and NH_4^+–N+P treatments in 2013, and there was a similar trend for stems.

3.2. Nutrient (N and P) concentration and accumulation at different stages

Localized application of NH_4:NO_3 (1:1)+P or NH_4^+–N+P significantly increased N concentration in shoots at 31 (35) DAS and grain at 140 (145) DAS in 2012 (2013), and in leaves at 75 and 100 DAS in 2013 compared with CK or localized NO_3^-–N+P or P only (Fig. 1). Similarly, in comparison with CK, localized NO_3^-–N+P or P alone treatments, shoot N concentration was markedly or at least slightly greater at 31 (35), in leaves in localized NH_4^+–N at 73 (75) and 98 (100), and in grain at 140 (145) DAS in 2012 (2013), except the treatment of localized NO_3^-–N+P at 98 DAS in 2012. There was no significant difference among localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments at any of the five stages in two years.

Compared with CK, localized NH_4^+–N, NO_3^-–N+P and P only treatments, P concentration was greater in shoots with localized NH_4^+–N+P at 31 DAS in 2012, higher in shoots with localized NH_4:NO_3 (1:1)+P at 35 DAS in 2013, and higher in leaves with localized NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments at 75 DAS in 2013. No difference in P concentration was found in leaves at 98 (100) DAS and in grain at 140 (145) DAS among the six treatments in 2012 (2013).

The localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P application significantly affected N and P accumulation in shoots at different stages (Fig. 2). Compared with the CK, localized P and NO_3^-–N+P treatments, shoot N content was higher in the localized NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments at 73 (75), 98 (100) and 140 (145) DAS in 2012 (2013), and was also markedly or slightly greater in localized NH_4^+–N. No difference was found in shoot N content among localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments at 51 (53), 73 (75), 98 (100) and 140 (145) DAS in 2012 (2013).

In comparison with CK, localized NH_4^+–N, P and NO_3^-–N+P treatments, shoot P content was higher with localized NH_4^+–N+P at 31 DAS in 2012 and greater with localized NH_4:NO_3 (1:1)+P at 35 DAS in 2013 (Fig. 2). The shoot P content at 73 and

75 DAS was greater in localized NH_4^+–N, NO_3^-–N+P, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments than CK and localized P treatments. Localized supply of NH_4:NO_3 (1:1)+P or NH_4^+–N+P increased shoot P content at 98 (100) and 140 (145) DAS in 2012 (2013) compared with CK, localized NH_4^+–N, P and NO_3^-–N+P (except the treatment of localized NH_4^+–N at 100 DAS in 2013).

3.3. Maize grain yield, kernel number per ear, agronomic N-use efficiency, as well as the relationship between kernel number per ear and PGR

Maize grain yield in localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments increased by 10-47% in 2012 and 17-48% in 2013 compared with CK, localized P and NO_3^-–N+P (Table 3). Kernel number per ear showed a similar pattern as maize grain yield. Harvest index was significantly lower in the localized NO_3^-–N+P than the other five treatments in 2012, and there was also a marked to slight decrease in localized NO_3^-–N+P in 2013. Agronomic N-use efficiency was two times higher in localized NH_4^+–N, NH_4:NO_3 (1:1)+P and NH_4^+–N+P than localized NO_3^-–N+P in each of the two years.

The kernel number per ear was positively and linearly correlated with plant growth rate from anthesis to 25 days after in both years (Fig. 3).

3.4. The contributions of different factors in NH_4^+–N+P treatment on maize growth

We examined the magnitude of influence of different factors in localized NH_4^+–N+P treatment on maize growth and grain yield in calcareous soil (Fig. 4). Results showed that NH_4^+–N was the major factor influencing (i) plant growth rate at the grain-filling stage (25 days after anthesis onwards), (ii) shoot dry weight, and (iii) grain yield at maturity, which accounted for over 50% and 60% of variation, respectively, in 2012 and 2013. In contrast, P supply explained little variation (<20%) in total for these three parameters. Similarly, the interaction between NH_4^+–N and P accounted for around 10% of variation in the above parameters, except 20% in plant growth rate at the grain-filling stage in 2012.

4. Discussion

4.1. Effect of localized application of N and P on maize growth at different stages

It has been recognized that adopting N-efficient management strategies, such as optimizing the forms and rate of N fertilization, can significantly affect the growth and development of numerous species, including maize (Binder et al., 2000; Hirel et al., 2007). In agreement, we found that localized supply of NH_4^+–N+P as starter fertilizer and as side-dressing at jointing significantly increased maize shoot dry weight at seedling and later stages in two years, even when the amount of NH_4^+–N applied was reduced by 50% or NH_4^+–N was supplied without P (Table 1). In contrast, most studies evaluating the effect of nitrate:ammonium ratio were done at the early growth stage and/or in hydroponic culture. Maize had greater biomass (shoot and root) in the seedling stage in the mixed-treatment (NH_4^+–N + NO_3^-–N) when compared with the NO_3^-–N only treatment (Irshad et al., 2008). Jing et al. (2012) also reported that localized supply of NH_4^+-N+P at sowing improved maize shoot dry weight and leaf area in the seedling stage compared with localized NO_3^-–N+P, but the positive effect disappeared at the 10-leaf stage. However, repeated (at the seedling and jointing stages) localized application of ammonium and P (or NH_4^+–N only) significantly improved shoot dry weight at a later stage in the present study, indicating the importance of continuous and high supply of available soil NH_4^+–N in the localized root zone for maize growth later in the season. However, application of N as ammonium to soil does not necessarily mean ammonium is taken up, with nitrification being dependent on soil conditions. Mechanisms underpinning enhanced maize growth by addition of NH_4^+–N in calcareous soils remain to be determined in further studies.

Localized supply of NH_4^+–N, NH_4:NO_3 (1:1)+P or NH_4^+–N+P significantly increased dry matter distribution to reproductive sinks (ears) compared with localized supply of NO_3^-–N+P at grain filling (25 DAS after anthesis) in both years (Table 2). It has been reported that increasing distribution of dry matter to kernels during the critical period of kernel establishment was important and beneficial for an increase in kernel number per ear (Lee and Tollenaar, 2007; Paponov et al., 2005). The possible explanation for the positive effect of banding NH_4^+–N, NH_4:NO_3 (1:1)+P or NH_4^+–N+P on maize growth may include: (1) plants can absorb both ammonium and nitrate. However, localized application of ammonium could hamper nitrification by inhibiting ammonium-oxidizing bacteria due to ammonium toxicity and local acidification (Kozlovský et al., 2009; Tong and Xu, 2012). In the present study, despite no evident ammonium toxicity symptoms on maize, relatively high ammonium concentrations in the fertilizer bands might, at least partially, have contributed to depressed bacteria turnover and thus inhibited nitrification. Root proliferation in the local site could increase uptake of ammonium before it being nitrified. Hence, in the present study, localized application of NH_4^+–N or NH_4^+–N+P could have resulted in most N uptake by maize roots occurring as NH_4-N, (2) ammonium uptake by plants not only saved energy (10 ATP per mol N) by obviating a need for nitrate reduction, but also increased CO_2 assimilation rate in comparison with nitrate-supplied plants, thus enhancing biomass accumulation (Guo et al., 2007; Marschner, 2012; Raab and Terry, 1995), (3) increased endogenous cytokinin in maize supplied with NH_4^++NO_3^- instead of NO_3^- may be associated with enhanced dry matter distribution to grain and decreased kernel abortion (Smiciklas and Below, 1992), (4) ammonium enhanced lateral root branching in a previous study (Lima et al., 2010). In our previous studies, root proliferation in the fertilizer zone with localized NH_4:NO_3 (1:1)+P or NH_4^+–N+P compared with localized NO_3^-–N+P (Jing et al., 2012) increased nutrient acquisition via expanding root absorption surface and enhancing nutrient uptake efficiency (Ma et al., 2013a). Furthermore, localized NH_4^+–N could enhance P availability in the soil by acidifying the rhizosphere of maize during uptake and prevent the applied P from being fixed, leading to increased P accumulation and enhanced maize development (Jing et al., 2010). These results suggested that ammonium-N may play an important role in promoting vegetative growth and reproductive development of maize.

4.2. Effect of localized application of N and P on nutrient accumulation and grain yield

In the present study, compared with the localized P or NO_3^-–N+P treatments, localized supply of NH_4:NO_3 (1:1)+P or NH_4^+–N+P significantly increased N content at seedling, anthesis, grain-filling and grain maturity stages, with a significant or at least slight increase in shoot N concentration in 2012 or 2013 (Figs 1 and 2). Teyker et al. (1991) reported that, compared with application of NO_3^-–N, maize supplied with NH_4^+–N plus a nitrification inhibitor had significantly increased shoot N concentration (32%) and N content (33%). Similarly, our previous study (Jing et al., 2012) found that NH_4^+–N+P supply markedly increased N and P content compared with NO_3^-–N+P, and no significant difference was found in maize growth and nutrient uptake between the treatments of NH_4^+–N+P and NH_4^+–N+P+NI (nitrification inhibitor). Moreover, there was a higher proportion of NH_4-N compared with NO_3 in the zone with localized application of NH_4^+–N+P in the field during the seedling stage, suggesting relatively ineffective nitrification (Jing et al., 2012). Other studies on maize also showed that a NH_4^+–N source was superior to NO_3^-–N for plant growth, which may be partly attributed to substantially increased concentration and availability of soil NH_4^+–N and sparingly soluble nutrients such as P and micronutrients (Jing et al., 2010; Ma et al., 2014; Ruan et al., 2000). Hence, localized supply of NH_4^+–N, NH_4:NO_3 (1:1)+P or NH_4^+–N+P as starter and as side-dressing at the jointing stage could be an effective N-efficient management strategy for maize by improving the availability of soil N, P and micronutrients in comparison to localized NO_3^-–N+P on calcareous soils.

Translocation and recycling of N taken up before anthesis is important for supporting kernel growth (Andrade et al., 1999). In the present study, the percentage of pre-anthesis N accumulation contributing to final N contents of whole plants ranged from 65 to 69 % in 2012 and 73 to 82 % in 2013 (data not shown). The results were in accordance with those by Ning et al. (2013). In other words, only 18-35% of total N of maize plants at maturity was from post-anthesis accumulation. However, many studies have found that increased post-anthesis N accumulation significantly contributed to a greater grain yield of new compared with old maize varieties (Echarte et al., 2008; Ning et al., 2013). In the present study, there was a greater grain yield in 2012 than 2013, which was associated with increased translocation of N accumulated before anthesis, and greater N accumulation post-anthesis (Table 2, Fig. 2). In addition, well distributed and sufficient rainfall in 2012 was higher on average than that in 2013, which facilitated P uptake via improved diffusion rates and higher root length density (root length density at 0-15 cm soil depth was 2-fold greater in 2012 than 2013). We concluded that adequate rainfall in 2012 may have increased N and P accumulation and translocation of pre-anthesis N, and improved dry matter accumulation and distribution to ear (from anthesis to 25 days afterwards), leading to a higher grain yield and harvest index (Table 3) compared with that in 2013.

Localized application of NH_4^+–N, NH_4:NO_3 (1:1)+P or NH_4^+–N+P supply on calcareous soil significantly improved maize grain yield and agronomic N-use efficiency in the two years (Table 3). There was a positive relationship between kernel number per ear and maize grain yield in the present study, which is in accordance with the earlier report (Andrade, 1999). Maize grains are formed after silking, and the kernel number is determined during a critical period around silking (15 d before to

15-20 d after silking) (Uhart and Andrade, 1995). There was a liner relationship between kernel number per ear and plant growth rate during the critical 2-week period around silking (Paponov et al., 2005). In contrast, the relationship between kernel number per ear and plant growth rate at the critical period bracketing silking was curvilinear (Andrade, 1999). In the present study, the kernel number per ear was positively and linearly correlated with plant growth rate at 25 days after the onset of anthesis in both years (Fig. 3). Therefore, higher grain yield and AEN in the localized NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments could be associated with higher plant growth rate during the period after the onset of anthesis.

The possible explanation for an increased grain yield and AEN under the localized NH_4:NO_3 (1:1)+P and NH_4^+–N+P treatments may include: (1) nitrogen source can significantly affect N uptake kinetics, and maize may take up NH_4^+ more efficiently than NO_3^- [eg. higher maximum uptake velocity ($V_{\max,}$ maximum uptake velocity at saturating NH_4^+ or NO_3^- concentration), lower half-saturation constant ($K_{0.5}$) and lower $C_{\min}$ (NH_4^+ or NO_3^- concentration at which net uptake starts) for NH_4^+–N than NO_3^-–N] (Piwpuan et al., 2013), (2) localized application of NH_4:NO_3 (1:1)+P and NH_4^+–N+P can be a source of both NH_4^+–N and NO_3^-–N (due to rapid conversion of NH_4^+ to NO_3^- by nitrifying microorganisms), which could enhance plant growth by affecting the cation/anion uptake ratio and lowering the NADPH consumption (Guo et al., 2007), (3) root-soil interactions in the rhizosphere with localized supply of NH_4^+–N or NH_4^+–N+P may markedly affect plant-availability of P and micronutrients in calcareous soils (Ma et al., 2014), and (4) ammonium could play a critical role in improving plant growth rate at the grain-filling stage and enhancing accumulation and distribution of shoot biomass, leading to increased grain yield (Fig. 4). Furthermore, ammonium uptake- or/and nitrification-induced acidification, higher mobilization of soil nutrients (such as P and micronutrients) and increased N/P influx per unit root length with localized application of NH_4:NO_3 (1:1)+P or NH_4^+–N+P may stimulate uptake and accumulation of nutrients (Hoffmann et al., 1994; Jing et al., 2012; Ma et al., 2014; Ortas et al., 1996). Therefore, localized application of NH_4^+–N, NH_4^+–N+P [or NH_4:NO_3 (1:1)+P] would be an effective N-management strategy for increasing maize grain yield and agronomic N-use efficiency on calcareous soils. Further research is needed to focus on the mechanisms underlying increased maize growth and kernel number per ear as well as soil processes in the treatments with localized NH_4:NO_3 (1:1)+P or NH_4^+–N+P.

Acknowledgements

This study was supported by the National Basic Research Program (No. 973-2015CB150405) and National Natural Science Foundation of China (Nos. 31210103906, 31330070, 31121062). ZR is supported by Australian Research Council (DP130104825).

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Table 1. Shoot dry weight of maize (g plant^{-1}) at different growth stages in 2012 and 2013.

Years	**Treatments**	**Days after sowing (DAS)**				
2012		**31**	**51**	**73**	**98**	**140**
	CK	2.7±0.1d	27±2c	100±5c	149±12c	203±4c
	NH_4^+–N	4.6±0.1b	43±2ab	114±3b	224±7ab	278±13a
	P	4.2±0.2b	42±1b	105±2bc	195±8b	244±11b
	NO_3^-–N+P	3.5±0.2c	42±2b	109±3bc	195±9b	305±11a
	NH_4:NO_3 (1:1)+P	4.4±0.1b	47±2a	127±4a	239±15a	285±26a
	NH_4^+–N+P	5.4±0.2a	45±1ab	125±4a	227±9a	279±9a
2013		**35**	**53**	**75**	**100**	**145**
	CK	3.1±0.4d	26±1c	82±3d	126±7c	161±4c
	NH_4^+–N	4.8±0.4bc	42±3ab	112±3a	207±5a	219±15a
	P	4.4±0.2c	35±3b	88±6c	144±12b	188±9b
	NO_3^-–N+P	4.5±0.2c	36±2b	101±1b	148±2b	211±3ab
	NH_4:NO_3 (1:1)+P	6.2±0.3a	47±4a	118±5a	202±3a	234±4a
	NH_4^+–N+P	5.5±0.1ab	42±3ab	118±2a	204±2a	229±5a

Each value is the mean of four replicates ±SE. Different letters in each column denote significant differences among treatments at a specific growth stage ($P \leq 0.05$) in 2012 or 2013. CK: no N and P, NH_4^+–N: localized ammonium nitrogen (as ammonium sulfate and mono-ammonium phosphate) as starter fertilizer and as side-dressing at jointing, P: localized superphosphate as starter fertilizer and as side-dressing at jointing, NO_3^-–N+P: localized nitrate nitrogen (as calcium nitrate) plus superphosphate as starter fertilizer and as side-dressing at jointing, NH_4:NO_3 (1:1)+P: localized ammonium nitrate plus superphosphate as starter fertilizer and as side-dressing at jointing, and NH_4^+–N+P: localized ammonium nitrogen (as ammonium sulfate and mono-ammonium phosphate) plus superphosphate as starter fertilizer and as side-dressing at jointing. DAS: days after sowing for years 2012 (2013) [31 (35) = seedling; 51 (53) = jointing; 73 (75) = anthesis; 98 (100) = grain filling; 140 (145) = grain maturity].

Table 2. Effects of different fertilizer treatments on plant growth rate, above-ground dry matter distribution among ear, stem and leaves at grain filling (25 days after the onset of anthesis) in 2012 and 2013.

Years	**Parameters**	**Treatments**					
		CK	**NH$_4^+$–N**	**P**	**NO$_3^-$–N+P**	**NH$_4$:NO$_3$ (1:1)+P**	**NH$_4^+$–N+P**
2012	Plant growth rate (g d^{-1})	1.5c	4.4a	3.6b	3.5b	4.8a	4.3a
	Ear (g plant^{-1})	81c	136ab	122b	117b	153a	140ab
	Stem (g plant^{-1})	32b	39a	38ab	37ab	41a	43a
	Leaves (g plant^{-1})	32b	49a	44a	48a	53a	48a
2013	Plant growth rate (g d^{-1})	1.8b	3.8a	2.1b	1.9b	3.4a	3.5a
	Ear (g plant^{-1})	40c	86a	52b	59b	92a	89a
	Stem (g plant^{-1})	41b	49a	43ab	39b	49a	48a
	Leaves (g plant^{-1})	43d	55bc	50cd	52c	61ab	67a

Each value is the mean of four replicates. Different letters in each row denote significant difference among treatments in 2012 or 2013 ($P \leq 0.05$). For explanation of the treatments, see Table 1.

Table 3. Effects of different fertilizer supplies on maize grain yield, kernel number per ear, harvest index and agronomic N-use efficiency in 2012 and 2013.

Years	**Treatments**	**Grain yield** (t ha^{-1})	**Kernel number** (per ear)	**Harvest index** (%)	**Agronomic N-use efficiency** (kg grain increase kg^{-1} N applied)
2012	CK	8.5±0.5d	396±17e	56±3a	—
	NH_4^+–N	11.7±0.2ab	477±7bc	57±3a	—
	P	10.2±0.2c	454±3cd	56±2a	—
	NO_3^-–N+P	10.6±0.3bc	440±5d	47±2b	2
	NH_4:NO_3 (1:1)+P	12.5±0.4a	522±10a	59±5a	13
	NH_4^+–N+P	11.9±0.6a	500±16ab	57±3a	10
2013	CK	6.1±0.3c	383±22c	51±3abc	—
	NH_4^+–N	8.8±0.4a	515±5b	54±2a	—
	P	6.5±0.3c	421±37c	46±1bc	—
	NO_3^-–N+P	7.5±0.4b	394±35c	45±2c	6
	NH_4:NO_3 (1:1)+P	9.0±0.3a	593±22a	53±1ab	15
	NH_4^+–N+P	8.8±0.4a	522±16ab	52±3abc	14

Each value is the mean of four replicates ±SE. Different letters in each column denote significant differences among treatments at a specific growth stage ($P \le 0.05$) in 2012 or 2013. For explanation of the treatments, see Table 1. Harvest index was calculated by dividing the amount of grain yield by the total above-ground dry matter at grain maturity. Agronomic N-use efficiency was calculated as kg grain increase produced per kg N applied.

Figure legends

Fig. 1. Effects of different fertilizer supplies on N (a, c) and P concentration (b, d) in maize shoots at seedling [31 DAS in 2012 (35 in 2013)] and jointing [51 (53) DAS], in leaves at anthesis [73 (75) DAS] and grain-filling [98 (100) DAS], and in grain at maturity [140 (145) DAS] in 2012 (a, b) and 2013 (c, d). Each value is the mean of four replicates (+SE). Different lower case letters at a given growth stage denote significant differences ($P \leq 0.05$) among the treatments in 2012 or 2013. DAS: days after sowing. For explanation of the treatments, see Table 1.

Fig. 2. Effects of different fertilizer supplies on N (a, c) and P content (b, d) in maize shoots at seedling [31 DAS in 2012 (35 in 2013)], jointing [51 (53) DAS], anthesis [73 (75) DAS], grain filling [98 (100) DAS] and grain maturity [140 (145) DAS] in 2012 (a, b) and 2013 (c, d). Each value is the mean of four replicates (+SE). Different lower case letters at a given growth stage denote significant differences ($P \leq 0.05$) among the treatments in 2012 or 2013. For explanation of the treatments, see Table 1.

Fig. 3. The relationships between plant growth rate (from anthesis to 25 days after anthesis) and kernel number per ear in 2012 (a) and 2013 (b). For explanation of the treatments, see Table 1.

Fig. 4. The contribution of different factors in NH_4^+-N+P treatment on maize plant growth rate (PGR) at the grain-filling stage, as well as shoot dry weight and grain yield at maturity in 2012 (a) and 2013 (b). For explanation of the treatments, see Table 1.

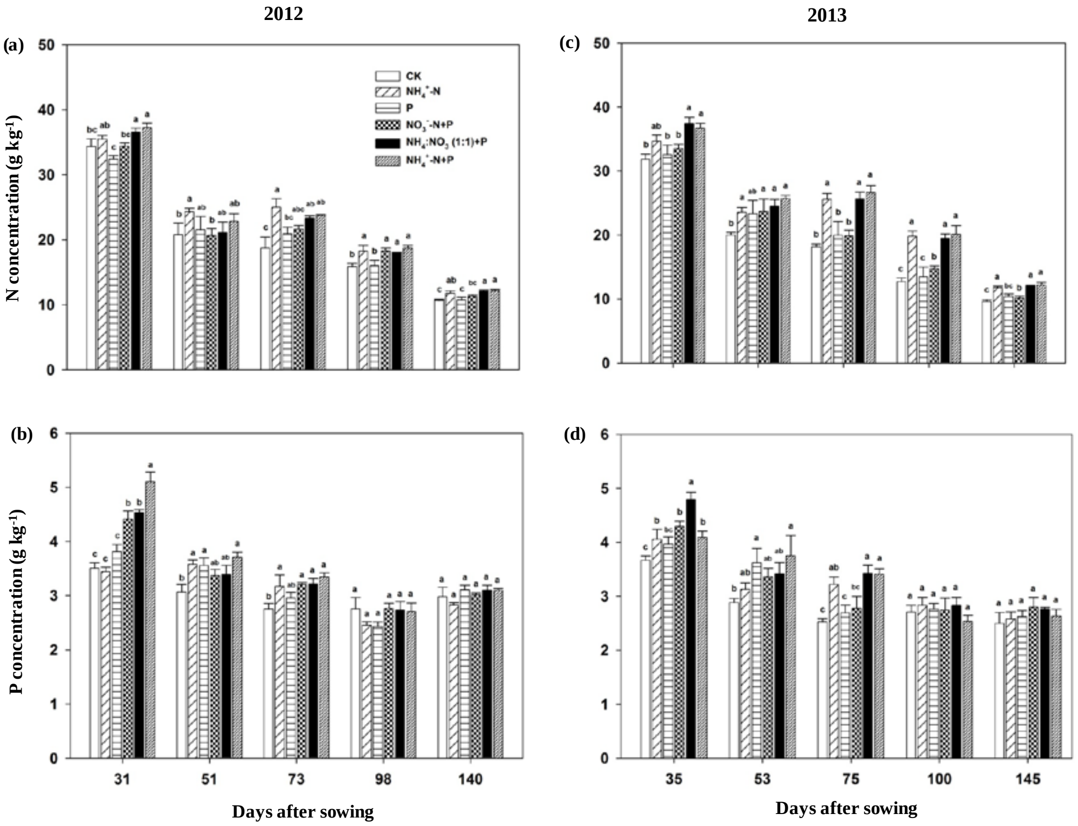
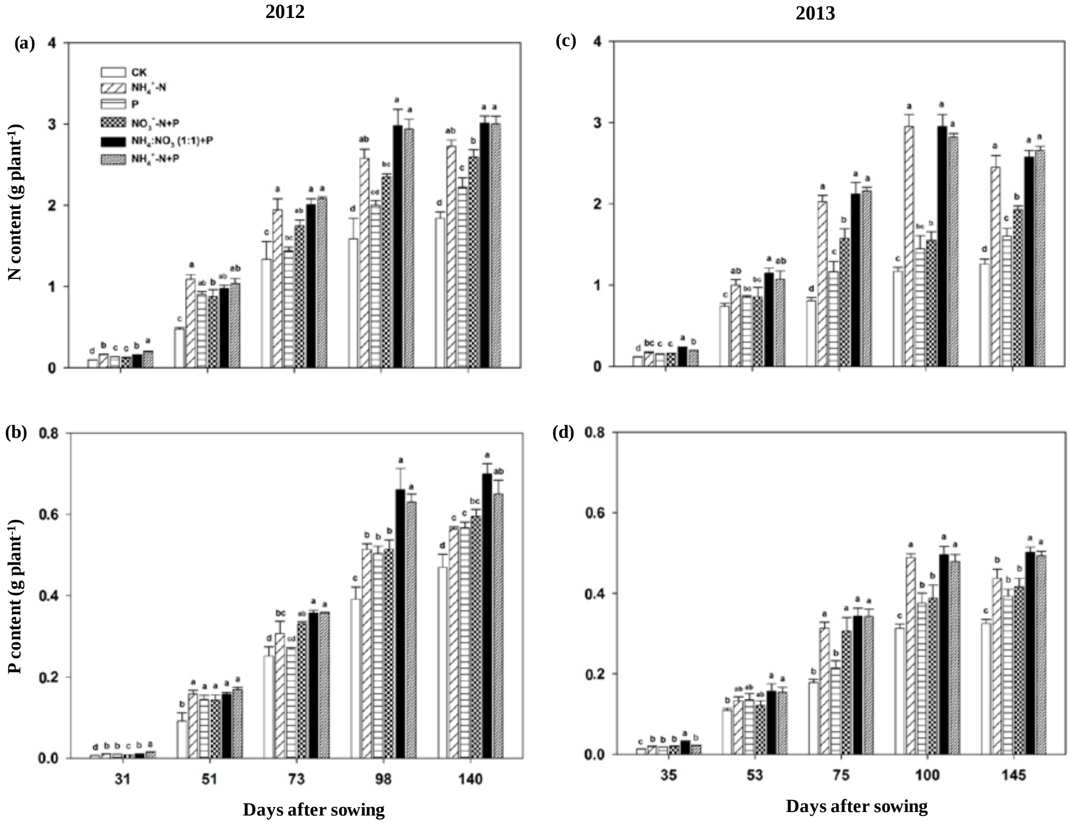

2012
2013
(a)
(b)
(c)
(d)
CK
NH$_4^+$-N
P
NO$_3^-$-N+P
NH$_4$:NO$_3$ (1:1)+P
NH$_4^+$-N+P
N concentration (g kg^{-1})
P concentration (g kg^{-1})
0
10
20
30
40
50
0
1
2
3
4
5
6
31
51
73
98
140
35
53
75
100
145
Days after sowing
Days after sowing

Fig. 1.


2012
2013
(a)
(b)
(c)
(d)
CK
NH_4^+-N
P
NO_3^--N+P
NH_4:NO_3 (1:1)+P
NH_4^+-N+P
N content (g plant^{-1})
P content (g plant^{-1})
Days after sowing
31
51
73
98
140
35
53
75
100
145

606

Fig. 2.

608

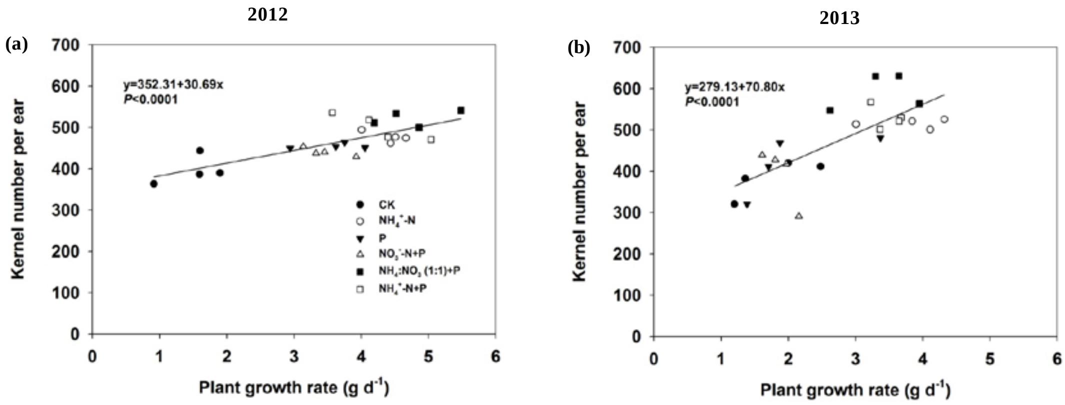
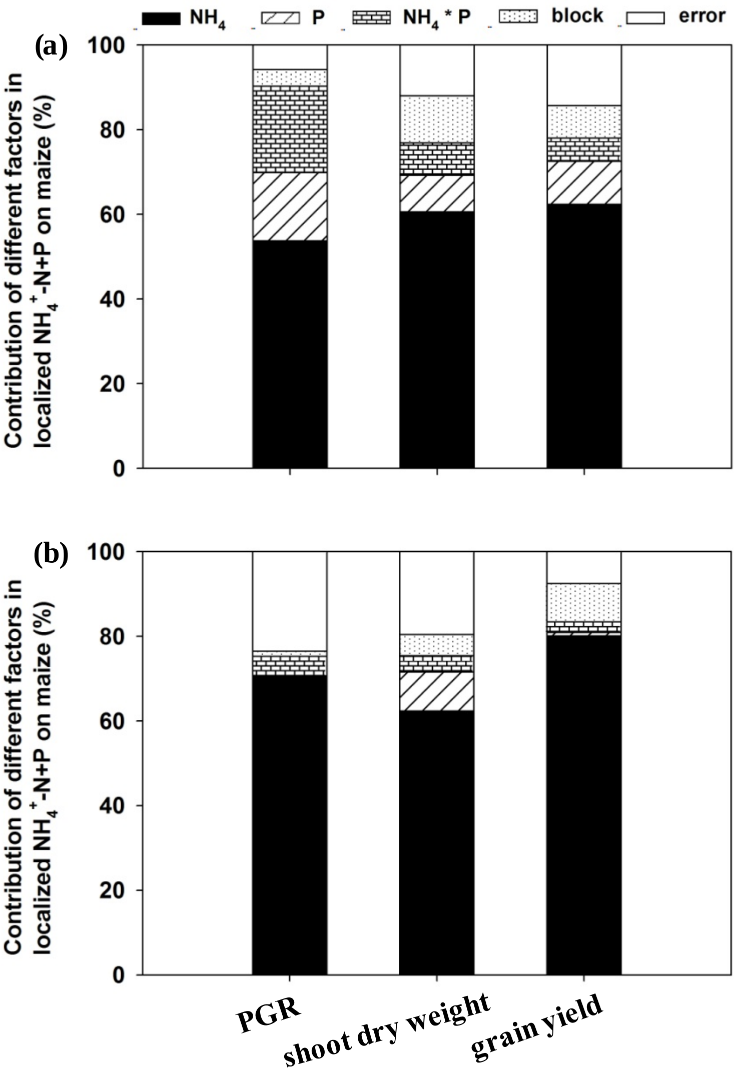

2012
(a)
y=352.31+30.69x
P<0.0001
Kernel number per ear
0
100
200
300
400
500
600
700
CK
NH4+-N
P
NO3−-N+P
NH4:NO3 (1:1)+P
NH4+-N+P
Plant growth rate (g d-1)
0
1
2
3
4
5
6
2013
(b)
y=279.13+70.80x
P<0.0001
Kernel number per ear
Plant growth rate (g d-1)

Fig. 3.


NH4
P
NH4 * P
block
error
(a)
(b)
Contribution of different factors in localized NH4+-N+P on maize (%)
0
20
40
60
80
100
PGR
shoot dry weight
grain yield

614

Fig. 4.

616