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ORIGINAL ARTICLE

Nitrogen and phosphorus nutrition and stoichiometry in the response of maize to various N rates under different rotation systems

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Abstract Better understanding of plant nutrition and nutrient interactions is of critical importance for developing best management practices in crop production. A three-year study was conducted to examine N and P nutrition and their association in maize as affected by rotation system, N application rate and hybrid maize. Rotation by N treatments were composed of maize–alfalfa (MA), maize–soybean (MS) and continuous maize (MM), fertilized in maize year at 0, 50, 100 and 150 kg N ha^{-1}, respectively. The two maize varieties were glyphosate-resistant (RR) non-Bt (non-Bt) and stacked RR + Bt near-isoline (Bt) hybrids. Our data showed that grain yield, stover, and total aboveground (or shoot) dry matter, N and P uptake (except for stover P) in amounts followed the order MA > MS > MM and were well responsive to N rates. Grain and shoot N and P contents of Bt maize was greater ($P < 0.05$) than those of non-Bt hybrid in MM. The N:P ratio was positively correlated with N application rates, and was greater in rotational maize than in MM. Both hybrids attained their maximum yields at approximately 201 kg ha^{-1} of grain N and 255 kg ha^{-1} of shoot N, corresponding to 36 and 43 kg P ha^{-1} in grain and shoot. Nitrogen harvest index, P harvest index and nutrient internal efficiency were responsive to N rates but were not different between the hybrids. This study revealed that the critical grain and shoot N content achieving maximum yield appeared to concomitantly result in high P contents.

Keywords N nutrition · N:P stoichiometry · Transgenic maize · Nutrient use efficiency · Crop rotation

Introduction

While N fertilizer is of crucial importance for increasing crop productivity, plant nutrient balance is recognized to be essential for maximizing crop growth and yield (Ciampitti and Vyn 2013). This is due to nutrient interactions that occur in soils and plants when the supply of N synergistically or antagonistically affects the absorption and utilization of other nutrients (Fageria 2001). The interactions of N with macronutrients in soils and plants have been examined and documented by Wilkinson et al. (1999) and Fageria (2001). In general, N supply was reported to increase P uptake in maize plants (Setiyono et al. 2010; Ciampitti et al. 2013). A stoichiometry study on cereal, legume and oilseed crops indicated that P uptake rather than N uptake is the main source of variations in the N:P stoichiometry (Sadras 2006). In the light of the relatively similar dilution of N and P in increasing shoot biomass, efforts have been devoted to

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establishing stoichiometric relationships between P and N concentrations in leaf and shoot biomass in maize which could then be used for in-season plant-based diagnosis of P deficiency (Belanger et al. 2011; Ziadi et al. 2007). However, the observed N and P stoichiometry expressed in both the N:P ratio and in the direct function of N uptake on P concentration (or uptake) at either the whole plant or single leaf level was not fully appraised for maize grown in contrasting cropping systems. Also, it is unclear if the responsive stoichiometry persists in modern transgenic hybrids, such as stacked glyphosate-resistant (RR) and Bt-insecticidal transgenic maize.

Cropping practices can affect soil mineral status by influencing soil physical, chemical and biological properties. It is generally acknowledged that crop rotation adds nutrients to soil, especially when legumes are included in the rotation, and/or enhances nutrient bioavailability in the soil (Sinclair and Vadez 2002; Ma et al. 2003; Riedell et al. 2009). Maloney et al. (1999) found that N uptake by maize following soybean was greater than that in continuous maize on two silt loam soils. The beneficial rotation effect of maize-soybean was not likely a result of N provided directly by soybean but rather an enhancement in the maize plant's ability to accumulate and utilize N released from soil mineralization (Ma et al. 2003; Wu et al. 2008). Riedell et al. (2009) investigated maize nutrition in response to crop rotation and N fertilizer rates and revealed significant interactions of N rate and rotation on N, P, K, Ca, Mg and Zn concentrations in plant shoots at the V12 growth stage. In spite of the well-documented effects of cropping practices on bioavailability of nutrients in soils, plant nutrient contents are mainly affected by crop uptake, which is dependent on crop genotype and uptake intensity (Rochester 2006). A high-yielding maize hybrid demonstrated a greater ability to take up soil available N later during grain-filling period with greater N use efficiency relative to conventional maize (Ma and Dwyer 1998; Ciampitti et al. 2013). Since the rapid adoption of transgenic crops (GM) in North America and worldwide in the 1990s, questions have been arising concerning the safety and nutritional balance of transgenic crops. While some studies indicate that there is no difference in N uptake between GM and non-GM crops (Ma and Subedi 2005; Subedi and Ma 2005, 2007), advancing knowledge of transgenic crop plants nutrition is still a prerequisite to fully discover the potentials in GM crop management and address any uncertainties. A recent review on GM crops (Duke et al. 2012) concluded that in most studies, mineral nutrition in GM crops is not affected by either GM trait or by application of glyphosate, although in some cases, negative effects of glyphosate on mineral nutrition in GM-soybean were reported.

Plant nutrient accumulation is genetically controlled, but affected by genotype-environment-management interactions. Rochester (2006) examined the impacts of genotype, environment, soil property and agronomic management practices on the expression of insecticidal crystalline σ-endotoxin protein (Cry1Ac) in cotton. He indicated that cultivar was the major source of variation in leaf Cry1Ac expression, and impaired crop nutrition could reduce the protein expression. On an inceptisols sub-tropical soil, Bt-cotton was found to constrain N availability but enhance P availability by regulating dehydrogenase enzyme activities and soil respiration in the rhizospheres (Sarkar et al. 2008). In maize, Bruns and Abel (2003) reported that σ-endotoxin concentration was positively associated with whole-plant N concentration. They therefore speculated that Bt hybrids acquire more N from the soil than non-Bt counterparts to synthesize the protein. However, studies with Bt versus non-Bt near-isolines grown side-by-side indicate that Bt and non-Bt hybrids were similar in leaf chlorophyll content, N concentration and content until silking (Ma and Subedi 2005; Subedi and Ma 2007).

Some recent reports appear to make a link between mineral deficiencies in GM crops with increased susceptibility to plant diseases (Yamada et al. 2009; Zobiole et al. 2010). Nevertheless, there is lack of knowledge about the interaction of N and P in plant nutrition and the response of modern transgenic maize hybrids to different N application rates, especially under contrasting crop rotation systems. It was hypothesized that Bt- and non-Bt maize hybrids would have responded similarly to N applications with similar N:P ratios under various rotation systems. Therefore, the objectives of this study were to (1) determine yield, N and P nutrient uptake responses of a roundup ready (RR) and its near-isoline stacked RR + Bt maize to N application rate under different rotation systems, and (2) quantify the relationships between grain P and N contents, shoot P and N contents, and plateau trends of grain yield over grain and shoot N contents.

Materials and methods

Experimental design

This study was imposed on a long-term rotation experiment, which was established on a Brandon loam soil (fine loamy, mixed, mesic Typic Endoaquoll), at the Central Experimental Farm of Agriculture and Agri-Food Canada in Ottawa, Ontario, Canada (45°22′N, 75°43′W) in 1992. The soil contained an average of 340, 270 and 390 g kg^{-1} of clay, silt and sand, respectively, with a water pH of 6.5 (Ma et al. 2003). The detailed soil chemical properties are given in Table 1. The original experiment consisted of an unbalanced rotation-by-N treatment and was arranged in a randomized complete block design (RCBD) with three replications. During this study (2008–2010), the original rotation-by-N treatment plot (16 m long and 9.14 m wide) was split into halves to host two maize hybrids and the experiment was considered a RCBD in split-plot arrangements. The original rotation-by-N application rate combination was considered in the main plot, and maize hybrid as the subplot. Rotation-by-N treatments were composed of maize in annual rotation with alfalfa (MA) or soybean (MS) or in a continuous monoculture (MM). In maize year, pre-plant N fertilizer was applied as urea at rates of 0 (N0), 50 (N50), 100 (N100) and 150 (N150) kg N ha^{-1}, respectively in all cropping systems, plus two additional rates of 200 (N200) and 250 (N250) kg N ha^{-1} in MM. No N fertilizer was applied on alfalfa or soybean plots, but the entire field, including alfalfa and soybean plots, was fertilized with ample P and K fertilizers according to the soil test recommendations prior to maize planting each year. In this study, all the rotation phases were included so that MA, MS and MM appeared each year. All the data collections and analysis were focused on maize crop.

The two maize hybrids tested were, ‘Pioneer 38N87’, with the stacked roundup ready (glyphosate resistant; RR) and the *Bacillus thuringiensis* (expression of the insecticidal lepidopteran-active crystalline protein (Cry1Ab) endotoxin to control European corn borer; Bt) traits (RR + Bt), and ‘Pioneer 38N85’, a near isoline hybrid containing only the RR trait (RR and non-Bt). The hybrids were planted at a density of 75,000 plants ha^{-1} on 30 May 2008, 20 May 2009 and 13 May 2010. The subplot consisted of 6 rows of maize crop with a row spacing of 0.762 m. Post-emergence glyphosate herbicides were used to control weeds. Alfalfa was harvested three times during growing season and the final cut was ploughed down after the biomass was recorded. Soybean crop was planted at 0.50 m spacing, generally about 4–7 days after maize planting. The crop was combine-harvested for grain yield and the non-seed residues were returned to the soil.

At harvest, maize grain yield was determined by harvesting the middle two rows (12.2 m^2). Grain moisture content was recorded at the time of yield determination. Grain yield was reported on a 155 g kg^{-1} water basis.

Determination of harvest index, plant N and P concentrations and calculation of N and P use efficiency indices

Shortly after physiological maturity (0 milk line or black layer stage), five maize plants in a row were sampled, separated into stover and grain, and dried in a draft-oven to a constant weight, and weighed for the determination of harvest index (HI) by dividing the grain weight over the whole plant weight. The stover and grain samples were ground first with a coarse grinding mill, and a subsample was then taken and re-ground with an analytical grain grinder to pass through a 1-mm sieve. The ground subsamples were digested by the Kjeldahl method and analyzed for N and P concentrations, with a flow injection autoanalyzer (QuikChem® 8000 Flow Injection Analyzer, Zellweger Analytics, Inc., Lachat Instruments, Milwaukee, WI, USA). Nutrient uptake was calculated on the DM basis. In this case, grain yield was converted to grain DM (kg ha^{-1}), and then multiplied by grain N (or P) concentration (g kg^{-1}) to get grain N (or P) content (kg ha^{-1}). Using the grain DM and HI data, stover DM was calculated and reported in kg ha^{-1}. Stover N (or P) content was also calculated as the product of stover DM and stover N (or P) concentration. The aboveground total DM (or shoot DM) of maize was the sum of the grain DM and stover DM, and the total N (or P) uptake or aboveground shoot N (or P) content of maize was the sum of grain N (or P) and stover N (or P) content.

Nitrogen use efficiency (NUE) was expressed as the increased grain yield per unit of fertilizer N applied, relative to N0 treatment (Ciampitti and Vyn 2012). Nutrient harvest indices of N and P (NHI and PHI) were calculated as the ratios between grain nutrient

Table 1 The initial soil physical and chemical properties of the experimental field measured in 1991

Horizon (cm)	OC (g kg^{-1})	TN (g kg^{-1})	Exchangeable cation (meq/100 g)				CEC (meq/100 g)
			Ca	Mg	K	Al	
A (24)	21.4	2.1	15.6	2.0	0.5	0.3	18.2
B (24–90)	2.7	0.3	15.0	2.1	0.7	0.07	17.8
C (>90)	0.9	0.2	10.8	2.0	0.7	0.0	13.5

contents and whole-plant nutrient contents. Nutrient internal efficiencies (NIE and PIE) were determined by dividing grain yields by whole-plant nutrient contents (Ciampitti et al. 2013).

Statistical analysis

The overall experiment was in an unbalanced factorial design with the unbalanced N level in rotational maize and monoculture maize. Therefore, the analysis of variance (ANOVA) on all the data was completed with the MIXED procedure of SAS in two steps: ANOVA was first run for the balanced rotation maize consisting of three rotation systems (MA, MS and MM), two hybrids and 4 N levels, where rotation, hybrid and N rate were considered the fixed effects, and replication, year and their interactions as random effects. After sorting the data, ANOVA was then performed on the maize monoculture where maize hybrid and N application rate (all 6 N levels) were arranged in a split-plot design with hybrid and N rate as the fixed effects and replication, year and their interactions as random effects. In each analysis, the *t* test letter grouping was done at 95 % confidence level, if the ANOVA results were significant ($P \leq 0.05$). The quantitative (linear and quadratic) relationships between N rate and dependent variables were first tested by the ESTIMATE statement in the MIXED procedure; if significant, the linear and non-linear relationships including plateau trend between variables were then established by running the NLIN procedure in SAS. All statistical analyses were performed at 5 % level of significance.

Results and discussion

Dry matter (DM) and grain yield production

In the balanced rotation, there was a significant rotation-by-N application interaction on grain yield and aboveground shoot DM production (aboveground DM or total DM) of maize (Table 2). Both grain yield and total DM production increased significantly with N application up to 150 kg N ha^{-1} in MM, but significant differences in grain and shoot DM occurred only between N0 and N50 or between N100 and N150 treatments in MS system, or in the grain, stover or shoot DM between N0 and N50 in the MA system (Table 3). Orthogonal contrasts showed a significant difference in stover, grain and shoot DM production among the three rotation systems (Table 2). At the same N input level, MA produced the greatest grain yield and shoot DM, while MM had the lowest values (Table 3). However, the difference in productivity amongst rotation systems tended to narrow with the increased N rate. For example, at N0, grain yields of maize averaged 8.60 Mg ha^{-1} in MA and 5.85 Mg ha^{-1} in MS, which were 351 and 239 %, respectively, of that in MM (2.45 Mg ha^{-1}). In contrast, at N150, the MM maize yielded 8.16 Mg ha^{-1}, or only 29 and 22 % lower than the MA and MS systems, respectively. This suggests that the beneficial effects of maize rotation with alfalfa or soybean on DM production and grain yield were progressively overridden by the increased fertilizer N input. In addition, multiple comparisons indicated, at N150, that there were no significant differences in DM or yield between MA and MS (Table 3). Polynomial contrasts demonstrated a general linear relationship between N rate with stover, grain and shoot DM production for both of the balanced rotation maize and continuous monoculture maize (Table 2).

The yield benefits of maize in MA and MS were likely due to the N credits created by the symbiotic N_2 fixation during the previous soybean and forage legume cultivation, which confirmed the findings of Ding et al. (1998) from an earlier study. The comparable maize yield and DM production in MM at N250 with rotation maize (MA or MS) at N150 (Table 3), indicated the great fertilizer N replacement value

Table 2 The ANOVA p values for the fixed effects of rotation, N application and maize hybrid on dry matter (DM) production, N and P contents and N:P ratio in stover, grain and aboveground shoot (grain + stover), N use efficiency (NUE), nutrient harvest index (NHI and PHI) and nutrient internal efficiency (NIE and PIE), for the balanced rotation maize and continuous monoculture maize (only $P < 0.05$ are presented)

Source	DM			N			P			N:P		NUE	NHI	NIE	PHI	PIE
	Stover	Grain	Shoot	Stover	Grain	Shoot	Stover	Grain	Shoot	Grain	Shoot					
Balanced rotation maize																
Rotation (R)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006	0.0003	0.0081	<0.0001	<0.0001
Hybrid (H)								0.0131					0.0016		0.0004	
R × H																
N rate (N)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001		0.0006	<0.0001	<0.0001	<0.0001
R × N	0.0004	<0.0001	<0.0001					<0.0001	0.0014			0.0216	0.0012		<0.0001	<0.0001
H × N																
R × H × N																
MM versus MA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0001	0.0036	<0.0001	<0.0001
MM vc MS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	0.0011	<0.0001		0.0021		<0.0001	<0.0001
MA versus MS	0.0210	<0.0001	0.0020	0.0003	<0.0001	0.0020	<0.0001	<0.0001	<0.0001			0.0049		0.0153		0.0011
N Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001
Monoculture maize																
Hybrid (H)					0.014	0.019		0.0107	0.0347				0.0383			
N rate (N)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
H × N																
N Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	0.0285	<0.0001	<0.0001

Table 3 The least square means (LS-means) of stover, grain yield (at 155 g kg^{-1} water) and total aboveground shoot (grain + stover) dry matter, as affected by rotation, N application rate and maize hybrid, averaged across 3 years (2008–2010)

Treatment	Stover ($Mg\ ha^{-1}$)	Grain	Shoot
Maize-alfalfa (MA)			
N0	6.2b	8.6c	13.4c
N50	7.2a	9.4bc	15.2b
N100	7.5a	9.9ab	15.8ab
N150	8.0a	10.5a	16.8a
Maize-soybean (MS)			
N0	4.7c	5.9c	9.6c
N50	6.2b	7.6b	12.6b
N100	6.6b	8.3b	13.7b
N150	7.8a	9.9a	16.2a
Maize monoculture (MM)			
N0	2.1e	2.5f	4.1e
N50	3.3d	3.6e	6.3d
N100	5.1c	6.8d	10.8c
N150	6.7b	8.2c	13.6b
N200	7.6a	9.3b	15.4a
N250	8.1a	10.4a	16.9a
Hybrid			
Pioneer 38N87 (RR and Bt-)	6.4a	8.1a	13.2a
Pioneer 38N85 (RR and Non-Bt)	6.1a	7.6a	12.5a

The least square means within a treatment in the same column followed by different letters are significantly different at the 5 % level of probability

when maize was rotated with alfalfa or soybean. This large fertilizer N replacement value could have originated directly from the legume crop breakdown or indirectly from the stimulated soil native OM mineralization. Maloney et al. (1999) and Riedell et al. (2009) reported that greater DM and grain yield produced in maize-alfalfa or -soybean rotation was attributed, in part, to a better synchrony between N mineralization and maize N uptake. The amount of N released from decomposition of native soil organic matter for plant uptake decreased over time (Raimbault and Vyn 1991; Ma et al. 1999; Wu et al. 2008), and this reduction was more severe in maize monoculture than maize-legume rotation (Ma et al. 2003), resulting in a sharp decline in productivity in unfertilized MM plots, as compared to unfertilized MA and MS plots (Table 3). The large difference in grain and total shoot DM between MM and rotational maize (MA or MS) at low N than at high N supply conditions indicate that continuous maize monoculture over 15 years since 1992 might have led to deficiency or imbalance in soil nutrients, and/or deterioration of soil physical, chemical and biological properties (Chan et al. 2013).

In this study, there was no difference in stover, grain or total shoot DM between the Bt and non-Bt hybrid maize (Table 3). Since there was no evident European corn borer (ECB) infestation observed from 2008 to 2010, the similar DM and grain yield between the hybrids in this study implies that the Bt beneficial gene was unable to enhance the crop productivity in the absence of ECB pressure. These results were in consistent with Graeber et al. (1999) and Ma and Subedi (2005), who reported that under low ECB infestation conditions, there were no benefits of Bt hybrids on grain yield and agronomic performance. This was also partially in agreement with Subedi and Ma (2007), who found a statistically significant difference between Bt and non-Bt near-isolines on grain yield but not on stover and shoot DM production.

In contrast to the reports of a significantly higher total plant DM and/or grain yield in Bt than non-Bt hybrids (Dillehay et al. 2004; Mungai et al. 2005; Fang et al. 2007), Jung and Sheaffer (2004) recorded 17.1 % less plant weight in one Bt-maize (N3030Bt) than its non-Bt near isoline (N3030). Clearly, the potential benefits of GM maize over non-GM hybrids are associated with the expression of the parental genetic backgrounds and the interaction of genetics and environment.

Nitrogen uptake

The N contents of stover, grain and the aboveground shoot in the balanced rotation maize were significantly affected by rotation system and N application rate, but not by hybrid or any interaction (Table 2). When N was applied up to 150 kg N ha^{-1}, shoot total N ranged from 106 to 174 kg N ha^{-1} in MA, 60.8 to 145 kg N ha^{-1} in MS, and 24.6 to 124 kg N ha^{-1} in MM, with larger differences in shoot N among cropping systems under low N than under high N treatments (Fig. 1). The larger maize crop N uptake under low N supply conditions in MA and MS than in MM was a clear evidence of N credits from the legume crops (Raimbault and Vyn 1991; Ding et al. 1998). Expressed as fertilizer N replacement value (FRV-N; an indicator of the amount of N requirement for monoculture maize to reach the equivalent grain yield of rotational maize at 0 N treatment), Ma et al. (2003) demonstrated that from 1993 to 1996, seasonal FRV-N was on average 68 kg N ha^{-1} for soybean and 133 kg N ha^{-1} for alfalfa in the same long-term rotation experiment. Our recent data of N uptake and maize yield confirmed the previous findings that growing maize in rotation that includes forage legume is a more sustainable practice than growing it in either monoculture or 2-year rotation with soybean (Ma et al. 2003, 2012; Ma and Biswas 2015; Riedell et al. 2009).

Under the balanced rotation system, maize hybrids did not differ ($P > 0.05$) in stover, grain and shoot N contents at physiological maturity (Table 2), reflecting the similarity in grain and DM production between the Bt and non-Bt genotypes (Table 3). In the MM system, Bt maize had higher grain and shoot N contents than the non-Bt hybrid (Table 2), while the overall difference across rotation-by-N combinations and years were not statistically significant. Nevertheless, for the monoculture maize in which two additional levels of N treatment were included, grain and shoot N contents in Bt maize were significantly ($P < 0.05$) greater than those in non-Bt maize, and the difference tended to be more evident along with the increased N rate (Table 2; Fig. 1). This suggests that there was greater N uptake and N remobilization from vegetative tissues to grain development in Bt than in non-Bt maize, though N contents in stover were comparable between the two hybrids. Subedi and Ma (2007) reported that there was

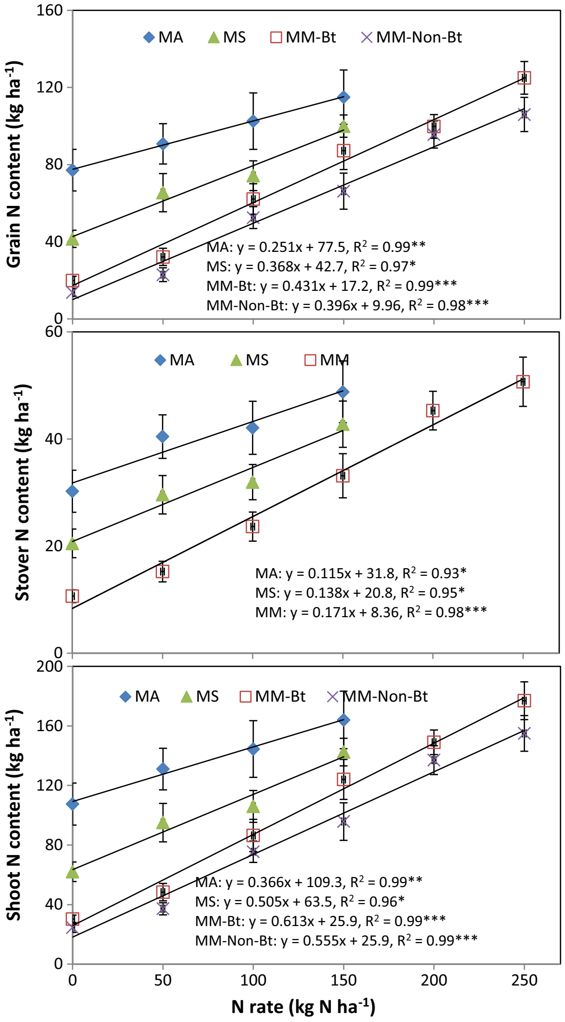

Fig. 1 Relationships of N contents in stover, grain and shoot with N application rate in maize as affected by rotation system and hybrid on a Brandon loam from 2008 to 2010. *Bars* on a marker are standard errors over nine combinations of block and year (n = 9). *MA* maize-alfalfa rotation, *MS* maize-soybean rotation, *MM* maize monoculture; *MM-Bt* Bt-maize in MM, *MM-non-Bt* non-Bt maize in MM; *single asterisk*, *double asterisks*, and *triple asterisks*, significant at $P < 0.05$, 0.01, 0.001, respectively

no indication that Bt maize accumulated more N than its non-Bt near-isoline hybrid until silking stage, and the greater N content of Bt hybrid at physiological maturity was associated with the greater DM in kernels and leaves. In the current study, the greater N contents in grain and shoot in Bt than in non-Bt maize were attributed to the combined effects of greater DM production (Table 3) and higher N concentration of the Bt hybrid (for example, Bt hybrid had an average of 1.12 % grain N, compared to 1.02 % in its near-isoline). However, neither DM nor grain N concentration differed significantly.

Phosphorus uptake

As an essential element for plant structure and energy metabolism, P in maize stover was solely affected by rotation system (Table 2), with notably greater P content (Fig. 2) in MA (6.66 kg ha^{-1}, pooled over N rate and hybrid) than in MS (4.82 kg ha^{-1}) and MM (5.22 kg ha^{-1}). Large variations led to an unclear trend of stover P with N application rates, except for a negative association between stover P contents and N application rates in MA (Fig. 2). In the balanced rotation system, rotation and N rate interactively affected maize grain and shoot P contents (Table 2), leading to the divergent responses of plant P uptake to N application. Stover and grain P contents were higher in MM than in MA and MS, although plant total P was greater in rotational than in monoculture maize (Fig. 2). This was associated with the improved soil nutrient availability in rotational maize (Ma et al. 2003; Riedell et al. 2009). For the monoculture maize, both grain and shoot P contents were affected by genotype and N rate (Table 2). Similar to the patterns in N uptake (Fig. 1), consistently greater grain and shoot P contents in Bt than its near-isoline in MM and greater grain P contents in MS or MA (Fig. 2) indicated a distinct P uptake pattern between Bt and non-Bt hybrids. This pattern likely reflected the differences in plant N uptake between the hybrids.

Several studies have reported positive interactions between N and P, which resulted in an increase in P absorption and higher crop yields (Wilkinson et al. 1999; Fageria 2001; Ciampitti et al. 2013). A number of soil and plant related mechanisms have been proposed. Wilkinson et al. (1999) reported that the enhanced P uptake in plants by N application was through increasing root growth and root's ability to absorb P and/or increasing solubility of soil P due to reduced soil pH as a result of absorption of NH_4^+. When N fertilizer is applied, it is likely that NH_4^+ ions compete with cations for fixation on interlaminar surfaces of clay minerals, beneficially releasing the P fixed on the oxide surfaces of clay minerals, thereby increasing soil P availability for plant uptake. Some

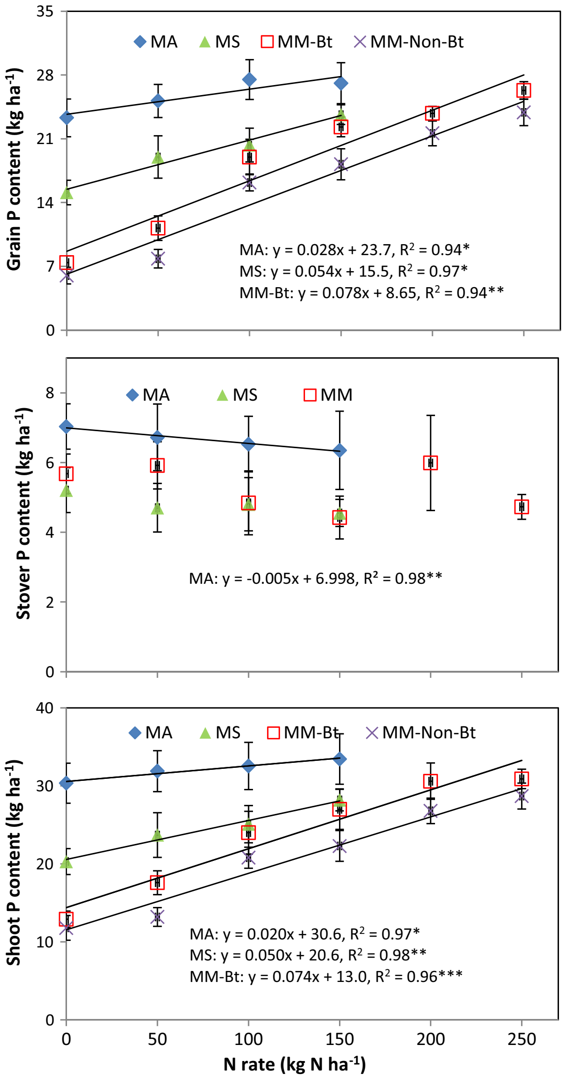

Fig. 2 Relationships of P contents in stover, grain and shoot with N application rate in maize as affected by rotation system and hybrid on a Brandon loam from 2008 to 2010. *Bars* on a marker are standard errors over nine combinations of block and year (n = 9). *MA* maize-alfalfa rotation, *MS* maize-soybean rotation, *MM* maize monoculture, *MM-Bt* Bt-maize in MM, *MM-non-Bt* non-Bt maize in MM, *single asterisk*, *double asterisks*, and *triple asterisks*, significant at $P < 0.05$, 0.01, 0.001, respectively

recent studies (Setiyono et al. 2010; Ciampitti et al. 2013) support our findings in this study that there is a positive response of plant P uptake to the amounts of N application, regardless of rotation system. The fact that grain P content at physiological maturity was significantly responsive to N rate (Fig. 2) indicates that there exists an N-fixed effect on P remobilization (Wilkinson et al. 1999).

Nitrogen and phosphorus interrelationships

The relationships between N and P were examined in two ways, specifically the N:P ratio and the P concentration (or content) as an envelope function of N concentration (or content). Grain and shoot N:P ratios followed similar trends and were affected separately by rotation system and by N rate, but not by hybrid (Table 2). At maturity, N:P ratios varied from 2.4 to 4.7 in grain and from 2.1 to 5.7 in stover and responded well to N application rates, regardless of rotation systems (Fig. 3). This reflects the higher N accumulation rate (larger slope of function) than that of P with increased N rate. For example, stover N content for MA maize increased by 0.37 kg ha^{-1} per unit N application as compared to 0.02 kg ha^{-1} of shoot P content (Figs. 1, 2). Moreover, grain and stover N:P ratios in rotational maize (MA, MS) were comparable and generally higher than those in MM, while the gap tended to diminish with increased N rate (Fig. 3). The relationship between relative yield and grain N:P ratio indicate that <4.0 of grain N:P ratio would more likely result in low relative yield (Sadras 2006; Belanger et al. 2012). In this study, out of the 252 data points across the three years, a lower grain N:P ratio of <4.0 was found in 175 cases, of which 95 % cases exhibited below 85 % of relative maize yields (data not shown). This indicates that the risk of having low relative yield is greater when N:P ratio in grain is less than 4.0, whereas this critical grain N:P ratio merits further verification in situations where different levels of N and P are examined. Also, even though a genotype effect on N and P accumulations in grain and in stover in MM system was present (Figs. 1 and 2), there was no genotype effect on N:P ratio, due to higher grain (or shoot) N and P contents in Bt maize than its near-isoline hybrid.

Recent efforts have been devoted to establishing the quantitative relationships between P and N in shoot biomass and uppermost collar leaf in maize, in the hope that this stoichiometry could be employed as an in-season plant diagnostic indicator of P deficiency (Ziadi et al. 2007; Belanger et al. 2011). In this study, the relationships between N and P in both grain and plant shoot were poor, irrespective of rotation systems (Fig. 4A, B). This was in agreement with Belanger et al. (2012) who indicated that the weak association and the small change in grain P concentration with increasing grain N concentration limited its potential use for a posteriori diagnostic assessment of P deficiency. However, this 'one-point' evaluation at physiological maturity differed from the 'time-course' appraisal of Ziadi et al. (2007) and Ciampitti et al. (2013) where a linear relationship between N and P concentrations in maize shoot was established by exploring the seasonal changes in N and P concentrations in shoot biomass.

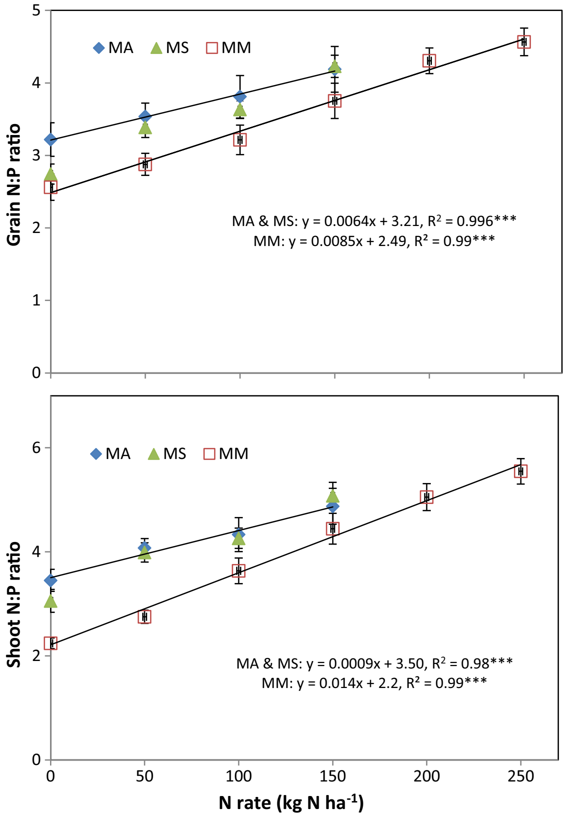

Fig. 3 Relationships of N:P ratios in grain and shoot with N application rate in maize under maize-alfalfa (MA), maize-soybean (MS) rotations and maize monoculture (MM) on a Brandon loam from 2008 to 2010. *Bars* on a marker are standard errors over nine combinations of block and year (n = 9). *Triple asterisks* significant at $P < 0.001$

When calculated on a nutrient-uptake (DM production × nutrient concentration) basis, grain and

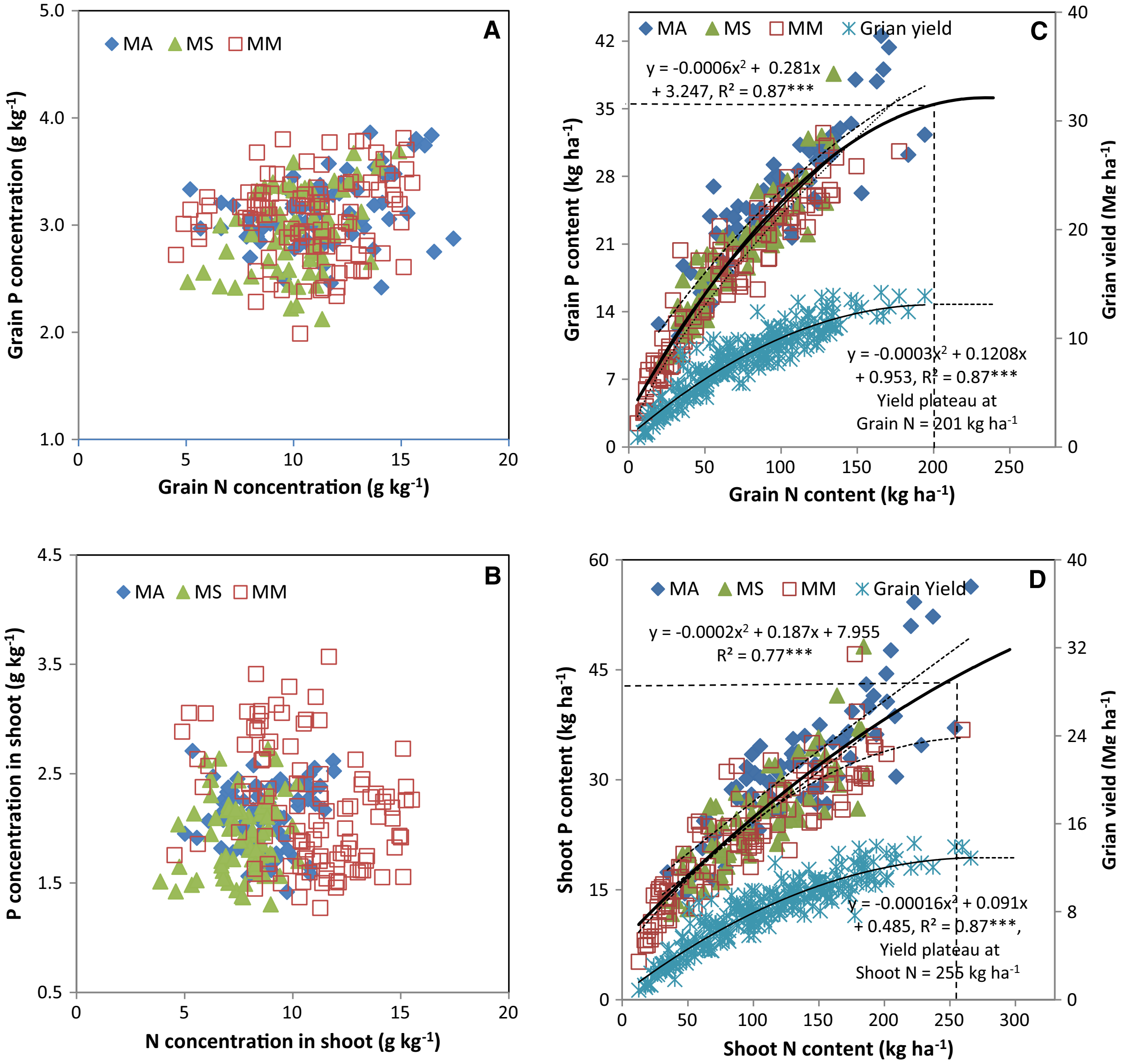

Fig. 4 Relationships between grain and shoot N and P concentrations (**A**, **B**), between grain and shoot N and P contents (**C**, **D**), and between grain yield and N contents in grain and shoot (**C**, **D**). *MA* maize-alfalfa rotation, *MS* maize-soybean rotation, *MM* maize monoculture. The *dotted curve lines* are nonlinear regression for MA (n = 72), MS (n = 72) and MM (n = 108), respectively; *solid curve lines* are nonlinear regression for all data (n = 252); *dash lines* indicate the critical N and P contents in grain and shoot that attained maximum grain yield by a plateau trend between yield and N content; *triple asterisks* significant at $P < 0.001$

shoot P contents were well related to grain and shoot N contents, with similar curvilinear trends among rotation systems (Fig. 4C, D). The curvilinear relationship between N and P uptake was evidenced earlier in Sadras (2006) and Setiyono et al. (2010). The different N and P stoichiometry in concentration and in content bases suggests that, the concurrent increases in N and P uptake with the increased N application (Figs. 1 and 2) was mainly regulated by factors other than nutrient concentration in this study. Furthermore, the relationships between grain yield and grain (and shoot) N contents demonstrated that yield reached a plateau of 13.1 Mg ha^{-1} when shoot and grain N accumulations were at 255 and 201 kg N ha^{-1}, respectively.

Table 4 The least square means (LS-means) of nitrogen use efficiency (NUE), nutrient harvest index (NHI and PHI) and nutrient internal efficiency (NIE and PIE), as affected by rotation, N application rate and maize hybrid

Treatment	NUE kg grain kg^{-1} N applied	NHI kg grain N kg^{-1} N uptake	NIE kg grain kg^{-1} N uptake	PHI kg grain P kg^{-1} P uptake	PIE kg grain kg^{-1} P uptake
Maize-alfalfa (MA)					
N0	.	0.71a	72.2a	0.77b	241b
N50	13.6a	0.69a	63.7b	0.79ab	254b
N100	9.8a	0.70a	61.2b	0.80ab	256ab
N150	10.5a	0.70a	57.5b	0.82a	273a
Maize-soybean (MS)					
N0	.	0.67b	82.3a	0.74c	243c
N50	29.2a	0.68ab	69.9b	0.80b	274b
N100	20.7a	0.70a	66.8bc	0.81ab	283ab
N150	23.0a	0.70a	59.0c	0.84a	300a
Maize monoculture (MM)					
N0	.	0.60b	74.3a	0.53c	164d
N50	18.8c	0.63b	69.9a	0.61b	191c
N100	36.4a	0.70a	72.9a	0.80a	259b
N150	32.2ab	0.69a	66.3a	0.82a	281ab
N200	28.9b	0.68a	55.5b	0.80a	282ab
N250	26.9b	0.70a	53.5b	0.84a	296ab
Hybrid					
Pioneer 38N87 (RR and Bt)	23.9a	0.69a	65.8a	0.78a	256a
Pioneer 38N85 (RR and Non-Bt)	21.6a	0.67a	66.3a	0.76a	258a

The least square means within a treatment in the same column followed by different letters are significantly different at the 5 % level of probability

Correspondingly, at the plateau yield level, shoot accumulated 43 kg P ha^{-1} and grain contained 36 kg P ha^{-1} (Fig. 4C, D). The calculated optimum N:P ratio for attaining maximum yield was about 6.0. Although these critical N, P and N:P values may be affected by other factors, including genotype-environment interactions, and need to be further verified, these values are still comparable with those reported earlier (Sadras 2006). By examining different crops, Sadras illustrated that for achieving maximum yield, all three types of crops, cereal, grain legume and oilseed, took up about 240 kg N ha^{-1} and 42 kg P ha^{-1}, with an optimum N:P ratio of 5.6 for cereal crops, including maize. In contrast, using QUEFTS model, Setiyono et al. (2010) estimated a linear increase in maize grain yield if nutrients are taken up in balanced amounts of 16.4 kg N, 2.3 kg P and 15.9 kg K Mg^{-1} of grain or an N:P ratio of 7.1. Their study involved a large database of nutrient uptake, including data obtained from irrigated and favorable rain-fed maize production environments, and large variations in N:P ratio are expected.

Nutrient use efficiencies

Nutrient use efficiency, nutrient harvest index and internal efficiency were calculated and are presented in Table 4. The NUE is an index reflecting the efficiency of using fertilizer N for grain production (Ma and Dwyer 1998). The NUE was affected by the interaction of rotation and N rate, with no difference among N treatments in MA and MS, but decreased linearly with increasing N rates in MM. The weak effect of N application on NUE for rotational maize was associated with the large N credits or FRV-N (68–133 kg N ha^{-1}) from the preceding legume crops (Ma et al. 2003). In general, NUE decreased with increasing N rates. Interestingly, NUE for N50 was lower than that of other N plots in monoculture maize. Actually, the N50 treatment was largely deficient in N nutrient in the soil after 15-year monoculture without N input. A small amount of N input (i.e., 50 kg N ha^{-1}) improved yield performance but had not led to a high NUE value, indicating N can boost carbohydrate accumulation without increasing N concentration under low N supply conditions.

Averaged across the three years, NHI varied narrowly from 0.60 to 0.71, while PHI ranged from 0.53 to 0.84 (Table 4). There were significant interactions of rotation system and N rate on these parameters (Table 1). Similar to NUE, NHI was relatively stable in MA and MS, but responsive to N rate in MM. Phosphorus is generally present as inorganic P or in mobile compounds in plants so that P is readily available for remobilization and transfer to developing seeds. PHI is commonly higher than NHI, but it is considered to be below 0.8 (Sinclair and Vadez 2002). However, in this study, we observed that it is possible to achieve >0.8 of PHI values in plots receiving 100 kg ha^{-1} or more N fertilizer (Table 4). While the overall NHI and PHI were not different significantly between Bt and non-Bt hybrids from the pooled data analysis (Table 4), NHI and PHI values differed significantly between the two hybrids when separate statistical analyses were performed for the balanced rotation and for the monoculture maize (Table 1). For example, NHI was significantly higher for Bt (0.70) than for the non-Bt maize (0.67). Similarly, the average PHI of 0.78 for Bt hybrid was statistically different from that for the non-Bt maize (Figs. 1, 2).

The NIE and PIE describe the efficiencies of crop plants using N and P for grain production. Rotation system and N treatment had significant effects separately on NIE and interactively on PIE (Table 2), with the lowest NIE in MA (63.6 kg grain kg^{-1} N uptake) and the lowest PIE in MM (224 kg grain kg^{-1} P uptake), in the balanced rotation system. The NIE and PIE were well responsive to N application rates but in opposite directions: negatively for NIE and positively for PIE. These divergent pathways were attributable to the greater amount of N uptake than of P uptake, supported by the positive relationship between plant N:P ratio and N rate (Fig. 3). Similar NIE and PIE associations as affected by N application rate were recently reported (Ciampitti et al. 2013; Caviglia et al. 2014). Using the fertility requirement modeling approach, Setiyono et al. (2010) also predicted a decrease in nutrient internal efficiencies with maize crop to approach the yield potential. There was no significant hybrid effect or any interaction on NIE and PIE.

Conclusions

This study revealed that grain yield, DM production, and N and P uptake by Bt and non-Bt maize were affected by rotation system and fertilizer N application rate, with the relative amounts of each variable in the order of MA > MS > MM. A significant genotype effect on grain and shoot N and P contents existed in MM, with greater contents in Bt than non-Bt hybrid, but this effect was not found in MA or MS. This suggests that the function of the transgene on nutrient uptake is somewhat regulated by cropping practices such as rotation systems and N application rates. Nitrogen to P ratios increased with increasing N rates, although the gap between MA and MS was leveled off at high N rates. There was a close curvilinear relationship between P and N contents in grain or in shoots. The maximum grain yield was attained at 201 kg ha^{-1} of grain N and 255 kg ha^{-1} of shoot N, corresponding to 36 kg ha^{-1} of grain and 43 kg ha^{-1} of shoot P. These critical N and P values and their potential for a posteriori nutrient diagnosis in maize warrant further studies by examining different N and P application rates under various environmental conditions. Rotation system and N rate affected nutrient use efficiency, either separately or in interaction, but the negligible difference in NUE and relatively stable NHI in rotational maize (MA and MS), indicated the great fertilizer N replacement values for maize preceded by alfalfa or soybean.

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