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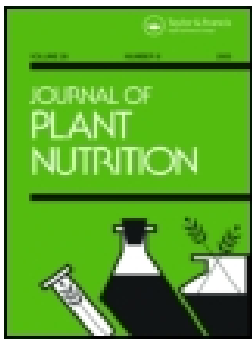
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# Slurry injection with nitrification inhibitor in maize: Plant phosphorus, zinc, and manganese status

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## ABSTRACT

Slurry injection below the maize (*Zea mays* L.) row may substitute a mineral nitrogen (N) phosphorus (P) starter fertilizer (MSF) and thus reduce nutrient surpluses in regions with intensive livestock husbandry. We investigated the plant P, zinc (Zn), and manganese (Mn) status compared to the current farm practice. In 2014 and 2015 field trials were conducted to evaluate plant nutrient status at different growth stages. Besides an unfertilized control, two slurry injection treatments ( $\pm$ nitrification inhibitor (NI)) were compared to slurry broadcast application plus MSF. In both experiments NI addition significantly increased nutrient concentrations during early growth (6-leaf 2015: +33% P, +25% Zn, +39% Mn). Under P deficiency due to cold weather conditions broadcast application showed higher P uptake until 6-leaf (36–58%), while it was lower at 8- (32%) and 10-leaf (19%) stage compared to slurry injection (+NI). Zn availability was enhanced for slurry injection (+NI) during early growth and Zn and Mn uptakes were higher at harvest. Slurry injection decreased P balances by 10–14 kg P ha<sup>-1</sup>, while Zn and Mn balances were excessive independent of treatments. Slurry injection (+NI) can substitute a MSF without affecting early growth and enhances the Zn and Mn status. This new fertilizing strategy enables farmers to reduce P surpluses.

## ARTICLE HISTORY

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## KEYWORDS

fertilizer placement; starter fertilizer; nutrient balances; rhizosphere acidification; micronutrients; recovery efficiency

## Introduction

In northwestern Germany, maize (*Zea mays* L.) is the dominating crop used as fodder for intensive livestock husbandry or as substrate for biogas production (Keckl 2015; Warnecke et al. 2011). The slurry serves as fertilizer for the next maize crop. In farm practice the slurries are broadcast applied using a splash plate or trailing hose applicator followed by immediate incorporation (e.g. using a disc harrow) prior to maize planting. In this region low temperature in April (mean air temperature 9.7°C) and May (mean air temperature 13.4°C) is critical for maize production, as optimum growth temperature for maize is about 25–30°C (Imran et al. 2013). Root zone temperatures below 15°C reduce root growth and impair chemical availability of sparingly soluble nutrients such as phosphorus (P) (Engels and Marschner 1990; Imran et al. 2013). Phosphorus deficiencies during early stages can strongly impact maize growth until harvest (Barry and Miller 1989). To overcome this critical period most farmers apply a mineral nitrogen (N) plus P starter fertilizer (MSF) at planting (Ohlrogge et al. 1957; Schröder et al. 2015). However, this current farm practice often causes P surpluses (Schröder et al.

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2011; Warnecke et al. 2011), resulting in soil P accumulation (Leinweber 1996) and as a consequence increased eutrophication of non-agricultural ecosystems due to surface runoff (Smith et al. 2001).

To reduce this nutrient surpluses, slurry injection below the maize row before maize planting to substitute the MSF is gaining interest by farmers in recent years. The addition of a nitrification inhibitor (NI) to the injected slurry seems to be useful to decrease N losses via leaching (Westerschulte et al. 2017) or denitrification (Dittert et al. 2001). Enhanced N and P nutrient use efficiencies due to slurry placement compared to broadcast application without MSF has been shown by Nkebiwe et al. (2016) and Schröder et al. (1997). With this improved nutrient efficiency, some studies suggest omitting MSF without a negative impact on final yields (Federolf et al. 2016; Schmitt et al. 1995; Schröder et al. 2015; Sutton et al. 1982). However, a better understanding is necessary of nutrient uptake processes following slurry injection with and without NI compared to broadcast application plus MSF during the early growth development and possible effects on yield and nutrient balances.

In this respect, N supply during early growth of maize was investigated in detail by Federolf et al. (2017), Sawyer et al. (1990), and Schmitt et al. (1995). Studies focusing on P supply in this context are scarcely available. Bittman et al. (2012) and Chen et al. (2010) reported that the distance between the maize row and a slurry band needs to be as small as possible, to optimize P availability for the roots and thus to avoid negative impacts on yields. However, they did not compare the injection treatments to a broadcast reference receiving a MSF. That was done by Petersen et al. (2010) for pig and cattle slurry injection in a field trial on sandy soil in Denmark. Both slurry injection treatments showed significantly lower P uptake ( $\approx 23\%$ ) during early growth compared to the reference with N P MSF, but biomass accumulation was not negatively affected. They concluded, that at that trial site P deficiency was not growth restricting during this growth period.

Using a NI leads to increased  $\text{NH}_4\text{-N}$  concentrations in the direct range of a slurry band during early growth of maize (McCormick et al. 1983; Schmitt et al. 1995; Westerschulte et al. 2017). Furthermore, it is known that an increased  $\text{NH}_4^+$  uptake by roots enhances the P availability due to reduction of the rhizosphere pH compared to uptake of  $\text{NO}_3^-$  (Hinsinger 2001; Jing et al. 2012). Thus, it seems to be possible to enhance P availability during early maize growing due to combining slurry injection with a NI.

Cold weather conditions during early growth of maize in northwestern Germany influence the availability of other nutrients. Engels and Marschner (1996) determined decreased net translocation rates of manganese (Mn) and zinc (Zn) in maize at low root zone temperatures without an effect of different shoot temperatures. This indicates a general restriction of the acquisition of these nutrients at low soil temperatures (Imran et al. 2013). On the other hand, maize is very susceptible for deficiencies of both nutrients (Fageria et al. 2002). Triggered by a fast Zn fixation after broadcast application, Zhang et al. (2013) found increased Zn uptakes of maize due to placement of  $\text{ZnSO}_4$  in a pot trial. They suggest that further investigations concerning Zn placement are necessary under field conditions. Caused by the strong pH depending solubility of these micronutrients (concentrations decrease 100-fold per pH unit; Fageria et al. 2002), NI addition may also improve their availability. Currently no studies are available, which investigated the Zn or Mn status of maize shoots after slurry injection with or without NI addition.

The objective of the present study is to examine the P, Zn, and Mn status of maize plants after slurry injection compared to broadcast application plus MSF, with a special focus on the early growth development. We hypothesize that slurry injection can substitute the mineral P fertilizer, leading to similar early growth and increased P recovery efficiencies (PRE). Furthermore, the availability of Zn and Mn will be promoted due to better spatial availability. Additionally, the effects of NI (3,4-dimethylpyrazol phosphate (DMPP)) addition to the injected slurry on availability of these nutrients were investigated. We expect that the enhanced  $\text{NH}_4^+$  uptake leads to increased availability of P, Zn, and Mn due to reduction of the rhizosphere pH.

## Material and methods

### *Experimental sites, soil characteristics, and weather conditions*

In 2014 and 2015, field trials were conducted at two adjacent fields in Hollage, Lower Saxony, north-west Germany ( $52^\circ 20\text{N}$ ,  $07^\circ 58\text{E}$ ). The altitude is  $\approx 65$  m above sea level (a.s.l.) and the region is

characterized by a maritime climate. Long term (1994–2014) mean annual precipitation is 799 mm and mean annual air temperature 10.0°C. The soil type is Plaggic Podzol (IUSS Working Group WRB 2014) with sandy soil texture at both sites (Table 1). The site in 2015 had somewhat higher organic matter and micronutrient contents.

Weather conditions during the vegetation season (April–October) were different between years. In 2014 the monthly mean temperatures were higher during the whole season (exception: August; Table 2). Thus, with respect to the early growth development of maize the thermal time from planting till the end of June was lower in 2015 (417°C) compared to 2014 (555°C) (Federolf et al. 2017). Furthermore, in 2014 precipitation events occurred regularly from April until mid-July (Table 2). The amount of precipitation decreased from the end of July and was very low in September. Per contrast, in April 2015 from the second half of May until the end of June a dry period occurred and thus, between April and the end of June 2015 the cumulative precipitation was lower by 119 mm compared to 2014. Later in the 2015 growing season the amounts of precipitation increased (Table 2). All in all warm and wet conditions were given during early growth stages of maize in 2014 compared to dry and colder conditions in 2015.

### Experimental design, treatments, and crop management

The field trial was set up in a randomized complete block design with four replicates and four treatments in both years. Each plot was 3 m wide and 25 m long with four maize rows (75 cm row spacing). The following treatments were compared:

- Control (C): without any fertilization,
- Broadcast (B): slurry application by trailing hose applicator followed by immediate incorporation with a disc harrow (0–10 cm deep; in less than 5 min after application) and additional application of a mineral, side-banded fertilizer (MSF) containing 10 kg P ha<sup>-1</sup> and 23 kg N ha<sup>-1</sup> (9.4 kg nitrate N ha<sup>-1</sup>, 13.6 kg ammonium N ha<sup>-1</sup>) at planting,
- Injection (I): slurry injection without MSF,
- Injection + NI (I(N)): slurry injection without MSF, but with addition of the NI DMPP (3,4-dimethylpyrazol phosphate; ENTEC<sup>®</sup> FL, EuroChem Agro GmbH, Mannheim, Germany) at a rate of 10 l ha<sup>-1</sup>.

This experimental design enabled a system comparison between slurry injection (with and without NI) and the current agricultural practice (broadcast). The slurry was injected by using a four row slurry injector (Xtill, Hugo Vogelsang Maschinenbau GmbH, Essen/Oldenburg, Germany) at a row spacing of 75 cm. The top of the slurry band was about 12 cm (2014) and 10 cm (2015) below the soil surface.

The fertilization rates were calculated according to the legal framework and local standards (i.e. the Chambers of Agriculture; Baumgärtel et al. 2010). Hence, the slurry application rate is defined by the recommended N rate of 180 kg N ha<sup>-1</sup> minus N applied as MSF and preplant soil mineral N (SMN; 0–60 cm; Table 1). Furthermore, site-specific conditions like recent organic fertilizer application and catch cropping are considered. This led to slurry application rates in treatments B, I, and I(N) of 23 m<sup>3</sup> ha<sup>-1</sup> in 2014 and 24 m<sup>3</sup> ha<sup>-1</sup> in 2015. Thus, the applied amount of P, Zn, and Mn depends on the nitrogen content of the pig slurry and varied slightly between the 2 years (Table 3). The omission of the MSF in the injection treatments resulted in a smaller nutrient input by 10 kg P ha<sup>-1</sup> and 23 kg N ha<sup>-1</sup>, without affecting the amount of Zn and Mn.

**Table 1.** Soil properties (soil layer 0–30 cm) for both experimental sites.

	Texture			pH CaCl <sub>2</sub>	Corg %	C/N	Total N %	P (CAL) mg 100 g <sup>-1</sup>	Zn (CAT) mg kg <sup>-1</sup>	Mn (CAT) mg kg <sup>-1</sup>	SMN <sup>1</sup> kg ha <sup>-1</sup>
	Sand %	Silt %	Clay %								
2014	91	8	1	5.3	1.14	13.0	0.09	8.0	6.5	27.7	35
2015	87	9	4	5.5	1.66	16.5	0.10	7.8	11.4	53.8	45

SMN = Soil mineral nitrogen (NH<sub>4</sub>-N + NO<sub>3</sub>-N); CAL = extracted with calcium-acetate-lactate solution; CAT = extracted with calcium chloride/DTPA solution; <sup>1</sup> = soil layer 0–60 cm.

**Table 2.** Monthly mean temperature and precipitation at the experimental sites in 2014 and 2015 compared to the long-term mean (1994–2014); mean or sum = mean or sum over growing season (April–October).

	Apr.	May	June	July	Aug.	Sept.	Oct.	Mean
Temperature (°C)								
1994–2014	9.7	13.4	16.2	18.5	17.8	14.1	10.1	14.3
2014	12.0	13.2	16.2	20.3	16.4	15.8	13.3	15.3
2015	9.0	12.3	15.9	18.9	19.3	13.5	9.1	14.0
Precipitation (mm)								Sum
1994–2014	41	59	66	76	79	79	70	457
2014	69	113	74	129	83	15	61	543
2015	54	40	43	133	187	71	62	589

The pig slurry from a regional pig fattening farm was applied on April 25 in 2014 and on April 22 in 2015 (Tables 3 and 4). About 1 week later maize (*Zea mays* L. cv. Ricardinio, KWS SAAT AG, Einbeck, Germany) was planted at a rate of 9.2 grains m<sup>-2</sup> (4.5 cm deep). For the injection treatments maize grains were positioned directly above the slurry bands. Further details concerning crop management practices are described by Federolf et al. (2017) and Westerschulte et al. (2017).

### Plant sampling and analysis

Plant samples were taken to determine the aboveground biomass and nutritional status at several developmental stages during both growing seasons (definition: Vn stage was reached when collar of n<sup>th</sup> leaf was visible after broadcast application; Table 4). In 2014 samples were taken at V6 and V10, tasseling (VT) and harvest. Based on the results in 2014, a higher sampling frequency was required to characterize the early growth. Thus, in 2015 additionally samplings were done at V3, V4, and V8. To obtain sufficient sampling material at V3 and V4 20 plants were cut at the stem base of both middle rows per plot, while for the samplings between V6 and VT 16 plants were taken. At silage maturity both middle rows were harvested over a distance of 7 m using a special plot forage harvester. For all samples fresh matter was measured and dry matter content was determined by drying a representative sample to constant weight at 80°C.

After grinding the samples (<0.5 mm), the plant material was digested using a microwave system (MARS Xpress, CEM GmbH, Kamp-Lintfort, Germany). In brief, an acid digestion with concentrated nitric acid and hydrogen peroxide was performed in a closed vessel. The temperature was raised to 180°C over a period of 25 min and held for 10 min, followed by a cool down period of 15 min. Subsequently the concentrations of P, Zn, and Mn were analyzed by using inductively coupled plasma—atomic emission spectroscopy (ICP-AES, DIN EN 15621 2012).

### Calculations and data analysis

All results presented in tables and figures are arithmetic means of the four replications. Nutrient balances and the apparent phosphorus recovery efficiency (PRE, according to Fageria 2009) were calculated

**Table 3.** Pig slurry properties and application rates.

Properties	2014	2015	Application rate	2014	2015
Total N (g kg <sup>-1</sup> )	7.2	5.4	Slurry (m <sup>3</sup> ha <sup>-1</sup> )	23	24
NH <sub>4</sub> -N (g kg <sup>-1</sup> )	5.5	3.5	NH <sub>4</sub> -N (kg ha <sup>-1</sup> )	127	84
P (g kg <sup>-1</sup> )	1.8	1.4	P (kg ha <sup>-1</sup> )	42	34
Zn (mg kg <sup>-1</sup> )	81.5	58.1	Zn (kg ha <sup>-1</sup> )	1.88	1.40
Mn (mg kg <sup>-1</sup> )	70.9	52.9	Mn (kg ha <sup>-1</sup> )	1.63	1.27
DM (%)	9.3	6.5			
C/N	4.7	4.7			
pH	7.7	7.6			

DM = Dry matter content.

**Table 4.** Plant sampling data.

	2014		2015	
	Date	DAP	Date	DAP
Slurry application	Apr. 11		Apr. 14	
Planting date	Apr. 25		Apr. 22	
V3 sampling	—		May 22	30
V4 sampling	—		Jun. 01	40
V6 sampling	Jun. 10	46	Jun. 08	47
V8 sampling	—		Jun. 19	58
V10 sampling	Jun. 30	66	Jun. 29	68
VT sampling	Jul. 22	88	Jul. 24	93
Harvest date	Oct. 09	167	Sep. 29	160

DAP = Days after planting; V3, 4, 6, 8, or 10 = vegetative leaf stage 3, 4, 6, or 10, VT = tasseling.

as follows:

$$\text{Nutrient balance} = (\text{Fertilized nutrient amount}) - (\text{Nutrient uptake})$$

and

$$\text{PRE} = \frac{(\text{P uptake of fertilized plot}) - (\text{P uptake of unfertilized plot})}{\text{Quantity of P applied}}$$

Statistical analysis were performed using R software version 3.2.2 (R Core Team, 2016). Normal distributions and variance homogeneity were tested visually using qq-plots followed by a Levene-Test ( $p < 0.01$ ). If the normal distribution or variance homogeneity of the original data was not given, a transformation (log, exp, or sin) was done, however, in all tables and figures the original values are shown. To check the interaction between year and treatment for dry matter accumulation, nutrient concentration and nutrient uptake, a linear mixed-model [lmer(parameter ~ treatment × year + (1|year:replication); package: lme4, Bates et al. 2015] was performed. For the single sampling dates the differences between the treatments (Table 5) were tested by a univariate analysis of variance (ANOVA) [aov(parameter ~ treatment + replication); package: stats, R Core Team, 2016]. When differences were considered significant ( $p < 0.05$ ), the Tukey honest significant differences (Tukey HSD,  $p < 0.05$ ) post hoc test was computed for comparing all possible pairs of means (package: agricolae, De Mendiburu 2016). The same procedure was used, to test the differences between the treatments for the nutrient balances and the PRE (Figures 1 and 2). The interaction between treatments and sampling dates for each trial year (repeated measurements over the growing season) for the dry matter accumulation, nutrient concentration, and nutrient uptake were tested by the model aov(parameter ~ treatment × sampling date + replication + error(ID)) (package: stats, R Core Team, 2016). In this respect, the sphericity was checked using the Mauchly test ( $\alpha = 5\%$ ) and if it was violated, the Greenhouse-Geisser correction was done (package: EZ, Lawrence 2013).

## Results

The interactions between treatments and years were significant ( $p < 0.05$ ) in all cases (exception: P uptake at harvest), thus in Table 5 and Figures 1 and 2 the main effects are not shown. In addition the interactions between treatments and sampling dates (repeated measurements over each growing season) were significant ( $p < 0.05$ ) for all parameters in both trial years (Table 5 and Figure 3).

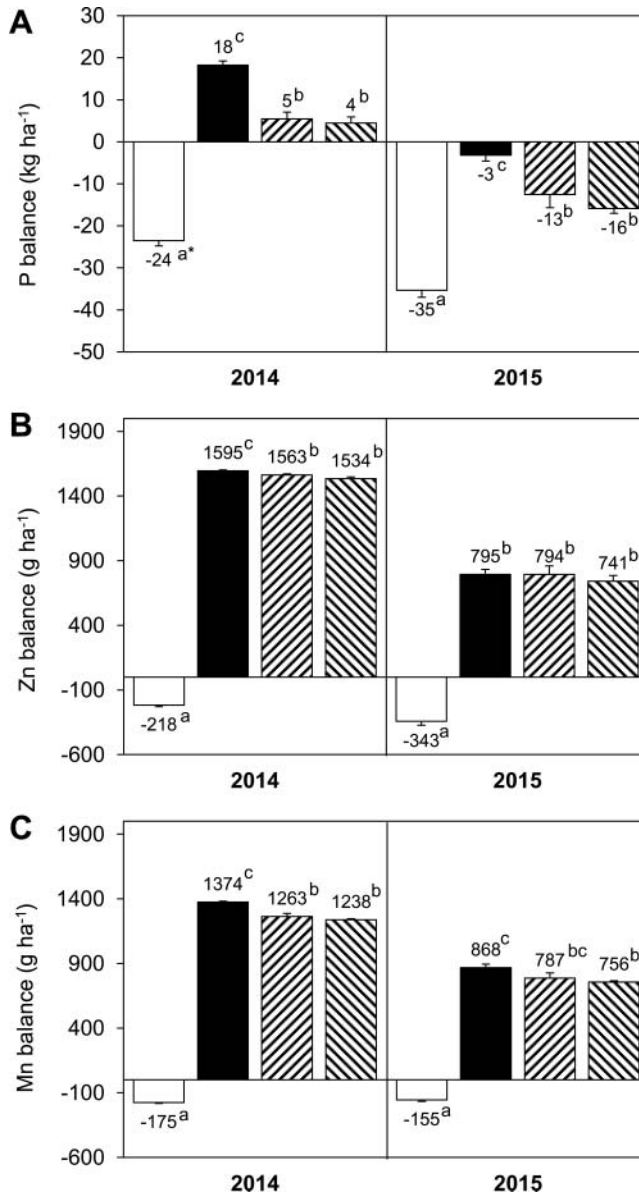
**Table 5.** Dry matter, nutrient concentration, and nutrient uptake in the field trial 2014 and 2015.

2014			V3	V4	V6	V8	V10	VT	Harvest
DM (kg ha <sup>-1</sup> )	C				169 a*		1044 a	5880 a	10291 a
	B				466 b		1860 b	8706 b	16188 b
	I				523 b		2803 c	12294 c	18134 c
	I(N)				569 b		3055 c	12835 c	18851 c
Nutrient concentration	P (g kg <sup>-1</sup> )	C			4.8 ab		3.9 b	2.7 b	2.3 b
		B			4.9 b		3.7 ab	2.6 ab	2.1 a
		I			4.5 a		3.3 a	2.2 a	2.0 a
		I(N)			5.3 c		3.6 ab	2.3 a	2.0 a
	Zn (mg kg <sup>-1</sup> )	C			27 a		32 ns	23 ns	21 b
		B			29 a		30 ns	23 ns	17 a
		I			34 b		34 ns	21 ns	17 a
		I(N)			37 c		37 ns	22 ns	18 a
	Mn (mg kg <sup>-1</sup> )	C			68 ns		42 ab	27 ns	17 ab
		B			68 ns		41 a	24 ns	16 a
		I			69 ns		45 b	28 ns	20 b
		I(N)			65 ns		52 c	28 ns	21 b
Nutrient uptake	P (kg ha <sup>-1</sup> )	C			0.8 a		4.0 a	16 a	24 a
		B			2.3 b		6.9 b	22 b	33 b
		I			2.4 b		9.2 bc	28 c	36 b
		I(N)			3.0 c		11.0 c	29 c	37 b
	Zn (g ha <sup>-1</sup> )	C			4.6 a		33 a	137 a	218 a
		B			14 b		56 b	197 b	280 b
		I			18 c		95 c	262 c	312 c
		I(N)			21 c		114 c	283 c	341 c
	Mn (g ha <sup>-1</sup> )	C			12 a		44 a	158 a	175 a
		B			32 b		76 b	211 a	256 b
		I			36 b		126 c	339 b	367 c
		I(N)			37 b		157 d	364 b	393 c
2015			V3	V4	V6	V8	V10	VT	Harvest
DM (kg ha <sup>-1</sup> )	C		8.7 ns	22 a	60 a	214 a	707 a	7509 a	16732 a
	B		9.6 ns	33 c	117 b	457 c	1617 c	10513 c	20785 b
	I		8.7 ns	23 ab	62 a	288 b	937 b	8293 ab	20077 b
	I(N)		9.9 ns	29 bc	103 b	455 c	1465 c	9212 b	20876 b
Nutrient concentration	P (g kg <sup>-1</sup> )	C	4.7 b	2.8 a	2.2 a	3.5 a	3.9 a	2.4 ns	2.1 a
		B	6.8 c	5.8 c	4.2 d	3.2 a	3.7 a	2.5 ns	2.3 ab
		I	4.2 a	3.0 a	2.7 b	4.5 b	5.0 b	2.6 ns	2.3 ab
		I(N)	4.9 b	4.0 b	3.5 c	4.7 c	5.0 b	2.4 ns	2.4 b
	Zn (mg kg <sup>-1</sup> )	C	45 a	35 a	34 a	45 a	48 a	30 a	20 a
		B	87 c	78 bc	71 b	69 b	59 b	39 b	29 b
		I	71 b	69 b	70 b	80 bc	75 c	42 b	30 b
		I(N)	87 c	86 c	86 c	86 c	68 bc	36 ab	31 b
	Mn (mg kg <sup>-1</sup> )	C	20 a	16 a	14 a	13 a	16 a	17 a	9 a
		B	88 b	97 c	90 b	71 b	67 b	31 b	19 b
		I	69 b	72 b	76 b	87 b	84 b	45 c	24 bc
		I(N)	78 b	100 c	97 b	86 b	70 b	38 bc	25 c
Nutrient uptake	P (kg ha <sup>-1</sup> )	C	0.04 ab	0.06 a	0.13 a	0.75 a	2.8 a	18 a	35 a
		B	0.07 c	0.19 c	0.49 c	1.47 b	5.9 c	26 c	47 b
		I	0.04 a	0.07 ab	0.16 a	1.28 b	4.7 b	22 ab	46 b
		I(N)	0.05 b	0.12 b	0.36 b	2.15 c	7.3 d	22 b	50 b
	Zn (g ha <sup>-1</sup> )	C	0.39 a	0.76 a	2.1 a	10 a	34 a	228 a	343 a
		B	0.84 c	2.64 c	8.3 c	32 c	96 c	406 b	600 b
		I	0.62 b	1.63 b	4.3 b	23 b	71 b	349 b	600 b
		I(N)	0.86 c	2.51 c	8.8 c	39 c	100 c	337 b	653 b
	Mn (g ha <sup>-1</sup> )	C	0.18 a	0.3 a	0.8 a	2.8 a	12 a	126 a	155 a
		B	0.85 c	3.2 c	10.6 c	33 bc	110 b	326 b	402 b
		I	0.60 b	1.7 b	4.7 b	25 b	79 b	378 b	483 bc
		I(N)	0.76 bc	2.9 c	10.0 c	39 c	103 b	353 b	514 c

C = Control treatment, B = Broadcast treatment, I and I(N) = Injection treatments without and with NI; V3, 4, 6, 8, or 10 = vegetative leaf stage 3, 4, 6, or 10, VT = tasseling;

\*Values for each parameter followed by different letters show differences according to the Tukey test ( $p < 0.05$ ) between treatments per sampling date (four values in each case), ns = not significant.



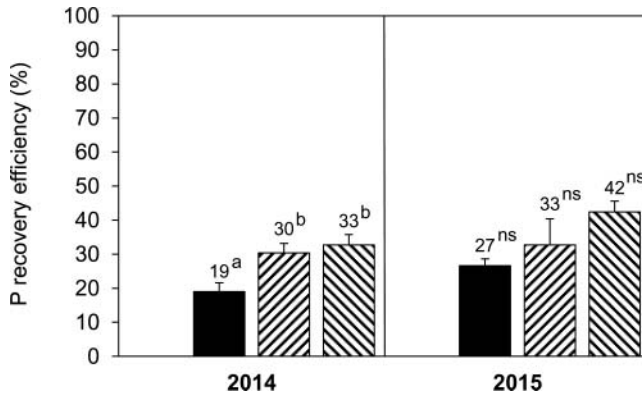


**Figure 1.** Phosphorus (A), zinc (B), and manganese (C) balances in 2014 and 2015; □ = control, ■ = broadcast, ▨ = injection without NI, ▩ = injection with NI; \*values for each trial year followed by different lowercase letters are different according to the Tukey test ( $p < 0.05$ ); whisker = standard error.

**Dry matter accumulation**

In 2014 the unfertilized control treatment showed a significantly lower dry matter (DM) accumulation compared to the fertilized treatments during the whole growing season (Table 5). No significant differences were given between the injection treatments, albeit injection with NI had slightly higher values at all sampling dates. In comparison the broadcast treatment showed significantly lower DM accumulation from V10 onward. At harvest, the DM yield of the broadcast treatment (16.2 t DM ha<sup>-1</sup>) was lower by 11–14% compared to the injection treatments [18.1 (I) and 18.9 t DM ha<sup>-1</sup> (I(N))].

In 2015 no significant differences could be detected at the first sampling date (V3). From V4 onward broadcast and injection with NI had a significantly higher DM accumulation compared to the control treatment, while the DM level after slurry injection without NI was similar to the unfertilized control



**Figure 2.** Phosphorus recovery efficiency in 2014 and 2015; □ = control, ■ = broadcast, ▨ = injection without NI, ▩ = injection with NI; \*values for each trial year followed by different letters are different according to the Tukey test ( $p < 0.05$ ); whisker = standard error.

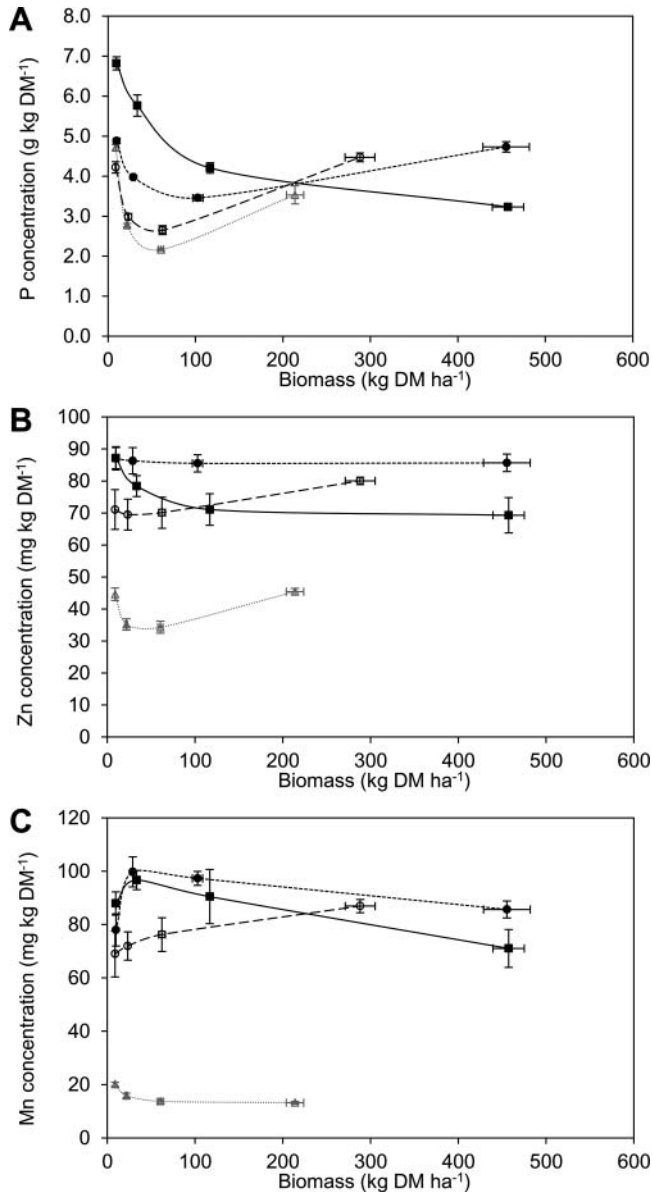
treatment until V8. Injection with NI had significantly larger DM values between V6 and V10 compared to injection without NI, but at VT and harvest there were no longer significant differences. The biomass accumulation of the broadcast treatment was similar to injection with NI during the whole growing season, with the exception that it was significantly higher by about 14% for broadcast application at VT. At harvest, for the fertilized treatments 20–21 t DM ha<sup>-1</sup> were determined, without significant differences.

### Phosphorus

The P concentrations decreased from 4.5–5.3 (V6) to 2.0–2.3 g P kg<sup>-1</sup> (harvest) for all treatments during the growing season in 2014 (Table 5). The only significant differences between the fertilized treatments occurred at V6: I(N) (5.3 g P kg<sup>-1</sup>) > B > I (4.5 g P kg<sup>-1</sup>). In the further course of the plant development, the control treatment showed the highest P concentrations. In respect to P uptake, the control treatment had the lowest and injection with NI the highest values during the whole growing season. At V6 slurry injection showed a lower P uptake by ≈20% (0.6 kg P ha<sup>-1</sup>) without using a NI, there was no longer a significant difference compared to I(N) from V10 onward. For broadcast application a significantly lower P uptake compared to I(N) occurred until VT (–7 kg P ha<sup>-1</sup> at VT). At harvest there was no significant difference between all fertilized treatments.

During the 2015 season a significantly lower P concentration was detected for slurry injection until V8, when no NI was added (Table 5). But like in the previous year no significant differences occurred from V10 onward. The broadcast treatment with additional mineral N P starter, showed the significantly highest P concentrations until V6 (+0.7–1.9 g P kg<sup>-1</sup> compared to I(N)). However, the values were significantly lower (≈1.3 g P kg<sup>-1</sup>) compared to both injection treatments at V8 and V10. In the end of the growing season (VT and harvest) significant differences occurred no longer between the fertilized treatments (harvest: 33–37 kg P ha<sup>-1</sup>). Regarding the P uptakes in 2015 no significant differences between the unfertilized control and the injection treatment without NI were detected until V6. Further onward, the control treatment had the significantly smallest uptake. Comparing the injection treatments, NI addition led to higher P uptake until V10. The broadcast treatment showed the significantly highest values until V6 and at VT (+18–58% compared to I(N)), however, at V8 and V10 it was significantly lower by 19–32% compared to I(N). As in 2014 no significant differences between the fertilized treatments were detected at harvest (46–50 kg P ha<sup>-1</sup>).

At the end of both growing seasons the P balances had the same significant differences between the treatments (Figure 1): C < I = I(N) < B. By application of slurry injection the balances were reduced by 10–14 kg P ha<sup>-1</sup>. Further on, in 2014 the PRE was significantly higher for slurry injection [+11% (I) and +14% (I(N)); Figure 2] compared to broadcast treatment (19%). In 2015 the PRE values were also higher for slurry injection [+6% (I) and +15% (I(N))],



**Figure 3.** Phosphorus (A), zinc (B), and manganese (C) concentrations depending on biomass accumulation from V3 to V8 in 2015;  $\triangle$  = control,  $\blacksquare$  = broadcast,  $\circ$  = injection without NI,  $\bullet$  = injection with NI; whisker = standard error; significant differences between treatments for all parameters at each sampling date are shown in Table 5; interactions between treatment and sampling date were significant in all cases (see section “Results”).

but due to the relative large standard error (SE) for the injection treatment, the treatment effect was not significant.

### Zinc

In 2014 plant Zn concentrations were on a similar level at V6 and V10 (27–37 mg Zn kg<sup>-1</sup>), before they decreased until harvest (17–21 mg Zn kg<sup>-1</sup>) (Table 5). Significant differences between the fertilized treatments were only present at V6: C = B (27 and 29 mg Zn kg<sup>-1</sup>) < I (34 mg Zn kg<sup>-1</sup>) < I(N) (37 mg Zn kg<sup>-1</sup>). In respect to Zn uptake the following significant differences occurred at all sampling

dates:  $C < B < I = I(N)$ . At harvest for the injection treatments  $312 \text{ g Zn ha}^{-1}$  (I) and  $341 \text{ g Zn ha}^{-1}$  I (N) were determined, while broadcast application had just a Zn uptake of  $280 \text{ g Zn ha}^{-1}$ .

During the whole growing season in 2015 the smallest Zn concentrations were detected for the unfertilized control treatment (Table 5). Already at V3 just  $45 \text{ mg Zn kg}^{-1}$  were found compared to  $71 \text{ mg Zn kg}^{-1}$  for the injection treatment and  $87 \text{ mg Zn kg}^{-1}$  for injection with NI and broadcast. In the further course of this growing season, the injection treatment with NI remained on the same level until V8, while the broadcast treatment decreased to  $69 \text{ mg Zn kg}^{-1}$  during this period. The injection treatment without NI remained at nearly  $70 \text{ mg Zn kg}^{-1}$  until V6 and increased until V8 to  $80 \text{ mg Zn kg}^{-1}$ . From V8 onward the Zn concentration declined for all fertilized treatments and were on the same level at harvest date ( $29\text{--}31 \text{ mg Zn kg}^{-1}$ ). In respect to Zn uptake in 2015 treatments showed the same significant differences between V3 and V10:  $C < I < B = I(N)$ . At VT and harvest significant differences between the fertilized treatments could no longer be detected (harvest:  $600\text{--}653 \text{ g Zn ha}^{-1}$  for fertilized treatments,  $343 \text{ g Zn ha}^{-1}$  for control treatment).

In respect to the Zn balances (Figure 1) very large surpluses were determined for all fertilized treatments in both trial years (2014:  $1.5\text{--}1.6 \text{ kg Zn ha}^{-1}$ ; 2015:  $0.74\text{--}0.80 \text{ kg Zn ha}^{-1}$ ). Injection treatments had a significantly lower surplus compared to broadcast application in 2014.

### Manganese

In 2014 the Mn concentrations decreased from  $65\text{--}69 \text{ mg Mn kg}^{-1}$  at V6 to  $16\text{--}21 \text{ mg Mn kg}^{-1}$  at harvest (Table 5). Significant differences between the treatments occurred at V10 and harvest date. At V10 injection with NI ( $52 \text{ mg Mn kg}^{-1}$ ) was significantly higher compared to injection without NI ( $45 \text{ mg Mn kg}^{-1}$ ), while after broadcast application a significantly lower Mn concentration was determined compared to both injection treatments ( $41 \text{ mg Mn kg}^{-1}$ ). At harvest, the injection treatments had the same level, while the broadcast treatment showed still significantly lower Mn concentrations. With respect to Mn uptake, the control treatment had the lowest values at all sampling dates (Table 5). The fertilized treatments did not differ at the first sampling date (V6), but further onward for the broadcast treatment significantly lower values for Mn uptake were detected compared to the injection treatments (51% at V10 and 35% at harvest compared to I(N)). NI addition just led to significantly higher Mn uptake at V10 (+25%).

During the whole growing season in 2015 the unfertilized control treatment showed the significantly lowest Mn concentrations ( $9\text{--}20 \text{ mg Mn kg}^{-1}$ ; Table 5). Comparing the fertilized treatments no significant differences occurred at V3, V6, V8, and V10 (range:  $67$  and  $97 \text{ mg Mn kg}^{-1}$ ). At V4 a significantly lower Mn concentration was detected for the injection treatment without NI ( $72 \text{ mg Mn kg}^{-1}$ ) compared to injection with NI ( $100 \text{ mg Mn kg}^{-1}$ ) and broadcast application ( $97 \text{ mg Mn kg}^{-1}$ ). At VT and harvest no significant differences were given for the injection treatments, while the broadcast treatment had significantly lower uptake. Similar to the course of the Mn concentrations, the control treatment showed the lowest uptake during the whole growing season in 2015 (Table 5). Broadcast application and injection with NI did not significantly differ until VT, however, at harvest the injection treatment with NI had significantly higher values. For slurry injection without NI significantly lower Mn uptake was given until V8. In the further course of the vegetation period this treatment rallied and at harvest it was in between the broadcast treatment and injection with NI:  $B (402 \text{ g Mn ha}^{-1}) \leq I (483 \text{ g Mn ha}^{-1}) \leq I(N) (514 \text{ g Mn ha}^{-1})$ .

Looking at the Mn balances very high surpluses for the three fertilized treatments occurred in both trial years (2014:  $1.2\text{--}1.4 \text{ kg Mn ha}^{-1}$ ; 2015:  $0.76\text{--}0.87 \text{ kg Mn ha}^{-1}$ ), but the surpluses were statistically significant lower for slurry injection (Figure 1).

### Discussion

Plant availability of soil nutrients depends extensively on annual weather conditions. This is particularly evident for the results determined in this field trial series with two very contrasting years. In 2014, maize development was greatly affected by N leaching due to heavy rainfall during the early growth

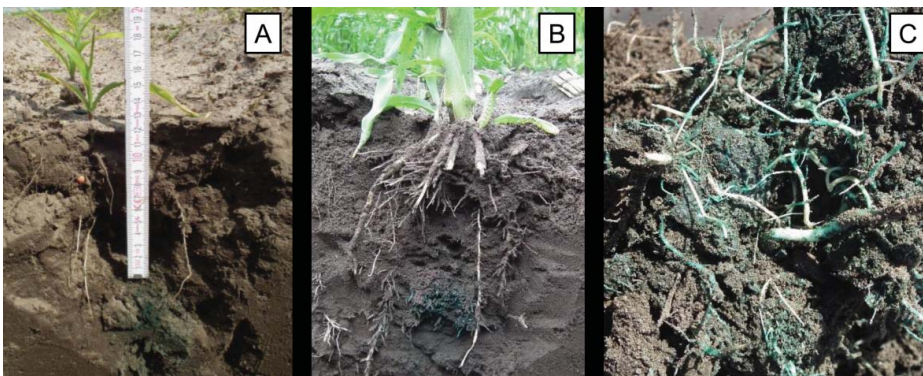
period (Westerschulte et al. 2017). A strong displacement of SMN occurred particularly after broadcast application, resulting in significantly lower DM accumulation compared to the injection treatments (Federolf et al. 2017). This different DM accumulation, induced by N deficiency, also affected the uptake of other, less mobile nutrients. By contrast, no N leaching occurred in 2015 (Westerschulte et al. 2017), because weather conditions were very dry and relatively cold from April to June (Table 2). Under these conditions, the nutrient acquisition of the young maize plants is notably limited for sparingly soluble nutrients, like P, Zn, and Mn (Imran et al. 2013; Grant et al. 2001; Engels and Marschner 1992; Engels and Marschner 1996). To compensate the lower P availability usually MSF is applied after slurry broadcast application in the current farm practice (Schröder et al. 2015; Nkebiwe et al. 2016).

### **Early growth: Effect of slurry placement**

The significantly higher Zn and Mn concentrations in the maize plants after slurry injection compared to the unfertilized control treatment at V3 in 2015 (Table 5 and Figure 4) prove, that the maize roots already took up nutrients from the slurry band, which has also been confirmed by Petersen et al. (2010) and Bittman et al. (2012). This is crucial for further plant growth, because the nutrient reserves of the maize seeds are more or less exhausted at V3–V4 (Cooper and MacDonald 1970). However, Figure 3 shows a distinctly better early growth development (V3–V8) of the broadcast treatment compared to injection without NI.

During this period significantly higher P and partially higher Zn (V3) and Mn (V4) concentrations were detected (Table 5). Campbell and Plank (2000) define sufficient ranges for P concentration of 4.0–6.0 g P kg<sup>-1</sup>, when the maize plants are < 10 cm ( $\approx$  V3 and V4) and 3.0–5.0 g P kg<sup>-1</sup> for the following growth period until VT. Thus, plant P concentration after slurry injection without NI was critical at V4 (3.0 g P kg<sup>-1</sup>) and V6 (2.7 g P kg<sup>-1</sup>). According to Barry and Miller (1989) a P deficiency during these early developmental stages can imply a negative impact on later plant growth and yield. Regarding Zn and Mn concentrations, all fertilized treatments were in an adequate or high range during the whole period. However, the so-called “adequate ranges” as defined by several references (e.g. Campbell and Plank 2000; Reuter and Robinson 1986; Fageria 2009) are fairly wide.

The improved availability of P, Zn, and Mn at early growth stages seems to induce the significantly higher DM accumulation of the broadcast treatment compared to slurry injection without NI (Figure 3). Even at V6 in 2014 the broadcast treatment had a higher P concentration compared to injection without NI (Table 5). Both treatments received a fertilizer placement (MSF or slurry band) containing N and P, which attracts maize roots (Drew 1975; Duncan and Ohlrogge 1958; Nkebiwe et al. 2016). As the availability of immobile nutrients is strongly affected by root length density (Ma et al. 2013; Neumann and Römheld 2012), a rapid and intensive root proliferation within the zone of high nutrient concentrations is necessary. Considering that the MSF is located in an area of



**Figure 4.** Root proliferation within a slurry band with nitrification inhibitor in 2015. A = 3-leaf stage; B and C = 10-leaf stage; the slurry was colored using food coloring (brilliant blue, E 133).

approximately 1 cm<sup>2</sup>, while the extent of the slurry band is at least 25 cm<sup>2</sup> ( $\approx 5 \times 5$  cm), the different spatial expansion of this placement zones in combination with the P amounts applied (10 kg (B) versus 34 kg P ha<sup>-1</sup> (I)) results in very different soil P concentrations. This might be another reason for the different availability for plants.

Furthermore, different solubility of mineral P versus slurry P might also have an influence on plant P uptake during the early growth development. However, Eghball et al. (2005) investigated the P availability from pig and cattle slurry in different soils and concluded similar plant availability of applied slurry P compared to P applied via mineral fertilizer.

Additionally, lower availability of P, Zn, and Mn could have been induced by a higher soil pH (Fageria 2009; Neumann and Römheld 2012; Syers et al. 2008) around the injected slurry (pH 7.6), while the surrounding soil had a site-typical pH of 5.5 (Table 1). Comfort et al. (1988) and Sawyer et al. (1990) showed, that pH differences between an injected slurry band (cattle or beef slurry) and the surrounding soil can be detected for several weeks.

Concentrations of P, Zn, and Mn in maize plants in the injection treatment without NI increased until V8 and reached similar (Mn and Zn) or higher (P) concentrations compared to broadcast treatment. This effect is most probably caused by an enhanced root proliferation into the slurry band and a decreasing slurry pH. However, it has to be kept in mind that these plant nutrient concentrations were strongly influenced by the distinctly lower DM accumulation (Figure 3).

### **Early growth: Effect of the nitrification inhibitor**

Due to NI addition the nutrient availability from the slurry band was enhanced during early growth stages in both trial years (Table 5). In 2015, significantly higher P, Zn, and Mn concentrations led to a much larger biomass accumulation (Figure 3), resulting in increased nutrient uptake (Table 5). These differences might be explained by several combined effects. Our previous study (Westerschulte et al. 2017) showed, that due to NI addition higher NH<sub>4</sub>-N concentrations occurred in the soil zone around the slurry band until V10. This induces the development of a more extensive fine root system (Bloom 1997; Drew 1975; Jing et al. 2012), resulting in a better availability of less mobile nutrients (Neumann and Römheld 2003).

Furthermore, the enhanced uptake of NH<sub>4</sub><sup>+</sup> compared to NO<sub>3</sub><sup>-</sup>, results in a lowering of the rhizosphere pH (Hinsinger et al. 2003; Neumann and Römheld 2003; Thomson et al. 1993). As already mentioned, the pH value in the direct range of the slurry band was most probably increased during early growth compared to the surrounding soil, leading to poor availability of P, Zn, and Mn (Neumann and Römheld 2012). The enhanced uptake of N as NH<sub>4</sub><sup>+</sup> in the treatment with NI very likely reduced the rhizosphere pH, leading to improved availability of these nutrients.

In addition, a more intensive root proliferation within the slurry band may promote nutrient availability due to enhanced root exudates (Figure 4; Hinsinger et al. 2009). For example, the secretion of carboxylates such as oxalate and citrate contributes to the acidification of the rhizosphere and furthermore mobilizes sparingly soluble P compounds. In this context, a root-induced enzymatic or chemical (e.g., excreted phenolics) Mn reduction as well as Mn reducing microorganisms could explain the higher Mn concentrations of the plants in the injection treatment with NI at V10 in 2014 (Table 5) and during early growth in 2015 (Figure 3). According to Neumann and Römheld (2003) the exudation of mucilage can facilitate the transport of Zn to the root surface, especially under dry conditions, like in the 2015 season.

### **Early growth: Slurry injection with nitrification inhibitor versus broadcast application**

Due to the above-mentioned improved nutrient availability, the injection treatment with NI showed an equivalent biomass development compared to the broadcast treatment during early growth stages in 2015 (Table 5 and Figure 3). A noticeable trend was given for the Zn concentrations from V3 until V8 (Figure 3). Starting at about 87 mg Zn kg<sup>-1</sup>, the concentration for the injection treatment with NI remained on the same level until V8, while it dropped down to a significantly lower level (69 mg Zn kg<sup>-1</sup>) for the broadcast treatment. A similar trend was given for the Mn concentration, but without a

significant difference (Figure 3). The placement of these nutrients in the slurry band with NI, seems to result in a better spatial availability (Figure 4) compared to broadcast application.

The plant P availability was also significantly lower for the injection treatment with NI until V6 (Table 5). At V4 (4.0 g P kg<sup>-1</sup>) and V6 (3.5 g P kg<sup>-1</sup>) P concentrations were close to the critical range according to Campbell and Plank (2000) (<4.0 g P kg<sup>-1</sup> (V4) or <3.0 g P kg<sup>-1</sup> (V6)). But from V6 onward, this treatment showed an increasing trend, leading to significantly higher P concentrations and uptake at V8 and V10 (Figure 3 and Table 5). At V8 the P concentration in the broadcast treatment (3.2 g P kg<sup>-1</sup>) was close to the lower boundary of the sufficient range (Campbell and Plank 2000). This reverse trend can be explained by the increased root proliferation into the slurry band during the early growth period (Figure 4), resulting in an enhanced P availability. On the other hand, Federolf et al. (2017) and Westerschulte et al. (2017) could show, that the nitrogen, applied via MSF, was completely consumed by the plants until V8. Thus, it can be assumed that the positive interaction of the combined placement of N and P did no longer persist (Jing et al. 2010; Ma et al. 2013). However, there was still enough N (even as NH<sub>4</sub><sup>+</sup>) and P around the slurry band in the injection treatment with NI (Westerschulte et al. 2017). Because roots are mainly located below the maize row ( $\pm 15$  cm) until V10 (Schröder et al. 1997), most of the slurry nutrients applied in the interrow space of the broadcast treatment seem to be less available until this date.

In 2015 maize plants reached V8 and V10 after a severe dry period (Table 2; Westerschulte et al. 2017). In this context it should be kept in mind, that the injected slurry was located somewhat deeper ( $\approx 5$  cm) compared to the MSF, most probably associated with higher soil moisture. Additionally, the slurry band has a higher water retention capacity due to a larger organic matter content related to the surrounding soil (Comfort et al. 1988). Both aspects result in a higher P solubility and thus an improved availability (Neumann and Römheld 2003). During further plant development, rainfall was sufficient (Table 2, July 2015) and the roots increasingly exploit into the interrow space (Lichtenegger et al. 2009). Thus, P applied via broadcast slurry became increasingly available, explaining the higher P uptake and DM accumulation at VT in 2015.

### **Nutrient status and balances at harvest**

At later growth stages differences between the fertilized treatments decreased in both years (Table 5). The effect of the NI on plant P, Zn, or Mn status during the early growth stages finally did not result in any significant differences between the injection treatments at harvest (Table 5). Compared to broadcast application slurry injection resulted in equal (2015) or even significantly higher (2014) DM yields. The lower input of P ( $-10$  kg P ha<sup>-1</sup>) combined with similar P uptake until harvest (Table 5) resulted in higher apparent PRE (+6–15%; Figure 2). These results are consistent with findings by Schröder et al. (2015). In addition, slurry injection led to significantly lower P balances by 10–14 kg P ha<sup>-1</sup> (Figure 1). Thus, this fertilizing strategy seems to be a suitable option for the farmers in northwest Germany to lower the partly high P surpluses (Warnecke et al. 2011). In 2014, higher Zn uptake following slurry injection remained significant until harvest, however, the differences were not significant at harvest date in 2015. The Mn uptake was higher for slurry injection at harvest in both field trials (Table 5). These results suggest, that slurry placement compared to broadcast application leads to a better availability of the applied micronutrients, due to the above mentioned increased spatial availability.

Independently of the fertilizing scheme extraordinary high surpluses for Mn (756–1374 g Mn ha<sup>-1</sup>) and Zn (741–1595 g Mn ha<sup>-1</sup>) appeared (Figure 1). Thus, repeated application of pig slurry, containing high Zn and Mn contents, lead to an excessive accumulation of these elements over years (Mattias et al. 2010; Nicholson et al. 2003). Zn mainly accumulates in form of hydroxides in the topsoil layer (L'Herroux et al. 1997), with possible long-term negative impact on plants and microorganisms (Mattias et al. 2010). High Mn surpluses increase the carbonate Mn fraction and may result in increasing leachate Mn concentrations (L'Herroux et al. 1997).

In conclusion, slurry injection instead of a MSF can result in critical plant P, Zn, and Mn status during early growth of maize under conditions, which limit the root growth and nutrient solubility. The

addition of a NI does distinctly enhance the nutrient availability resulting in similar early growth like for broadcast application. Thus, farmers who need to decrease their P surpluses can substitute the MSF by slurry injection combined with a NI. However, both fertilization strategies showed advantages and disadvantages depending on weather conditions and growth stages. An improved nutrient supply during early maize growth may possibly be assured by slurry injection plus a reduced rate of N and P via MSF, which might even be applied directly into the seed furrow. It has to be kept in mind that on sites with high soil nutrient contents the impact of adverse growing conditions during the early maize development on final yield is rather small.

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