

**Slurry injection to optimize
nutrient use efficiency in maize:
Soil nitrogen dynamics and plant nutrient status**

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List of Abbreviations

a.s.l.	above sea level
ADP	adenosine diphosphate
AMO	ammonia monooxygenase
ANOVA	analysis of variance
ATP	adenosine triphosphate
AU	auger
AUA	agriculturally used area
AU _{bSM3}	auger below the third soil monolith
AU _{tsb}	auger through slurry band
B	broadcast
BGR	Bundesanstalt für Geowissenschaften und Rohstoffe
BMR	below maize row
C	control
CAN	calcium ammonium nitrate
Cfb	warm temperate, fully humid, warm summer (according to Köppen-Geiger climate classification)
CV	coefficient of variance
d	range of error expressed as a fraction of the mean per plot
DAA	days after application
DAI	days after injection
DAP	days after planting
DCD	dicyandiamide
DM	dry matter
DMPP	3,4-dimethylpyrazol phosphate
DüV	Düngeverordnung (= 'Fertilizer Application Ordinance')
EU	European Union
HSD	honest significant difference
I	injection
I(N)	injection + NI
ICP-AES	inductively coupled plasma - atomic emission spectroscopy
IRS	interrow space
IUSS	International Union of Soil Sciences
LS	Lower Saxony (= 'Niedersachsen')
LWK	Landwirtschaftskammer (= 'Chamber of Agriculture')

M	middle
MSF	mineral starter fertilizer
MW	megawatt
NEC	national emissions ceiling
NI	nitrification inhibitor
NRE	nitrogen recovery efficiency
NRW	Nordrhein-Westfalen (= 'North Rhine-Westphalia')
NUE	nutrient use efficiency
NVZ	nitrate vulnerable zone
PRE	phosphorus recovery efficiency
Rep	replication
S	slurry
SH	Schleswig-Holstein
SM	soil monolith
SMN	soil mineral nitrogen
SOM	soil organic matter
T	top
Vn	vegetative leaf stage; n th leaf visible
VT	tasseling stage
WRB	world reference base
Zn	zinc

Chapter 1

General Introduction

1.1 Background and objectives

Biogeochemical flows of nitrogen (N) and phosphorus (P) are listed among the top nine planetary boundaries relating to manmade environmental changes. Both plant nutrients have a very high risk of affecting the performance of the earth system (Steffen et al. 2015). Thus, closing these nutrient cycles is a key factor for sustainable land use.

The release of plant available nutrient forms from organic manures after field application is difficult to predict (Bachmann et al. 2011; Gutser et al. 2005; Schröder et al. 2005). Consequently, many farmers apply excessively high amounts of manure and often use additional mineral fertilizers. This may lead to nutrient surpluses and thus to increased pollution of non-agricultural ecosystems, groundwater, and the atmosphere (Buckwell and Nadeu 2016; Dungait et al. 2012).

Project background

Agriculture in northwestern Germany is dominated by intensive livestock husbandry and biogas production (Guenther-Lübbbers and Theuvsen 2015; Otten 2013). The accumulating liquid manure is mainly used for the fertilization of maize. Usually these slurries are applied broadcast using a trailing hose applicator followed by incorporation into topsoil. To overcome the limited nutrient availability during the early growth stages a mineral N P starter

fertilizer (MSF) is mostly added at planting. This fertilizing scheme often leads to high nutrient surpluses. The research project '*Optimizing the nitrogen and phosphate use efficiencies from liquid manure by slurry injection to reduce environmental pollution*' performed at the University of Applied Sciences Osnabrück focused on developing a holistic fertilizing strategy based on slurry injection below the maize row to substitute the MSF. It was conducted in cooperation with the Chambers of Agriculture of North Rhine-Westphalia, Lower-Saxony, and Schleswig-Holstein. The present thesis is based on data collected in the context of this project.

Objectives

The main objective of this thesis is the comparison of the new approach to fertilization, 'slurry injection below the maize row' and the current fertilizing strategy in farm practice with respect to:

- (I) Characterization of the spatial and temporal soil mineral nitrogen (SMN) dynamics with special focus on changes in the direct range of the slurry band.
- (II) Evaluation of the plant phosphorus, zinc, and manganese status, highlighting the early growth development of maize.

Furthermore, the effect of the addition of a nitrification inhibitor (NI) into the slurry band is investigated regarding both of the above issues.

1.2 Structure and methodology of the thesis

Following the general introduction, Chapter 2 'Slurry injection: Soil mineral nitrogen and plant nutrient status' includes three peer-reviewed publications. This is followed by the general discussion and conclusion.

After slurry injection below the maize row, accurately locating the fertilizer band from above the soil surface is not feasible. Thus, standardized soil sampling strategies are not suitable to take samples from the direct range of

the slurry band. For this reason a new sampling strategy was developed to enable a reliable characterization of the spatial and temporal SMN dynamics after slurry injection (published in *Journal of Plant Nutrition and Soil Science* 178, 923–934). To achieve this, three consecutive field trials were conducted. Subsequently, this sampling strategy was used to characterize the SMN dynamics after slurry injection (+/- NI addition) compared to the current regional farm practice (published in *Nutrient Cycling in*

Agroecosystems 107, 1–17). Therefore, in 2014 and 2015 trials with a randomized complete block design were conducted at two adjacent fields. In addition to soil sampling, plant samples were simultaneously collected from these trials at several dates throughout the whole maize growing season in order to characterize the crop development. The results of the plant nitrogen status were also published in *Nutrient Cycling in*

Agroecosystems (107, 19–31) as a co-authored paper (abstract in the annex). Aside from the nitrogen dynamics, the plant phosphorus, zinc, and manganese status is important for evaluating the different fertilizing schemes. Thus, based on the aforesaid plant samples, this topic was investigated and evaluated in context of the 3rd publication (accepted for publication by *Journal of Plant Nutrition*).

1.3 Situation in northwestern Germany

Natural environment

According to the Köppen-Geiger climate classification, northwestern Germany is located in the warm temperate climate zone, fully humid, with warm summers (Cfb; Peel et al. 2007). The long-term average precipitation (1980 – 2010) ranges from about 740 – 880 mm per year (May to September: \approx 320 – 390 mm) and the long-term average air temperature (1980 – 2010) varies between 8.6 – 10.4°C (May to September: 13.8 – 15.4°C; Federolf et al. 2016).

The study region, which covers parts of the Federal States NRW, LS, and SH, can be characterized by three characteristics: prevalent sandy soils, large accumulation of liquid manures, and maize cropping. The main natural areas from the south to the north within this region are: the Ost-, Kern-, and Westmünsterland, which are part of the Westfälische Bucht, the Dümmer-Geestniederung, the Ems-Hunte-Geest, the Ostfriesisch-Oldenburgische Geest, the Stader Geest, and the Schleswig-Holsteinische Geest (Meynen et al. 1959).

Geologically and morphologically the region is characterized by the base moraine plates and the terminal moraine as well as the lowlands and glacial valleys within the old moraine areas of northern Germany (BGR 2008). The most dominant soil types are Haplic or Gleyic Podzols and Dystric or Stagnic Cambisols, and in some smaller areas Haplic Arenosols (BGR 2005). Figure 1 depicts the texture of the topsoils within the region, which is predominantly pure sand

(ss) or loamy sand (ls). The soil value with respect to the agronomic yield potential, which ranges from 0 to 100, is mostly less than 40 (Roßberg et al. 2015).

Land use: Livestock husbandry, biogas production, and maize cropping

Due to the predominantly sandy soils yield potential for most arable crops was rather low and, consequently, livestock husbandry has always had a major agro-economic share in northwestern Germany (Otten 2013). Additionally, during the course of the structural changes in German agriculture, which were characterized by specialization and intensification, livestock husbandry gained further in importance in the region (Bäurle and Tamásy 2012). To support this production, enterprises in upstream and downstream sectors were established (Deimel and Theuvsen 2010). As a result of this historic development, the agriculture and food sector evolved to be the most important economic sector in northwestern Germany (Otten 2013).

In relation to the total German livestock husbandry, about 53% of the cattle (BMEL 2016), 63% of all pigs (Statistisches Bundesamt 2016) and 64% of the poultry production (LfL 2013) are currently located in NRW, LS, and SH. Within these three Federal States, the livestock density is highest in the area north of the Ruhr region, in western LS and in northwestern SH (Figure 2).

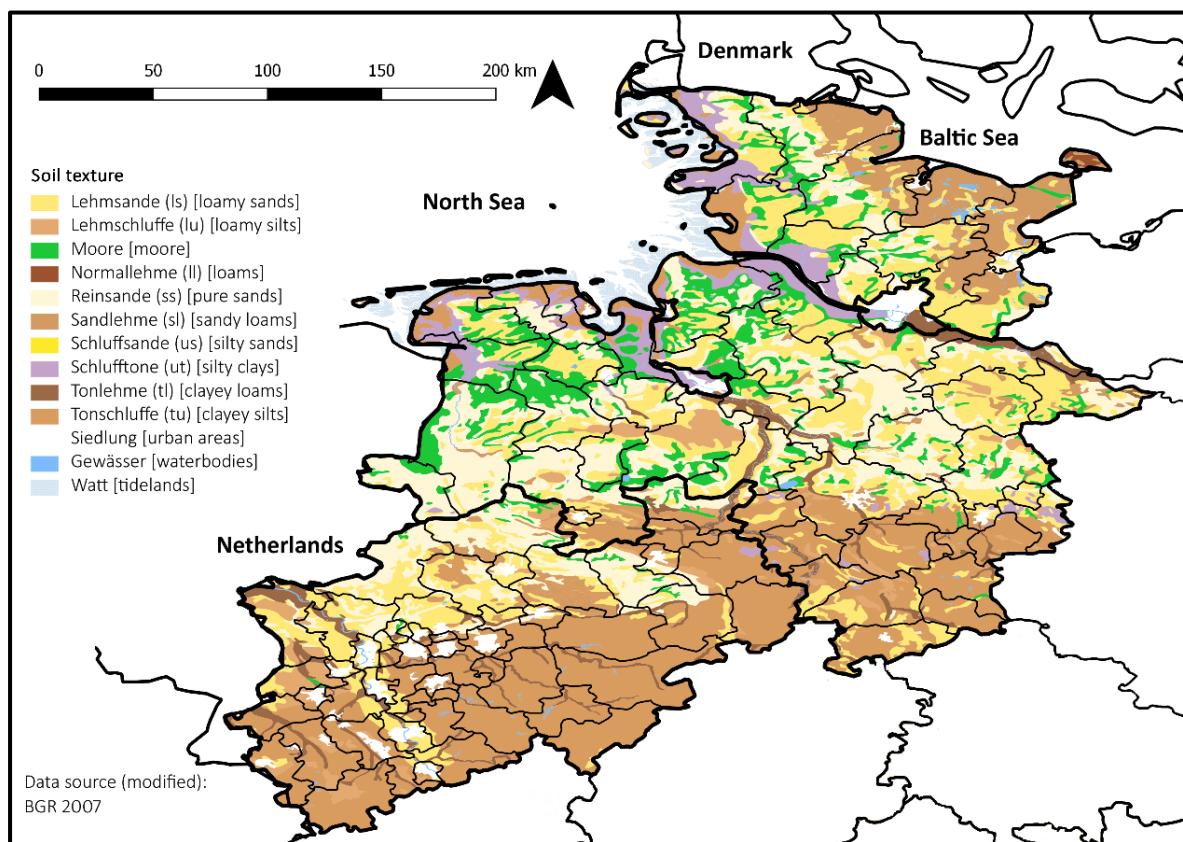


Figure 1: Soil texture of the topsoils in northwestern Germany (Federal States from south to north: North Rhine-Westphalia, Lower Saxony, and Schleswig-Holstein).

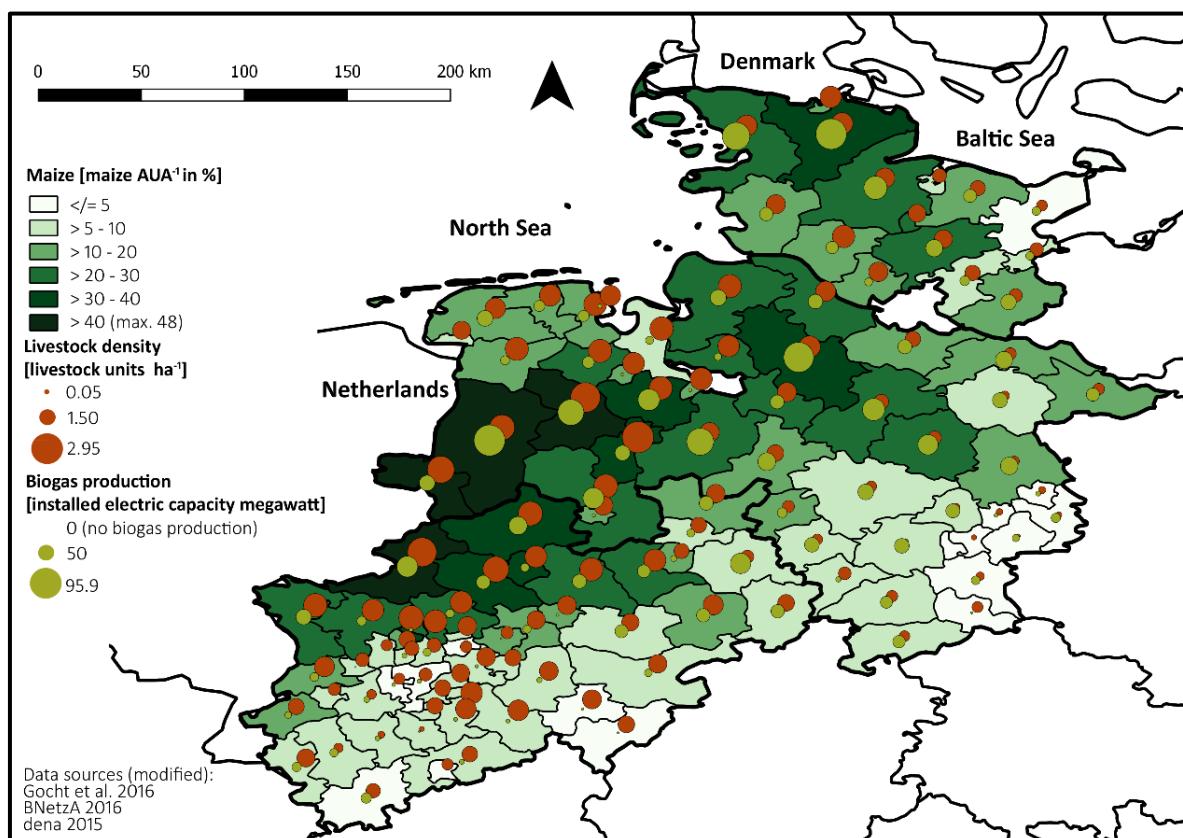


Figure 2: Maize cropping, livestock husbandry, and biogas production in northwestern Germany (Federal States from south to north: North Rhine-Westphalia, Lower Saxony, and Schleswig-Holstein); AUA = Agriculturally used area.

With respect to the different branches of animal husbandry the following core areas can be differentiated: In the south of the aforementioned region (e.g. Coesfeld and Borken) pig husbandry is dominant, whereas beef and dairy farming prevails in the north along the North Sea. Poultry, meat, and egg production can mostly be found in the Oldenburger Münsterland (Cloppenburg and Vechta) and the Emsland (Bäurle and Tamásy 2012). The highest livestock density per hectare of the agriculturally used area (AUA) occurs in the southwestern portion of LS and northeastern portion of NRW (Figure 2).

In line with the Renewable Energy Act biogas production markedly expanded in Germany from 1,050 (65 MW) to 8,151 (3,352 MW) biogas plants between the years 2000 and 2012 (Guenther-Lübbbers and Theuvsen 2015). Thus, Germany became the world's major biogas producing country (Weiland 2010). However, in recent years the amount of new installations distinctly decreased, mainly due to changes within the Renewable Energies Act in 2014, resulting in ca. 9,000 (4,166 MW) biogas plants in 2016 (Fachverband Biogas 2016a,b). The existing plants will affect the agricultural sector at least for the next 10 to 20 years. As in the case of livestock husbandry, regional hotspots developed during the biogas energy boom. Due to favorable local conditions, availability of substrates, and the good combination with other production branches, northwestern Germany became one of the most important biogas-producing regions in Germany (Guenther-Lübbbers and Theuvsen 2015). In 2014 38% of the total German biogas production was located in the three aforesaid Federal States, most notably LS, with the nationally highest installed electric capacity (884 MW; Fachverband Biogas 2016a,b). Thus, in addition to animal husbandry, biogas production has a high agro-economic relevance in the region. Figure 2 illustrates the correlation between high livestock densities and significant biogas production within administrative districts.

Due to intensive livestock husbandry and biogas production, large amounts of manure

accumulate. In the following section this will be characterized exemplarily on basis of the nutrient report 2014/2015 of LS (LWK Niedersachsen 2016). Livestock husbandry led to 47.6 million t of manure per year (38.8 million t slurries and 8.8 million t solid manures), containing 265,498 t N (after deduction of stable and storage losses), and 139,383 t P₂O₅. In the same period, the amount of biogas digestates summed up to 18.8 million t, containing 107,834 t N and 54,600 t P₂O₅. Considering that a portion of the slurries and solid manures was used as substrate for biogas production, in total 59.6 million t of manure were generated (326,446 t N, after deduction of stable and storage losses, and 165,308 t P₂O₅). This corresponds to 127 kg N and 64 kg P₂O₅ per hectare farmland in LS, keeping in mind that obvious major spatial differences exist (Figure 2). Furthermore, import and export of manure needs to be considered. On the one hand, The Netherlands exported about 97,778 t manure (1,848 t N and 2,385 kg P₂O₅) to LS, while further 326,867 t (3,196 t N and 2,271 t P₂O₅) were imported from neighboring Federal States. On the other hand, 1.1 million t (12,645 t N and 9,248 t P₂O₅) were exported from LS during the same period. All in all, the large amounts of manure produced within the region accompanied by intensive trading of manure (Warnecke et al. 2011), demonstrates the major challenge with respect to nutrients in organic fertilizer products.

Maize is the most important fodder and substrate for biogas production in the region. In animal husbandry maize silage, corn cob mix, or grain maize are used as fodder for beef and dairy cattle, pigs, and poultry (Nußbaum 2013). Furthermore, maize is used as substrate in 98% of all biogas plants in NRW, with a share of close to 50% in the total substrate input (Lohmann 2016). In Germany, the farmland used for maize cropping increased from 1.5 to 2.5 million ha between 2000 and 2011 and was more or less constant in recent years. 41% of this area (1.05 million ha; 16% grain maize and 84% silage maize) was grown in the three Federal States of northwestern Germany (average from 2014 to 2016; DMK 2017a). Figure 2 reveals the

close linkage between livestock density, biogas production, and the importance of maize cultivation. In areas with intensive livestock husbandry and biogas production maize is mostly grown on > 30% (in some regions even more than 40%) of the AUA. Grain maize prevails in areas where pig husbandry is dominant, while silage maize dominates in areas with high percentage of cattle farming. When comparing Figure 2 with Figure 1 it becomes obvious that intensive maize production largely corresponds with the presence of sandy soils. Among others, this is due to the fact that maize has low requirements regarding the soil quality. An additional benefit is the rather low demand of maize with respect to crop protection leading to an overall easy crop management (Herrmann et al. 2011). This is especially important for farmers, who generate their income mostly by livestock husbandry or biogas production. Typical pre-crops in the region are cereals, like barley, triticale, and rye on fields with low soil quality or wheat on fields with a higher yield potential, predominantly followed by catch crops. In regions with a very high amounts of maize production the pre-crop is often also maize.

Nutrient balances and the environmental impact
Germany is one of the European countries with the highest yields per hectare, but it is also one of the six countries with the highest national nitrogen surpluses (Taube et al. 2013). However, in the last decades a downward trend was observed. Since 1990 the nitrogen surplus decreased from 110 kg ha⁻¹ to 67 kg ha⁻¹ (average from 2011 to 2013; Bach et al. 2016; BMUB and BMEL 2016). As in case of livestock density or biogas production, there are large regional differences. In Germany a range of 24 – 123 kg N (ha AUA)⁻¹ exists depending on the region. The fact that a great share of northwestern Germany shows large N surpluses can be deduced from the aforesaid land use. It has to be kept in mind that nitrogen balances can considerably vary depending on the calculation approach (BMUB and BMEL 2016). Figure 3 illustrates the current state of scientific

knowledge for calculating N surpluses of the land area balance according to Bach et al. (2016). Besides the complete mineral and organic N input and removal from the fields, the import and export of manures according to the current nutrient reports were considered for NRW and LS. In northwestern Germany the N surpluses range from 45 to 119 kg N (ha AUA)⁻¹. The lowest surpluses occur in southern NRW and eastern LS. All in all, the correlation between sandy soils, accumulation of manure from intensive livestock husbandry or biogas production, cultivation of maize, and high N surpluses is obvious. Nearly 20% of all administrative districts show N surpluses of more than 100 kg N (ha AUA)⁻¹. It should be taken into account that surpluses at farm level may be even higher.

These N surpluses can pollute the environment in numerous ways. One of the consequences is the enrichment of water bodies with nitrate, contributing to eutrophication (Taube et al. 2013). In 2010 about 70 – 80% of the nitrogen pollution of surface waters originated from agricultural land via different pathways like drainage water or runoff (BMUB and BMEL 2016). The quality status of many lakes and rivers ranges from 'good' to 'satisfactory', however, the quality status of coastal water bodies is much worse (Taube et al. 2013). With respect to ground-water, between 2012 and 2014 28% of all monitored groundwater bodies in Germany were above the limit of 50 mg l⁻¹. In principle these are located all over Germany, but regional hotspots are evident. One such hotspot occurs in northwestern Germany, due to the sandy soils and high N surpluses at farm level (BMUB and BMEL 2016).

Furthermore, 95% of ammonia (NH₃) emissions in Germany are caused by agricultural activities (Wissenschaftlicher Beirat 2016). Emissions stemming from stables and those released during storage or application of manure are the most relevant sources (Flessa 2014). NH₃ leads to wet and dry deposits of nitrogen, resulting in acidification of soils and eutrophication (Taube et al. 2013).

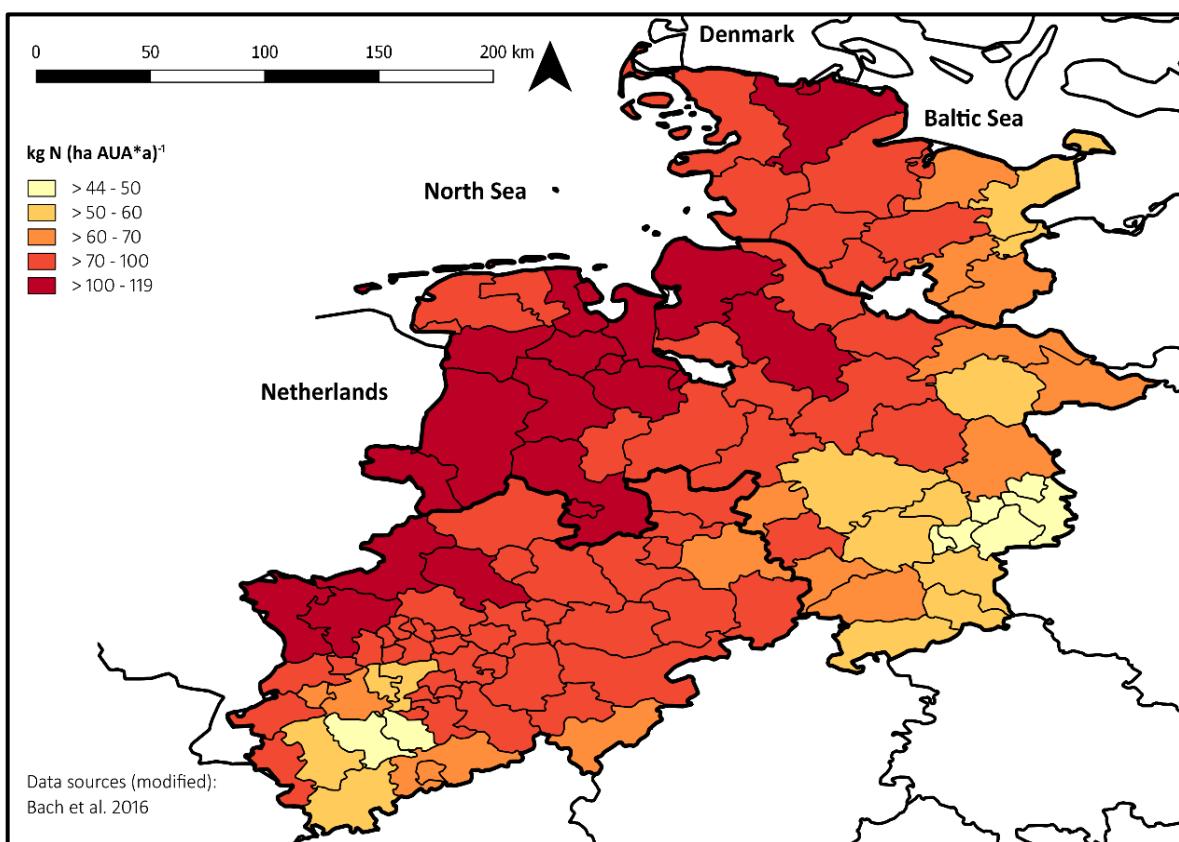


Figure 3: Nitrogen surplus of the land area balance in northwestern Germany (Federal States from south to north: North Rhine-Westphalia, Lower Saxony, and Schleswig-Holstein); AUA = Agriculturally used area.

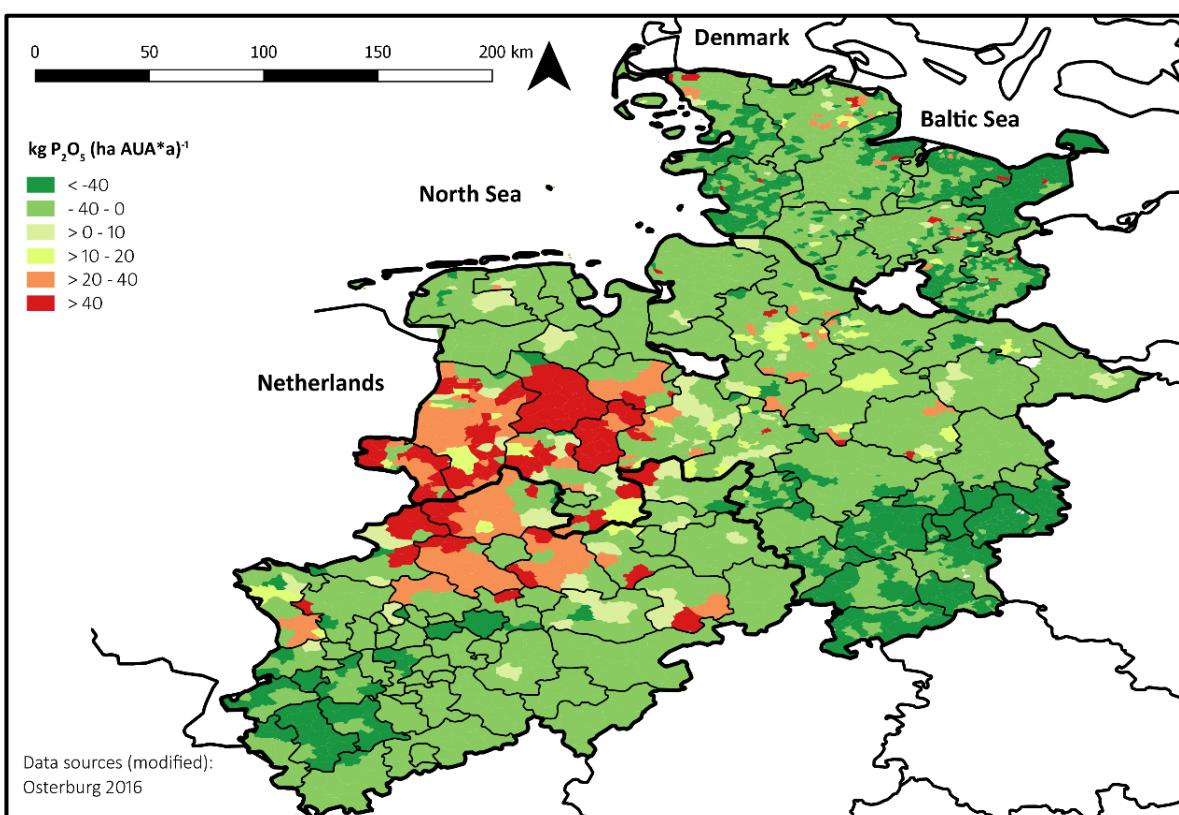


Figure 4: Phosphorus balance (animal P excretions minus plant P removal) of northwestern Germany (Federal States from south to north: North Rhine-Westphalia, Lower Saxony, and Schleswig-Holstein); without export of manures and without poultry manure; AUA = Agriculturally used area.

Another pathway for nitrogen losses from agriculture is through nitrous oxide (N_2O) emissions. N_2O has a very high greenhouse gas potential (about 310 times higher than CO_2) and thus it is of great importance for climate change effects. 7.2% of the climate-relevant gas emissions of Germany are attributable to agriculture (Taube et al. 2013). The N_2O emissions of German agriculture sum up to about 39.4 million t of CO_2 equivalents and thereof nearly 70% are caused by utilization of organic or mineral fertilizers (Dämmgen 2005).

One third of all agriculturally used soils in Germany are classified as high to very high in P status ('D' or 'E' according to the German classification with a total range from 'A' to 'E'). When solely considering administrative districts with > 1.5 Livestock Units (ha AUA) $^{-1}$ even 60% of the soils reach classification 'D' or 'E' (Osterburg 2012). Figure 4 shows the P balance of northwestern Germany at communal level, including P input by manures or biogas digestates and plant P removal, according to Osterburg et al. (2016). Calculation of a complete P balance was not possible, since regional data for P input via mineral fertilizers are not

available. Furthermore, export of manures was not considered. However, these values were calculated without taking poultry manures into account, as these are often transported over large distances (Osterburg et al. 2016). Thus, a complete land area balance, as shown for N in Figure 3, would lead partially to even higher P balances, especially considering mineral P fertilizers. However, it becomes obvious that in the northwestern region of NRW and the southwestern region of LS, very high P surpluses occur. As stated previously, these regions are mainly dominated by pig fattening farms associated with considerable feed imports (Osterburg et al. 2016).

Efficient use of P as fertilizer is of large relevance due to its environmental effects and because of worldwide limited resources. P enriched soils significantly impact the pollution of water bodies, considering that P has the largest eutrophication potential of all nutrients for surface and coastal waters (Taube et al. 2013). In 2010 about 50% of the P loads of German surface waters could be attributed to agricultural land. The main pathways were erosion, runoff, and groundwater (BMUB and BMEL 2016).

1.4 Legal framework

At the European Union (EU) level, the disrupted nutrient cycles and the related environmental consequences have been recognized (Buckwell and Nadeu 2016). Hence, a considerable number of regulations regarding nutrient use have been created. Key elements are to regulate nutrient use in livestock production and arable farming, the treatment of manures and other organic wastes, and air and water pollution (Buckwell and Nadeu 2016). In respect to the aforesaid situation in northwestern Germany, the Water Framework Directive (2000/60/EC) sets the basic conditions for the protection of surface and groundwater. This directive states that lakes, rivers, and coastal waters, as well as groundwater must reach the so-called 'good quality status' by the year 2027 at the latest (BMUB and UBA 2016). Furthermore, the

Nitrates Directive (91/676/EEC) ensures the protection of ground and surface waters against pollution caused by nitrates from agricultural sources. Among other things, an application limit of $170 \text{ kg N ha}^{-1} \text{ year}^{-1}$ via manures from livestock is set for nitrate vulnerable zones (NVZ). In addition, the Groundwater Directive (2006/118/EC), the Surface Water Directive (75/440/EEC), the Drinking Water Directive (98/83/EC), and the Marine Strategy Framework Directive (2008/56/EC) are relevant for the protection of different water bodies. A quality standard of $50 \text{ mg NO}_3^- \text{ l}^{-1}$ is defined for groundwater bodies (Buckwell and Nadeu 2016). Because this requirement has not been met in many regions to date, the EU Commission sent a letter of formal notice to Germany in 2013 as first step of the infringement

case. Following receipt of the notice, the German administration failed a second time in initiating the appropriate actions. Consequently the second step of the infringement process followed in 2014. If Germany does not react appropriately, the Commission will be entitled to take legal action at the European Court of Justice (Bach et al. 2016).

The Clean Air Policy Package resulted from a review of the EU air policy between 2011 and 2013. A part of this package is to set new objectives for the EU air policy for the period 2020 to 2029 as well as from 2030 onwards (EU 2017). Thus, the current EU National Emissions Ceilings Directive (NEC Directive 2001/81/EC) will be replaced by the Directive 2016/2284/EU from 2018 onwards. For five pollutants, e.g. nitrogen oxides and ammonia, which are responsible for ground-level ozone pollution, eutrophication, and acidification, national reduction commitments are defined. For Germany the following targets are set (reference year 2005): The annual ammonia emissions have to be decreased by 5% between 2020 and 2029 for each year and decreased by 29% from 2030 onwards; the annual nitrogen oxide (including N₂O) emissions must be lowered by 39% between 2020 and 2029 for each year and lowered by 65% from 2030 onwards.

The national authorities have to adapt their laws and legislation to the given framework. For the agricultural sector the EU Member States were required to set up 'rules of best practice'. In Germany these have been implemented into the 'Fertilizer Act'. The practical implementation of the Fertilizer Act takes place within the 'Fertilizer Application Ordinance' (DüV 2012). It is the central instrument in the German action program for implementing the Nitrates Directive (Taube et al. 2013). The key elements of the current ordinance can be summarized as follows:

- fertilizer requirements of crops have to be calculated before fertilization;
- maximum of 170 kg N ha⁻¹ year⁻¹ via animal excretions (farm average);

- maximum of 40 kg ha⁻¹ NH₄-N or 80 kg ha⁻¹ total N in autumn; no N application for following crops, which have no N requirements before winter;
- liquid manures, other liquid organic or organic-mineral fertilizers, and poultry excretions have to be immediately incorporated after application on bare soil;
- blocking period from 01 November until 31 January for the application of fertilizers with relevant N contents on arable land;
- ban of application for fertilizers with relevant N or P contents for not receptive soils;
- annual calculation of nutrient balances for N and P for each farm; maximum surpluses of 60 kg N ha⁻¹ year⁻¹ (3-year average) and 20 kg P₂O₅ ha⁻¹ year⁻¹ (6-year average); larger P surpluses are only allowed if the soil content is < 20 mg P₂O₅ per 100 g soil.

Currently the Fertilizer Application Ordinance is under revision. Considering this, an improved implementation of the Nitrates Directive (91/676/EEC) is of particular importance, especially due to the ongoing infringement case (BMEL 2015). The main changes based on the publicly available draft of the regulation will most likely be as follows:

- new data basis for calculation of fertilizer requirements of crops;
- maximum of 170 kg N ha⁻¹ year⁻¹ including all organic and organic-mineral fertilizer (i.e. also biogas digestates);
- reduction of autumn application rates down to 30 kg ha⁻¹ NH₄-N or 60 kg ha⁻¹ total N;
- more requirements concerning manure incorporation into the soil (e.g. band application or direct incorporation of liquid manures or organic-mineral fertilizers on cultivated fields from 2020 onwards);
- extension of the blocking period for N fertilizer application over winter time;
- new rates and data basis concerning calculation of annual nutrient balances for N and P from 2020 onwards: maximum surpluses of 50 kg N ha⁻¹ year⁻¹ (3-year average) and 10 kg P₂O₅ ha⁻¹ year⁻¹ (6-year average); if the soil content is > 20 mg P₂O₅

per 100 g soil, a maximum fertilization according to P uptake by the crop is allowed.

In addition, the Federal State governments are allowed to issue further regulations, especially for regional hotspots with groundwater nitrate concentrations $> 50 \text{ mg NO}_3^- \text{ l}^{-1}$ or $> 40 \text{ mg NO}_3^- \text{ l}^{-1}$

with increasing trends (BMEL 2015). This seems to be very likely for NRW and LS. All in all, it becomes obvious that the revised ordinance aims to reach higher nutrient efficiencies leading to lower nutrient losses finally resulting in decreased environmental pollution.

1.5 Maize: Nutrient requirements and fertilizing schemes

In northwestern Germany maize cropping yields ca. 10 – 11 t DM ha⁻¹ grain maize and ca. 45 t FM ha⁻¹ silage maize (average from 2011 to 2015; DMK 2017b). Of course this level can only be reached at adequate nutrient supply.

N is the most abundant mineral nutrient in plants (2 – 4% of plant dry matter) and major sources of N taken up by roots are NH₄⁺ and NO₃⁻ (Roy 2006). Within the plant, N is a substantial component of proteins, nucleic acids, and an essential component of chlorophyll (Mengel et al. 2001). N uptake by maize is about 30 – 40 kg (10 t FM)⁻¹ (Herrmann et al. 2011).

P is much less abundant in plants with a concentration in dry matter of about one-fifth to one-tenth of that of N. However, P is of great importance for cell division, seed and fruit development, growth, and ripening. Furthermore, P is crucial for energy transfer processes within the plant as an essential part of adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Maize roots take up P as orthophosphate, either as HPO₄²⁻ or H₂PO₄⁻, depending on the soil pH (Roy 2006). However, P is mainly bound in plant unavailable forms in the soil and it is particularly sparingly soluble under cold conditions (Grant 2001; Imran et al. 2013). Thus, P is especially growth-limiting during the early growth development of maize in northwestern Germany. According to Herrmann et al. (2011) P uptake of maize is about 40 – 50 kg P ha⁻¹.

Furthermore, maize is very susceptible to Zn and Mn deficiencies (Fageria et al. 2002). Both are taken up from soil as divalent cations (Zn²⁺, Mn²⁺). Zn has special functions for several

enzyme systems, auxins as well as protein synthesis, and seed production. Mn is also involved in activating enzymes and functions as an auto-catalyst. In addition, it is essential for splitting water molecules during photosynthesis (Roy 2006). Like P, Zn as well as Mn are less soluble under cold weather conditions (Imran et al. 2013). There are certainly further macro- and micronutrients essential for maize development, but these are not the focus of this thesis.

The current farm practice for maize fertilization is predominantly based on the recommendations given by the Chambers of Agriculture (LWK NRW 2016; LWK NS 2016). They advise a target value of 180 kg N ha⁻¹, which has to be adjusted as follows:

- preplant SMN (0 – 60 cm) at the end of March or beginning of April must be subtracted
- site-specific corrections:
 - long-term application of organic fertilizers: -40 kg N ha⁻¹
 - soil-specific high or low soil nitrogen mineralization: up to +/- 20 kg N ha⁻¹
 - previous catch-crop: up to -20 kg N ha⁻¹

This results in the necessary N fertilization rate. Taking into account the applied mineral fertilizer rate the allowed amount of N via slurry can be deduced. Thereby 70% of the total N content or 100% of the NH₄-N content have to be considered (LWK NRW 2016).

Nowadays in practical farming slurry is mostly applied by trailing hoses several days before maize planting in April. Afterwards the surface

applied slurry must be incorporated into the soil by using a disc harrow, cultivator or plow. For soils that are particularly susceptible for N leaching LWK NRW (2016) recommends a splitting of the slurry rate. In this case, application of the second slurry rate should be carried out between the 6- and 8-leaf developmental stages using trailing hoses. However, the application of this slurry rate is based on a second determination of the SMN content at the end of May. The 'splitting strategy' is also used by several farmers, especially in NVZ. Additionally, the application of a MSF is recommended at planting, because of insufficient P acquisition by maize roots during early growth combined with the fact that P is mostly bound in soils in plant unavailable forms (LWK NRW 2016). However, the soil P content must be considered, as aforementioned in the 'Legal Framework' Section. Due to the positive interaction of phosphate and ammonium (uptake of NH_4^+ lowers the rhizosphere pH leading to enhanced P availability; Ma et al. 2013), fertilizers containing $\text{NH}_4\text{-N}$ and water-soluble P are recommended. This MSF application (5 cm below and 5 cm aside the seed-corn) is a standard measure at planting in current farm practice. Typically used fertilizers are 20-20 N P, di-ammonium phosphate (DAP, 18-46 N P) or a blend of DAP and calcium ammonium nitrate (CAN, 23-23 N P).

To substitute this additional MSF the new fertilization approach focuses on slurry injection below the maize row before planting. It is assumed that due to slurry placement the interface between nutrients and surrounding soil is distinctly decreased. Thus, nutrient absorption and/or fixation declines, especially for P and Zn, most possibly resulting in an increased chemical and spatial availability for maize roots. Furthermore, slurries contain water-soluble P and $\text{NH}_4\text{-N}$, so that a similar effect as described for N P MSF can be expected. Appropriate slurry injectors have been developed by different manufacturers in recent years and are ready for farm use.

Pre-tests of the Chambers of Agriculture and the University of Applied Sciences of Osnabrück

showed that the following aspects must be taken into account. At first, to substitute the MSF, it is important that the distance between the seed-corn and the injected slurry band is appropriate. If the slurry band is too far away from the seed-corn, the so-called primary roots of the maize plant would reach the nutrients too late. On the other hand, if the seedling is in direct contact to the slurry, etching effects due to high salt concentrations are possible. Based on field trials it was revealed that a distance of about 7 cm (comparable to the distance between seedling and MSF) is suitable (Figure 5). The time span between slurry injection and maize planting might vary between a few days and several weeks. However, field trials of the Chamber of Agriculture LS showed that this period should be as short as possible to minimize transformation processes within the slurry band. After injection of slurries with rather low dry matter contents, some delay is required to ensure good soil conditions for the planting process.

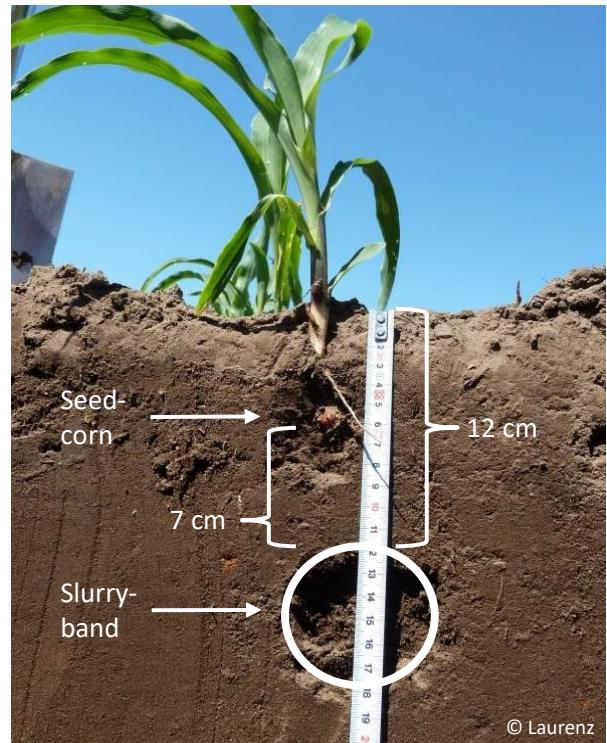


Figure 5: Slurry injection below the maize row.

At planting it is important to place the seedling preferably perpendicularly above the slurry band to ensure the suitable spatial distance. Additionally, the quality of slurry injection

depends on the soil texture. Best working quality is ensured on pure sands or loamy sands, so-called 'free-flowering' soils, because of satisfactory closure of the injection slots. The more clayey the soils are, the more challenging are the requirements concerning the technical setup of the machinery. Above a certain clay content, slurry injection cannot be recommended, because the injection slots will not be closed, resulting in an inadequate seedbed.

It might be useful to expand this fertilizing approach by addition of a NI to the slurry. These substances inhibit the transformation from NH_4^+ to NO_3^- during the nitrification process. In contrast to NH_4^+ , NO_3^- is at risk to be leached out of the rooting zone. Additionally, it is the initial substance for denitrification, which can lead to

N_2O emissions (Ruser and Schulz 2015, Subbarao et al. 2006). Thus, NIs seem to be appropriate to stabilize the NH_4^+ applied by slurries resulting in lower N losses. This could be especially valuable on the sandy soils of northwestern Germany, which are prone to nitrate leaching. Most of the available NIs inhibit the enzyme ammonia monooxygenase (AMO, EC 1.14.99.39; Placzek et al. 2017), which is responsible for the first enzymatic step of the nitrification. Within the field trials of the present thesis, the product ENTEC® FL (EuroChem Agro GmbH, Mannheim, Germany) containing the active substance 3,4-dimethylpyrazol phosphate (DMPP) was used. Its AMO inhibition mechanism is related to the chelating of copper (Cu) (Ruser and Schulz 2015). Furthermore, it is relatively immobile in soils and thus maintains spatial contact with the applied NH_4^+ (Yu et al. 2007).

Chapter 2

Slurry Injection: Soil Mineral Nitrogen Dynamics and Plant Nutrient Status

2.1 Soil nitrogen dynamics after slurry injection in field trials: Evaluation of a soil sampling strategy

Author Contributions

Matthias Westerschulte developed the experimental design, conducted the field trials, sampled and analyzed all data, performed the statistical evaluation, and wrote the manuscript

Carl-Philipp Federolf contributed to the development of the experimental design, conduction of the field trials and samplings as well as the evaluation

Herbert Pralle contributed to the development of the experimental design, conduction of the field trials and statistics as well as the evaluation

Dieter Trautz supervised Matthias Westerschulte

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Soil nitrogen dynamics after slurry injection in field trials: Evaluation of a soil sampling strategy

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ABSTRACT

Slurry injection below maize seeds is a rather new application technique developed to improve the nitrogen use efficiency of liquid organic manure. To enable the characterization of the spatial and temporal soil mineral nitrogen (SMN) dynamics after slurry injection the present study aims to develop an appropriate soil sampling strategy. Three consecutive experiments were conducted. The first testing of the soil sampling approach was conducted in an existing field trial where the slurry was injected down to a depth of 12 cm (upper rim) below the soil surface. The soil profile (75 cm wide) centered below the maize row was sampled grid-like to a depth of 90 cm. Around the injection zone, soil monoliths (SM) were sampled using a purpose-built soil shovel. Below the SMs and in the interrow space (15 and 30 cm distance to the row) a standardized auger procedure was performed. The second experiment aimed at improving the sampling strategy with a focus on sample homogenization quality and necessary sample sizes per pooled sample. Furthermore, the risk of a carryover of slurry components along the soil core due to drilling an auger through a slurry band was analyzed. In the third experiment this improved sampling strategy was validated. Results from the first testing of the sampling procedure showed that the strategy is suitable, although some problems occurred (especially the high spread in values among the replications causing high coefficients of variation (CV) of mostly 40 – 60%). The improvement trial revealed that due to the high gradient of SMN concentration in the direct range of the injection zone an intensive homogenization of these samples is required. Suitable sample sizes (twelve auger samples and six soil monolith samples per pooled sample) have to be collected to obtain reliable SMN values. Drilling an auger through a slurry band to sample subjacent soil layers has to be avoided. Following this enhanced sampling strategy in the final validation trial the spread in values was considerably reduced and resulted in CV values of mostly < 20%. The developed sampling strategy enables the characterization of the spatial and temporal SMN dynamics when slurry has been band-injected below a maize row. The method can be transferred to other row crops and different slurry injection spacings.

KEY WORDS

Band application; slurry injection; mineral nitrogen; soil monolith; soil sampling method

INTRODUCTION

Slurry injection has become an increasingly common fertilization method for maize because it had been shown that the traditional broadcast surface application of slurry causes problems such as ammonia volatilization (Carozzia et al. 2013; Misselbrook 2002) or surface runoff (Ceretta et al. 2010; Smith et al. 2001a,b). Several studies revealed that slurry injection close to the maize row leads to enhanced nitrogen use efficiency (Ahmed et al. 2013; Schmitt et al. 1995; Schröder et al. 2013; Sutton et al. 1982). However, improved understanding of the soil mineral nitrogen (SMN) dynamics when using slurry injection techniques is essential (Dell et al. 2011).

The main challenge for soil sampling after banding fertilizer are the huge differences in nutrient concentrations around the injection zone compared to the unfertilized soil zone. Standardized random sampling strategies are not suitable for the characterization of the soil nutrient status (Kitchen et al. 1990; Tewolde et al. 2013; Van Vuuren et al. 2000). For a reliable description of the soil nutrient status after harvest, Kitchen et al. (1990; extractable P) and Tewolde et al. (2013; total C, total N, and extractable P, K, Mg, Cu, Mn, Fe, and Zn) recommended to take auger samples in a defined volume ratio from directly above the fertilizer band and perpendicular to the band towards the unfertilized interspace. Similar approaches were used to characterize the spatial and temporal SMN dynamics after urea ammonium nitrate injection (Clay et al. 1995) or slurry injection (Assefa and Chen 2007). However, when using an auger it is not possible to take samples accurately in the direct surrounding of the fertilizer band, because it is not feasible to locate the band precisely from above the soil surface.

Therefore, in other studies soil pits perpendicular to the fertilizer bands were trenched to enable very small-scale grid-like sampling in the direct range of the injection zone as well as (at least partially) in the unfertilized interspace (Comfort et al. 1988; McCormick et al.

1983; Petersen et al. 2003). Van Vuuren et al. (2000) used a metal sampler with 4 cm × 4 cm grids for sampling to a depth of 28 cm and concluded that this method is not practicable for extensive studies. Comfort et al. (1988) conducted a slurry-injection field trial without crops and used a backhoe to excavate a ≈ 1 m deep trench perpendicular to the slurry-injection bands, and sampled the soil profile in a grid-like 5 cm × 5 cm to a depth of 90 cm. However, this procedure is not practical for ongoing field trials. Sawyer et al. (1990b) conducted maize field trials with injected beef slurry over 4 years. In the first 3 years they used different auger sampling methods (comparable to Clay et al. 1995 and Assefa and Chen 2007) to characterize the SMN dynamics. In the last year, they located the injection zone precisely by digging a trench across the application area and used plastic vials for sampling directly the injection zone disregarding the unfertilized interspace. But finally, no practicable soil sampling strategy was suggested.

ZebARTH et al. (1999) determined the spatial and temporal SMN dynamics in a maize field receiving a mineral nitrogen fertilizer band as side dress. They collected the complete soil material in a trench 40 cm long (perpendicular to the maize row), 10 cm wide, and 30 cm deep and segmented it into 5 cm × 10 cm × 15 cm soil monoliths. For the 30–60 cm soil layer a standardized auger sampling procedure was used. This very high sampling effort was only performed in one field trial to deduce a soil sampling strategy for estimating the SMN status in the root zone for fertilizing recommendations at a given date.

Based on these findings the objective of this study was to develop and evaluate a practicable soil sampling strategy to enable a reliable characterization of the spatial and temporal SMN dynamics in field trials fertilized with slurry band-injection below the maize row. Transformations of SMN in the direct range of the injection zone, SMN distribution in the unfertilized interrow space, and the sampling depth down to 90 cm were considered.

MATERIAL AND METHODS

Experimental sites, soil conditions, and slurry properties

Three consecutive experiments were conducted at different sites in Lower Saxony (LS), northwestern Germany in 2013 and 2014 (Table 1). A first testing of the sampling procedure (further on called 'test trial') was conducted at the Wehnen site (Table 1) in 2013. This field trial was part of a study where a new technology for slurry band-injection was evaluated for maize fertilization and one of the eleven treatments was selected to test the soil sampling strategy. Based on these results a so-called 'improvement trial' was conducted to enhance the sampling method at the Lechtingen site (Table 1) in autumn 2013. To implement and verify the findings a 'validation trial' was performed at the site in Hollage in 2014 (Table 1). This latter one was also executed in one of six treatments in an ongoing maize trial.

Long-term average annual air temperature was $\approx 9.5^{\circ}\text{C}$ and average annual precipitation $\approx 850 \text{ mm}$. The soil textural class of the topsoil was sand at all sites. According to IUSS Working Group WRB (2014) the soil type in Wehnen is a Gleyic Podzol, in Lechtingen a Gleysol, and in Hollage a Plaggic Podzol (Table 1). Pig slurries from regional pig fattening farms were used in the three experiments (Table 1). Dry mass of slurries ranged from 3.0 to 9.3% and the total nitrogen from 3.7 to 7.2 g kg $^{-1}$. The dry matter content was analyzed gravimetrically by oven-drying at 105°C to constant weight. Total N

concentration in the slurries was determined using the Kjeldahl method after nitrate reduction with Devarda's alloy (DIN EN 15476 2009), while the ammonium concentration was determined with titration after direct distillation of the slurry with magnesium oxide (according to Bremner and Keeney 1966).

Soil sampling and experimental set-up

Soil sampling method

The intent of the sampling strategy was to sample the soil profile grid-like to a soil depth of 90 cm (Figure 1). That was accomplished by combining a standardized auger (1.8 cm inside diameter) procedure with a purpose-built metal shovel (15 cm wide, 15 cm high and 10 cm deep; Figure 2). Using this sampling shovel yields rectangular soil monoliths (SM).

In the interrow space samples were taken with an auger at a distance of 15 cm and 30 cm perpendicular to the rows (auger (AU) 15 / 30; Figure 1). Respective samples from the left and the right side of a maize row were pooled. Each sample was divided into three layers termed top (T) (0 – 30 cm), middle (M) (30 – 60 cm) and bottom (B) (60 – 90 cm). Samples underneath the row were taken using the metal shovel and an auger. For this purpose a small pit was dug (about 35 – 45 cm deep) to ensure a precise localization of the banded slurry. After that a metal sheet was hammered into the ground in front of the shovel (Figure 2). At first one monolith was taken from above the injected slurry (SM 1).

Table 1: Site characteristics, soil and slurry properties of the field trials.

Trial	Site	Site characteristics		Altitude (m a.s.l. ¹⁾)	Soil properties			Soil texture	pH (CaCl ₂)	Soil type (WRB, 2014)	Slurry properties		
		Coordinates N	E		Sand (%)	Silt (%)	Clay (%)				DM (%)	N (g kg $^{-1}$)	NH ₄ -N (g kg $^{-1}$)
1	Wehnen	53°11'	8°08'	9	88	9	3	sand	5.4	Gleyic Podzol	3.0	3.7	2.6
2	Lechtingen	52°20'	8°01'	87	96	2	2	sand	6.1	Gleysol	7.9	6.8	4.2
3	Hollage	52°20'	7°58'	65	91	8	1	sand	5.3	Plaggic Podzol	9.3	7.2	5.5

¹a.s.l. = above sea level; DM = dry mass; 1 = test trial; 2 = improvement trial; 3 = validation trial

Then a second soil monolith (SM 2), which contains the complete slurry band (S), was sampled (Figure 2) and finally a third one was taken from the area directly below the slurry band (SM 3). For sampling the two layers underneath SM 3 again an auger was used up to a depth of 90 cm (AU 0)

Testing the sampling approach

The field trial at the Wehnen site used for the first testing of the procedure had eleven treatments in a randomized complete block design with four replications. Previous crop was winter triticale and the field was plowed in spring 2013. Each experimental plot was 3 m wide, 15 m long and included four maize rows with a row spacing of 75 cm. In the treatment selected for our soil sampling experiment the slurry (Table 1) was applied with a four-row slurry injector (XTill, Hugo Vogelsang Maschinenbau GmbH, Essen/Oldenburg, Germany) at a row-spacing of 75 cm on 7 May 2013. The application rate was $48 \text{ m}^3 \text{ ha}^{-1}$.

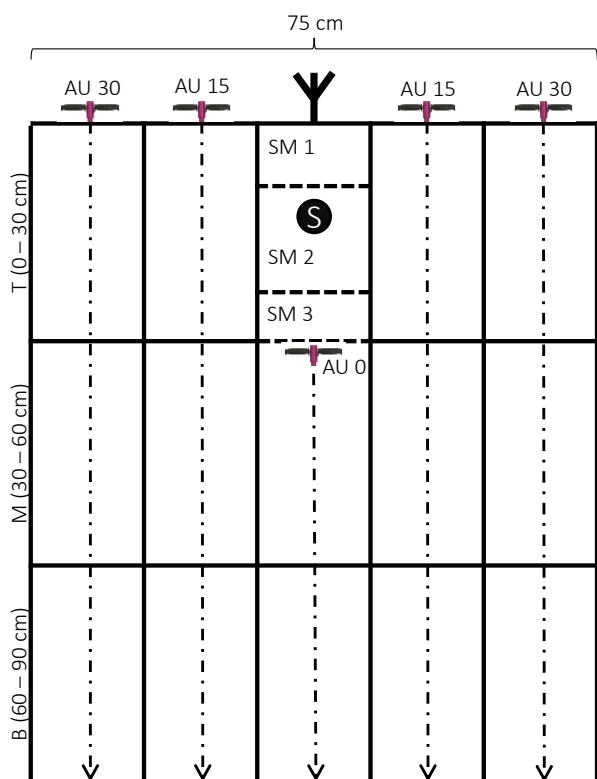


Figure 1: Basic scheme of the soil sampling method; AU 0 / 15 / 30 (—) = auger 0 / 15 / 30 cm distance to the maize row; SM 1 / 2 / 3 = soil monolith 1 / 2 / 3; T = top; M = middle; B = bottom; S = slurry band; M = maize row).

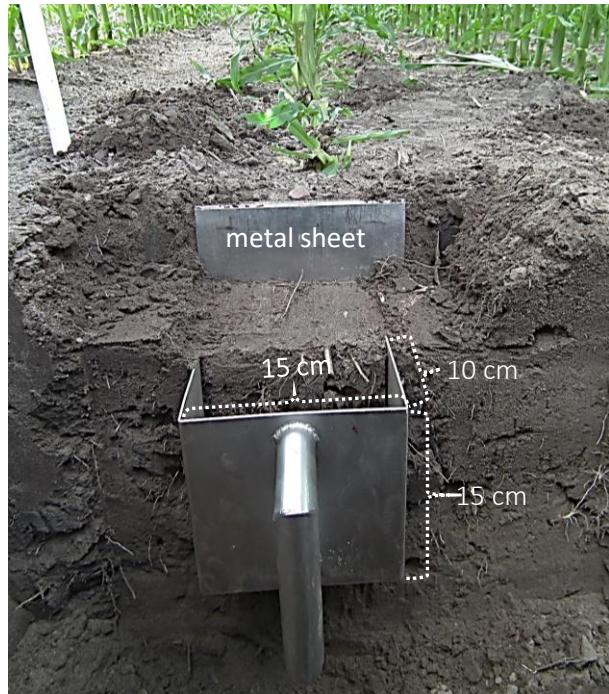


Figure 2: Usage of the soil shovel to obtain the soil-monolith sample around the banded slurry (SM 2); metal sheet = used to limit the SM in front.

The slurry band was $\approx 6 \text{ cm}$ wide and $\approx 8 \text{ cm}$ high and its upper rim was about 12 cm below the soil surface. Sowing of maize was carried out at drilling depth of 5 cm and a sowing rate of 9.0 grains per m^2 on 10 May 2013. The maize seed (variety: LG 30222, Limagrain GmbH, Ede-missen, Germany) was placed vertically above the slurry bands.

Soil samples were collected post emergence (May 30, 2013, 23 days after injection (DAI)) and at the 10-leaf developmental stage (July 10, 2013, 64 DAI). The soil samples were taken as described in Figure 1 from one of the middle rows per plot in order to avoid edge effects. One auger on the left and one on the right side of the row were collected for each interrow location (AU 15 and AU 30). The two auger samples per location were pooled for each soil layer (top, middle, and bottom, respectively). Soil monoliths were taken at depths of 0 – 10 cm (SM 1), 10 – 25 cm (SM 2), and 25 – 40 cm (SM 3) in the same row as the augers. One monolith was sampled per depth. To obtain the samples for AU 0, two augers per plot were pooled. On the first sampling date these augers (AU 0) were drilled directly through the banded slurry. At the second sampling date they were drilled into the

planar of the third soil monolith after sampling SM 1 – 3 and thus did not get into contact with the slurry band. All soil samples were collected in buckets and then homogenized intensively by hand and using a 5 mm sieve. Afterwards 200 (AU) – 300 (SM) g of soil were packed into plastic bags and immediately frozen until analyzed.

Improving the sampling strategy

After harvesting grain maize, a single slurry band was injected with a purpose-built single-row injector at the Lechtingen site on November 29, 2013. The application rate of the pig slurry (Table 1) was $25 \text{ m}^3 \text{ ha}^{-1}$. The slurry band was 16 m long, $\approx 5 \text{ cm}$ wide, $\approx 7 \text{ cm}$ high and its upper rim was about 17 cm below the soil surface.

Soil sampling was carried out 5 DAI (December 4, 2013) but only to a depth of 60 cm (top and middle soil layers, Figure 1). To obtain sufficient soil material for analysis, soil from two auger samplings close to each other were pooled. This procedure resulted in about 200 – 220 g of soil per sample. In total, 64 auger samples were collected in the interrow space (left and right side of the slurry band). They were distributed evenly along the 16 m long slurry band. In addition, 48 auger samples were taken from a position perpendicular through the slurry band to a depth of 60 cm. Furthermore, soil monoliths (SM 1 – 3; Figure 1) were collected at eight positions at depths of 0 – 10 cm (SM 1), 10 – 25 cm (SM 2), and 25 – 30 cm (SM 3). To sample the 30 – 60 cm layer underneath the eight soil monolith positions (AU, Figure 1), an auger was used. The 48 auger samples and eight soil monolith positions were also distributed evenly along the 16 m slurry band.

The further processing of all soil samples was carried out as described for the test trial. However, the total amount of soil from the monolith samples was split into subsamples. This resulted in six subsamples for SM 1, ten subsamples for SM 2, and five subsamples for SM 3. Each subsample contained ≈ 250 – 350 g of soil. This was done to test the quality of the homogenization.

Validating the sampling approach

The field trial at the Hollage site with six treatments had a randomized complete block design and four replications. Previous crop of this maize trial was silage maize and the stubble cultivation was performed twice (March, 5 and 27, 2014) with a disc harrow (working depth $\approx 10 \text{ cm}$). One experimental unit was a plot $3 \text{ m} \times 24 \text{ m}$ long with four maize rows at a spacing of 75 cm. For the selected treatment, the pig slurry (Table 1) was applied with the same slurry injector as used in the test trial at an application rate of $23 \text{ m}^3 \text{ ha}^{-1}$ on April 11, 2014. The slurry band was placed 12 cm (upper rim) below the soil surface. Maize (*Zea mays* L., cv. Ricardinio, KWS SAAT AG, Einbeck, Germany) was sown on April 25, 2014 (drilling depth: 4.5 cm, sowing rate: 9.2 grains m^{-2}).

Soil samples were collected before emergence (May 5, 2014, 24 DAI) and at the 10-leaf developmental stage (July 11, 2014, 81 DAI) in the two middle rows per plot as described in Figure 1. The soil of twelve single auger samples per interrow location (AU 15 and AU 30) was combined into a pooled sample per plot. The locations of the twelve augers were distributed evenly on the left and right side of the two middle maize rows over a row length of approximately 0.5 m using a template. The samples were collected in three buckets (top, middle, and bottom, respectively) per interrow location (AU 15 and AU 30) and thus six buckets per plot. The soil monoliths (Figure 1) were taken at depths of 0 – 8 cm (SM 1), 8 – 23 cm (SM 2), and 23 – 30 cm (SM 3). Six monoliths were sampled per depth and per plot (three from each maize row). Each of the six monolith samples was collected in a separate bucket. Thereafter, twelve augers were taken below the planar of the third soil monolith for the middle and bottom soil layers (AU 0; Figure 1) and pooled into one sample per layer and plot replicate.

All auger samples were immediately homogenized with a typical household electric hand mixer (using the whisks). Each monolith sample was first manually homogenized thereby removing all visible roots. Afterwards, the electric hand mixer was used for further

homogenization. After that, subsamples of about 300 mL per monolith from each of the six buckets were pooled and again intensively homogenized with the electric hand mixer. Finally, samples were passed through a 5 mm sieve and about 300 – 400 g of soil per sample were packed into plastic bags. To further check the homogenization process of SM 2 (Figure 1) two subsamples were generated. All samples were immediately frozen until analyzed.

Soil analysis

The frozen soil samples were thawed at 4°C. Then the field-moist samples were extracted with a calcium chloride solution ($c(\text{CaCl}_2) = 0.0125 \text{ mol l}^{-1}$) at a ratio of soil to solution of 1 : 4 (mass : volume; DIN 19746 2005). Subsequently the concentrations of ammonium and nitrate were determined spectrophotometrically.

Data analysis

Arithmetic means, standard deviations, and coefficients of variation were computed to evaluate the spread in values between the replications. Outliers were defined according to Grubbs (double-sided at a 5% level of significance; Grubbs, 1950). The normal distribution (Kolmogorov-Smirnov test), homogeneity of variances (Levene test), and the independent-samples t-test (5% level of significance) were performed with SPSS statistical software (SPSS 22, Inc., Chicago, USA). To obtain representative samples and thus to minimize the spread in values, the necessary sample sizes per plot were calculated according to Gomez and Gomez (1984):

$$n = \frac{Z_\alpha^2 \times s^2}{d^2 \times \bar{x}^2}$$

where n is the required sample size, Z_α is the value of the standardized normal variate corresponding to the level of significance α , \bar{x} is the arithmetic mean, s is the standard deviation, and d the margin of error expressed as a fraction

of the mean. The original formula was transformed using the coefficient of variance (CV) instead of the quotient from s^2 and \bar{x} :

$$n = \frac{Z_\alpha^2}{d^2} \times \frac{\text{CV}(\%)^2}{10000}$$

RESULTS

Testing the sampling approach

Soil mineral nitrogen (SMN = $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) concentrations (mg kg^{-1}) for the soil samples of the test trial are shown in Table 2 for both sampling dates. At the first sampling date (23 DAI), the effect of slurry injection on SMN concentrations for the various sampling grids of the soil profile became evident. For the second soil monolith, which contains the slurry band, a mean concentration of 237 mg kg^{-1} was found. SMN mean concentration of SM 1 (57.4 mg kg^{-1} , including the outlier of rep. 4 of SM 1) was higher than of SM 3 (29.1 mg kg^{-1}). Auger samples showed a decrease of SMN concentrations with increasing depth and from the maize row (AU 0) towards the interrow locations (AU 15 and 30) for top and middle layers. The concentration in grid SM 3 (25 – 40 cm) was about 22 mg kg^{-1} lower than of the middle layer (30 – 60 cm) of AU 0. Until 64 DAI, SMN concentration in the soil monolith around the slurry band (SM 2) declined clearly down to $\approx 19 \text{ mg kg}^{-1}$. However, the concentration directly beneath the slurry band (SM 3) increased by a factor of 2. At the second sampling date, the sampling strategy was slightly adapted that way that AU 0 was sampled down the planar of SM 3. Compared to the first sampling date, SMN concentrations of the third soil monolith were higher than in the middle layer of AU 0. In the interrow locations lower concentrations were found in the top layer compared to the first sampling date. However, SMN concentrations of the middle and bottom layers increased. Furthermore, SMN concentration of AU 15 was higher compared to AU 30 concerning these layers.

Table 2: Soil mineral nitrogen (SMN = NH₄-N + NO₃-N) concentration (mg kg⁻¹) of the test trial. SM (1 – 3) = soil monolith samples (1 = 0 – 10 cm; 2 = 10 – 25 cm; 3 = 25 – 40 cm); AU 0 / 15 / 30 = auger samples with a distance of 0 / 15 / 30 cm from the maize-row; Rep. = replication; s = standard deviation; CV = coefficient of variation; * = outlier according to Grubbs.

Soil layer (cm)	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	s	CV (%)	
<i>Pre-emergence (23 DAI)</i>								
SM (1 – 3)	0 – 10	31.7	39.0	24.0	135.0*	57.4	52.1	91
	10 – 25	263.8	143.1	226.2	316.2	237.3	72.9	31
	25 – 40	33.6	30.0	32.1	20.7	29.1	5.8	20
AU 0	0 – 30	181.2	171.9	169.3	213.3	183.9	20.3	11
	30 – 60	82.9	12.6	25.2	85.2	51.5	38.0	74
	60 – 90	4.5	3.3	1.7	2.1	2.9	1.3	44
AU 15	0 – 30	54.5	13.6	35.2	54.3	39.4	19.4	49
	30 – 60	35.0	12.1	5.2	27.4	19.9	13.7	68
	60 – 90	7.1	3.3	1.4	3.1	3.8	2.4	64
AU 30	0 – 30	20.7	17.4	28.3	29.5	24.0	5.9	25
	30 – 60	16.0	7.9	6.2	20.7	12.7	6.8	54
	60 – 90	4.3	4.0	39.8*	4.0	13.0	17.8	137
<i>Ten-leaf stage (64 DAI)</i>								
SM (1 – 3)	0 – 10	4.0	4.3	4.8	4.5	4.4	0.3	7
	10 – 25	54.3*	3.1	4.5	13.8	18.9	24.0	127
	25 – 40	100.7	7.6	31.2	106.7	61.5	49.7	81
AU 0	40 – 60	27.6	26.7	24.3	21.0	24.9	3.0	12
	60 – 90	16.7	20.0	11.2	3.1	12.7	7.4	58
AU 15	0 – 30	23.1	6.2	12.6	12.4	13.6	7.0	52
	30 – 60	34.0	21.7	31.9	58.6	36.5	15.6	43
	60 – 90	14.0	22.1	7.6	23.1	16.7	7.3	44
AU 30	0 – 30	9.8	7.6	17.1	16.0	12.6	4.6	37
	30 – 60	32.1	21.7	26.4	18.3	24.6	6.0	24
	60 – 90	20.2	16.0	5.5	6.2	12.0	7.3	61

The coefficients of variance illustrate a fairly wide scattering of the single values for the four replications. Most of them (seven values) ranged from 40 to 60%. Three extremely high coefficients of variance (91, 127, and 137%, respectively) were caused by outliers and three CV values were lower than 20%.

Improving the sampling strategy

Homogenization quality

The homogenization process of the soil monolith samples was evaluated in the improvement trial. The means of the eight replications ranged from 3.6 to 9.3 mg kg⁻¹ for SM 1 and 1.6 to 5.6 mg kg⁻¹ for SM 3, respectively (Figure 3). For soil monolith 2, which contained the banded slurry, the SMN level was much higher and ranged from about 115 to 248 mg kg⁻¹.

With only one exception (SM 1, Rep. 1) the range of all subsamples within a single replication was much smaller than 1 mg kg⁻¹ for SM 1 and SM 3. In contrast, the subsample values scattered

considerably for the soil monolith samples of the slurry injection zone (SM 2) ranging from more than 40 mg kg⁻¹ up to 119 mg kg⁻¹. An exception was replication 7 with a range of only 16 mg kg⁻¹.

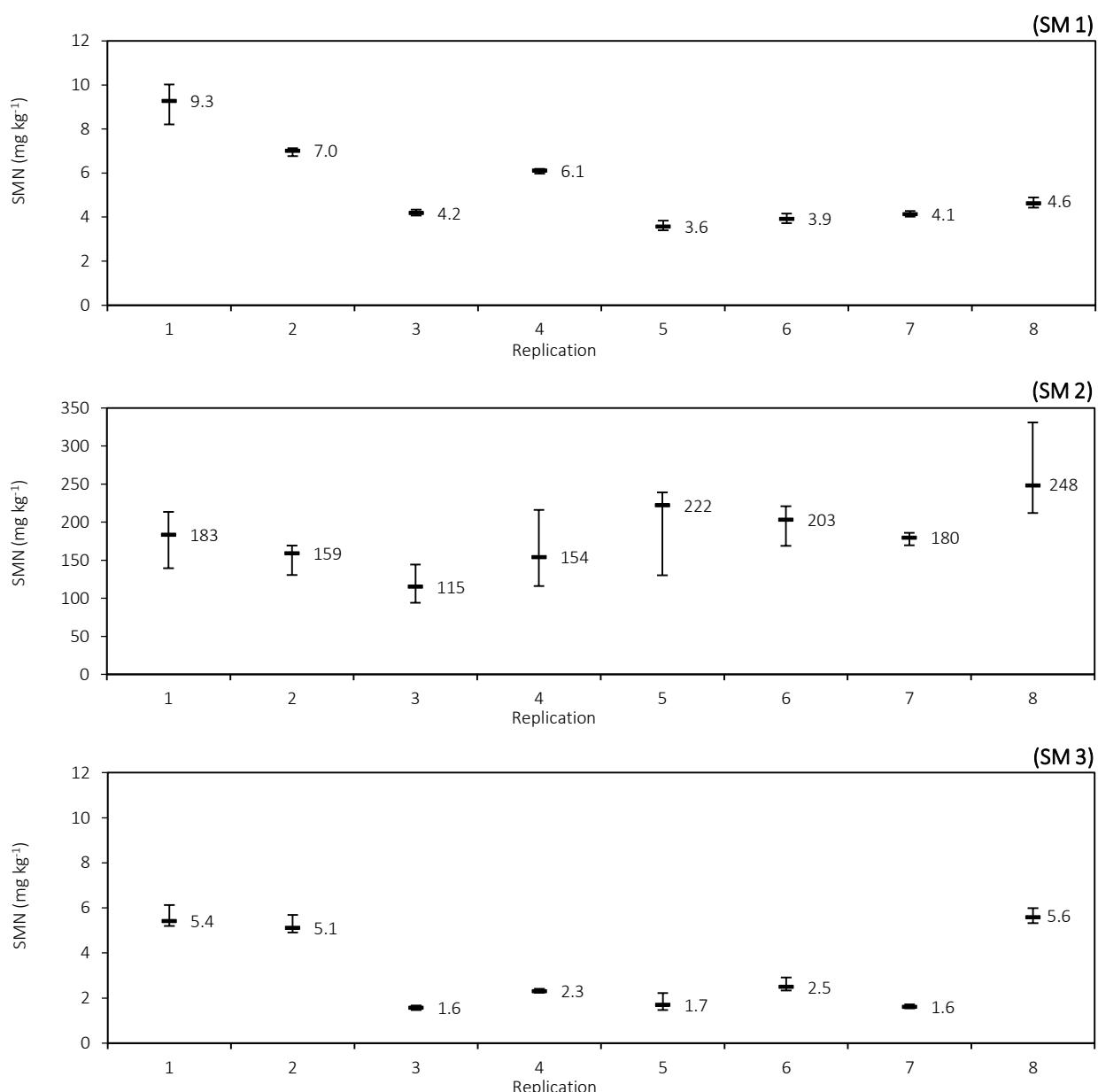
Coefficients of variation to calculate the sample size

In order to obtain a representative sample for each location the necessary sample size per pooled sample had to be determined based on the specification of the sampling variance for SMN (Section 'Data analysis'). For this purpose the coefficients of variance were calculated for each layer and averaged for the entire soil core (0 – 60 cm) and for the three soil monolith samples (0 – 30 cm), respectively (Table 3). To achieve this, SMN values for each sampling location were averaged for the entire sampling depth followed by calculating the means for all 64 (auger samplings) and 8 (soil monoliths) locations. For the auger samples from the interrow locations there was no relation between SMN values (both depths) and the row distance (15 or 30 cm).

Table 3: Coefficients of variation to calculate the sample size. AU (ir) = augers interrow; SM = soil monolith; AU (bSM) = augers below soil monoliths; n = sample size; s = standard deviation; CV = coefficient of variation.

	Depth (cm)	n	Mean (mg kg^{-1})	Min (mg kg^{-1})	Max (mg kg^{-1})	s	CV (%)
AU (ir)	0 – 30	64	7.2	2.7	17.7	3.1	44
	30 – 60	64	2.6	0.8	8.4	1.6	63
	0 – 60*	64	4.9	2.4	12.3	2.1	43
SM (1 – 3)	0 – 10	8	5.4	3.6	9.3	2.0	37
	10 – 25	8	185.0	129.7	248.4	38.6	21
	25 – 30	8	3.2	1.6	8.0	1.8	56
AU (bSM)	0 – 30	8	94.8	66.5	126.7	19.3	20
	30 – 60*	8	2.0	0.8	4.3	1.24	62

* = values for each sampling point were averaged for the entire sampling depth followed by calculating the means for all 64 (auger samplings) or 8 (soil monoliths) locations

**Figure 3:** Mean and range of soil mineral nitrogen (SMN = $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) concentrations (mg kg^{-1}) of the subsamples from the soil monoliths of the improvement trial. Whiskers = range; SM 1/2/3 = soil monoliths from a depth of 0 – 10 cm, 10 – 25 cm, and 25 – 30 cm.

Thus all interrow locations were combined in one data set (AU (ir), n = 64) to calculate the CV for each depth. That resulted in CV of 44% for the top layer, 63% for the middle layer, and 43% for the entire soil core (0 – 60 cm), respectively. The CV of the middle layer was confirmed by the CV of the eight auger samples taken below the soil monoliths (AU (bSM)). For the soil monolith samples CVs of 37% for SM 1, 21% for SM 2, 56% for SM 3, and 20% for the entire 0 – 30 cm layer were determined (Table 3).

Changes in SMN along the soil core due to drilling through banded slurry

Results of the test trial indicated SMN changes in soil core samples of the middle layers due to drilling the auger through the previously injected slurry band. Because of the distinctly higher SMN concentrations around the slurry injection zone compared to the middle layer (Section 3.1), it can be expected that even a slight carryover of slurry components from the injection zone along the soil core leads to incorrect SMN values for the middle layer. To verify this hypothesis, SMN values of the middle layer of 48 auger samples, which were drilled through the previously injected slurry band (AU_{tsb}), were compared with eight unaffected soil cores taken below the soil monolith samples (AU_{bSM3}; Figure 4).

For the AU_{tsb} samples six outliers according to Grubbs were determined (three of them extreme values), which were excluded from further calculations. The SMN mean of the middle soil layer of AU_{tsb} was 2.93 mg kg⁻¹ and thus significantly higher compared to the unaffected samples (AU_{bSM3}) with a SMN mean of 1.99 mg kg⁻¹.

Validating the sampling approach

The results of the validation trial confirmed the results observed in the test trial (Table 4). Slurry injection again resulted in extremely high SMN concentrations with a mean of 170 mg kg⁻¹ in SM 2 at the first sampling date.

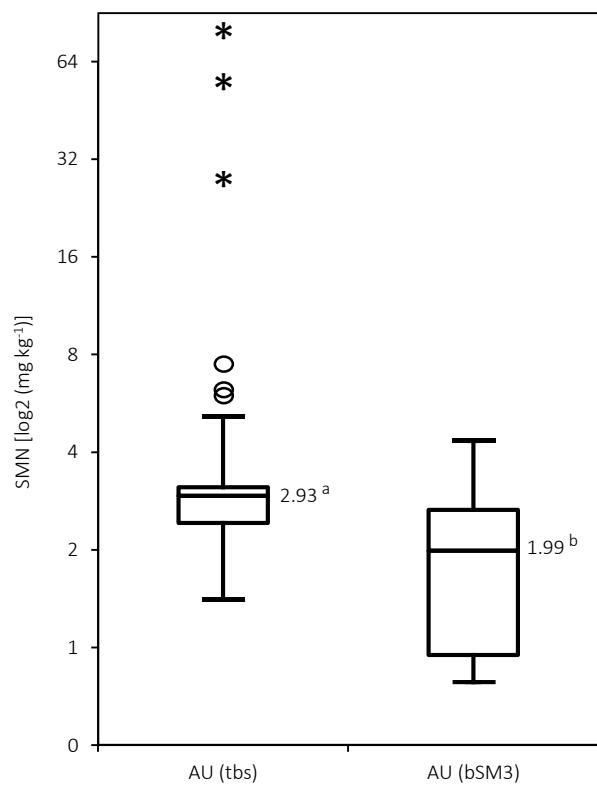


Figure 4: Soil mineral nitrogen (SMN = NH₄-N + NO₃-N) concentrations (mg kg⁻¹) of the middle layer (30 – 60 cm) based on 48 augers drilled through the slurry band [AU(tsb)] in comparison to eight samples taken from below the soil monolith samples [AU(bSM3)]; Boxplots: arithmetic mean without outliers (line inside the box), 1st and 3rd quartile (lower and upper hinge of the box); whiskers: minimum and maximum values; O and * = outliers according to Grubbs (O = values with a 1.5 to 3 times box height distance to nearest hinge; * = extreme values (distance nearest hinge is more than triple box height)); a/b = significant difference (t-test; P = 5%).

SM 3 (23 – 30 cm) showed also substantially higher SMN concentrations (34 mg kg⁻¹) compared to the other sampling grids. Furthermore, slightly higher SMN values were found for the AU 0 samples in the 30 – 60 and 60 – 90 cm soil layers compared to the same layers of AU 15 and 30. In the interrow locations decreasing SMN concentrations with increasing depth were noted. In addition, the SMN level declined with increasing distance to the row.

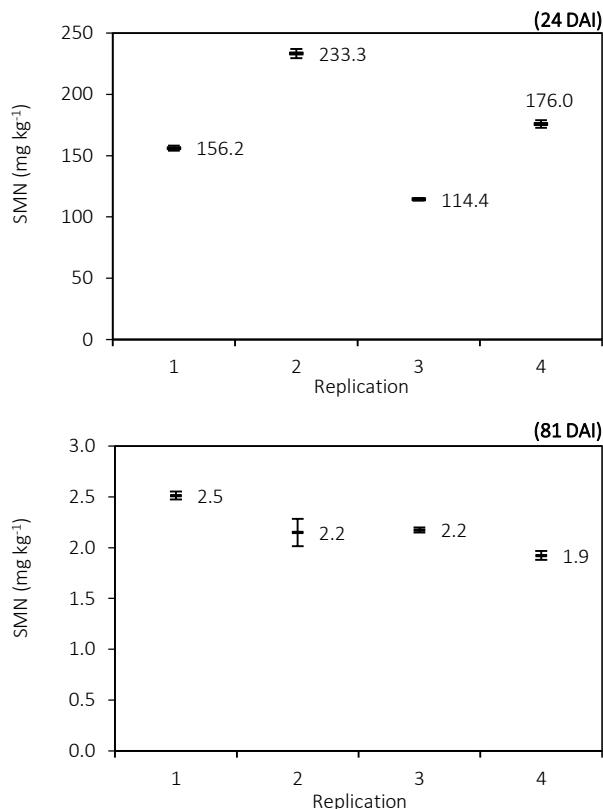
Until 81 DAI (10-leaf developmental stage) the SMN concentrations in the slurry injection zone (SM 2) and directly beneath this zone (SM 3) had sharply declined down to 2.2 mg kg⁻¹. Below the monolith samples, the SMN level had increased, resulting in the highest SMN content in the bottom layer of AU 0 (13.2 mg kg⁻¹).

Table 4: Soil mineral nitrogen (SMN; $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) concentration (mg kg^{-1}) of the validation trial. SM = soil monolith; AU 0 / 15 / 30 = auger with a distance of 0 / 15 / 30 cm to the maize row; Rep. = replication; s = standard deviation; CV = coefficient of variation; * = outlier according to Grubbs.

Soil layer (cm)	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	s	CV (%)
<i>Pre-emergence (24 DAI)</i>							
0 – 8	3.0	10.2	3.8	10.0	6.8	3.9	58
SM (1 – 3)	156.2	233.3	114.4	176.0	170.0	49.4	29
23 – 30	28.8	32.8	41.2	33.1	34.0	5.2	15
AU 0	30 – 60	4.3	4.4	6.1	5.4	1.2	23
	60 – 90	1.1	1.7	1.5	1.5	0.3	17
AU 15	0 – 30	4.7	6.6	7.9	6.5	1.3	20
	30 – 60	2.3	3.0	3.3	3.0	0.5	16
	60 – 90	1.1	1.2	1.2	1.2	0.1	7
AU 30	0 – 30	4.2	4.7	5.5	4.9	0.6	12
	30 – 60	2.5	3.3*	2.7	2.8	0.4	14
	60 – 90	0.9	1.2	1.3	1.1	0.1	13
<i>Ten-leaf stage (81 DAI)</i>							
0 – 8	6.0	5.8	6.7	5.7	6.1	0.4	7
SM (1 – 3)	2.5	2.2	2.2	1.9	2.2	0.2	11
23 – 30	3.8*	1.5	1.7	1.7	2.2	1.1	50
AU 0	30 – 60	10.0	9.1	6.0	9.2	2.3	25
	60 – 90	12.2	12.9	12.8	13.2	1.2	9
AU 15	0 – 30	3.7	3.0	3.2	3.1	0.5	16
	30 – 60	7.1*	3.6	3.2	4.4	1.8	41
	60 – 90	5.5	5.3	4.5	5.1	0.4	8
AU 30	0 – 30	2.7	2.6	2.5	2.6	0.1	2
	30 – 60	2.5	2.7	2.2	2.4	0.2	9
	60 – 90	3.2	2.8	2.5	2.9	0.3	11

With respect to the interrow locations the SMN concentrations of the top layer decreased, whereas higher concentrations were noted in the middle and especially in the bottom layer (exception: AU 30, middle). The coefficients of variance were mostly lower than 20%. The highest three CV values ranged from 40 to 60%. However, it has to be taken into account that two of them were influenced by outliers.

The homogenization process was tested for the second soil monolith at both sampling dates in all replications (Figure 5). At 24 DAI SMN values of the subsamples of each replication ranged from 2 to 7 mg kg^{-1} and at 81 DAI from 0.05 to 0.27 mg kg^{-1} . They were mainly less than five percentage of the mean values (exception: replication 2, 81 DAI).

**Figure 5:** Means and ranges of the soil mineral nitrogen (SMN = $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) concentration (mg kg^{-1}) of the subsamples of the soil monoliths of the validation trial in soil layer 8 – 23 cm (SM 2). DAI = days after injection.

DISCUSSION

The developed soil sampling strategy combines the use of a purpose-built soil shovel (Figure 2) in the direct range of the slurry injection zone and a standardized auger sampling-procedure below this zone and in the interrow space (Figure 1). This flexible method enables the investigator to obtain a reliable characterization of the spatial and temporal SMN distribution after banded slurry injection.

The results from the test trial show that the soil sampling strategy worked well, because SMN concentrations of the different sampling grids reflect the expected distribution after banded slurry injection. At the first sampling date (23 DAI) the slurry injection zone was characterized by very high SMN concentrations in the second soil monolith. With increasing distance from the injection zone to the interrow space and from top to bottom soil layers SMN concentrations decreased (disregarding the outlier in AU 30, 60 – 90 cm; Table 2). Until the second sampling date (64 DAI) the SMN concentration in the injection zone (SM 2) clearly declined. This is probably mainly due to vertical displacement of nitrate after nitrification of ammonium applied with the slurry into the middle and bottom soil layers and towards the interrow space (AU 15 and 30) of these layers, because the sandy Wehnen site (88% sand, 3% clay; Table 1) has a high risk of downward water movement. Similar results concerning SMN distribution were found by Clay et al. (1995) after band injection of mineral urea ammonium nitrate ($\text{CH}_4\text{N}_2\text{O} + \text{NH}_4\text{NO}_3$) fertilizer, by McCormick et al. (1983) after injection of pig slurry, by Sawyer et al. (1990b) after injection of beef slurry and by ZebARTH et al. (1999) around the injection zone of mineral ammonium nitrate (NH_4NO_3) fertilizer.

A basic advantage of using the purpose-built soil shovel is that it is possible to adapt the sampling depth of the single soil monoliths to the trial-individual slurry-injection depth, as done in the improvement and validation trials (see materials and methods ‘Improving the sampling strategy’ and ‘Validating the sampling approach’). Thus,

the complete slurry band can always be sampled in SM 2 (Figure 1). The overall sampling depth of the three soil monolith samples (SM 1 – 3) was limited to 30 cm below the surface in these trials to allow a better comparison between the sampling grids of the soil profile in the row within the interrow space. However, in the test trial several problems occurred, which were examined in the following experiments.

Carryover of slurry components

Unexpectedly, in the test trial the SMN value of the middle soil layer of AU 0, which was drilled through the banded slurry, was considerably higher compared to the third soil monolith at 24 DAI. This was most probably an unintended carryover of slurry components from the injection zone along the soil core into the middle soil layer of AU 0. This hypothesis was examined in the improvement trial. Obviously, the risk of such a carryover is high because the SMN mean of the middle layer of the augers, which were drilled through the banded slurry, is significantly higher compared to the unaffected samples taken from beneath the soil monolith samples (Figure 4). This is due to the very large differences in SMN concentrations between the injection zone and the soil layers below. McCormick et al. (1983) used a very small-scale sampling method around an injected pig-slurry band. Directly after slurry injection, they found SMN concentrations of nearly 500 mg kg^{-1} at a distance of 2.5 cm from the center of the banded slurry. Comfort et al. (1988) determined nitrate nitrogen concentrations up to $780 \text{ mg NO}_3\text{-N kg}^{-1}$ in the direct range of the injection zone of liquid dairy manure 26 DAI. Compared to the SMN concentrations of mostly less than 20 mg kg^{-1} , which can be expected in the middle soil layer (Tables 3 and 4; Comfort et al. 1988), the possible SMN changes, caused by a small carryover of slurry components along the soil core, is evident. As these results were found in a soil with 96% sand and only 2% clay (Lechtingen site; Table 1), we expect the problem of nitrogen contamination to be even more pronounced in soils with higher clay content. Obviously, drilling an auger through banded slurry for characteri-

zation of the spatial SMN distribution has to be avoided.

The high CV (i.e. the high spread in values among the four replications in relation to the mean) for each sampling location of the test trial (Table 2) were problematic. It is questionable that the soil sample is representative; this aggravates finding significant differences between treatments in field trials. It is possible that the homogenization quality was not sufficient or that the sample sizes were too small. The improvement trial was conducted to examine these aspects.

Homogenization quality

The range of the SMN mean values for the eight replications of soil monolith 1 (3.6 to 9.3 mg kg⁻¹) and 3 (1.6 to 5.6 mg kg⁻¹) of the improvement trial characterizes the heterogeneity of the SMN of the Lechtingen site. In comparison, the range of the subsamples of these sampling locations was rather low (mostly less than 1 mg kg⁻¹; Figure 3). Thus the homogenization of the soil-monolith samples (except the center from the injection zone, SM 2) by hand and using a 5 mm sieve was sufficient.

In addition to this site heterogeneity for the Lechtingen field the range of the SMN mean values for the eight replications of SM 2 (115 to 248 mg kg⁻¹) was influenced by the slurry application accuracy and slurry homogeneity. The scattering of the SMN values for the SM 2

subsamples (whiskers for SM 2; Figure 3) is even more pronounced. It can be hypothesized that soil-slurry compounds were formed which require a more intensive homogenization to obtain a representative sample. Based on these findings the homogenization process was improved by using an electric hand mixer in the validation trial, resulting in a markedly reduced scattering.

Sample sizes

For the calculation of a suitable sample size based on the formula from Gomez and Gomez (1984) (Section 'Data analysis' and Figure 6), the following three variables needed to be specified: The coefficient of variation (CV), the significance level (α), and the range of error expressed as a fraction of the mean per plot (d). Referring to Assefa and Chen (2007) and ZebARTH et al. (1999) the significance level (α) was set at 10%. For the auger samples, the CV of the entire soil core (AU (ir), 0 – 60 cm, CV = 43%; Table 3) was chosen for the calculation, because it is not practicable to differentiate the number of auger samples per soil layer. For the soil monolith samples the CV of SM 2 (SM 2, CV = 21%; Table 3) was used for the calculation, especially because nitrogen transformations around the slurry injection zone were of concern. For the d-value different scenarios are shown in Figure 6. Regarding soil sampling strategies in field trials the 'fitness of purpose' (Kuchenbuch et al. 2011) has to be taken into account.

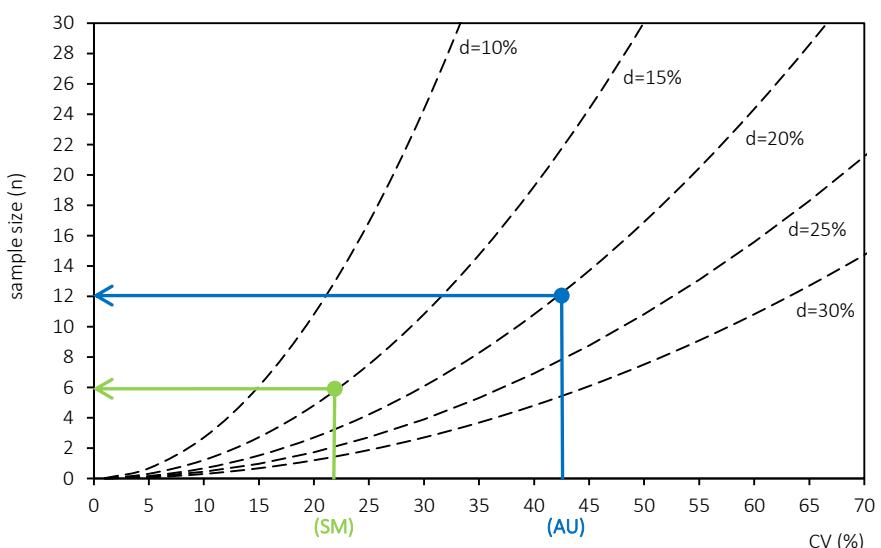


Figure 6: Schematic diagram for the calculation of the suitable sample sizes. (SM) = CV of soil mineral nitrogen (SMN; NH₄-N + NO₃-N) concentration of the soil monoliths (10 – 25 cm depth) of the improvement trial (Table 4); (AU) = CV of SMN concentration of the augers (0 – 60 cm depth) of the improvement trial (Table 4); d = range of error expressed as a fraction of the mean per plot; CV = coefficient of variance; significance level (P) = 10%.

Therefore, the d-value for the auger samples was set at 20%. This precision level was classified as practical for on-farm testing of SMN distribution by Clay et al. (1995) and ZebARTH et al. (1999) and leads to a sample size of twelve auger samples per pooled sample ((I); Figure 6). Because of the very high SMN concentration (Tables 2 and 4) around the slurry injection zone at the first sampling after slurry application a higher precision level with $d = 15\%$ is reasonable, leading to six soil-monolith samples per pooled sample ((II); Figure 6). These sample sizes were implemented in the validation trial.

Lower precision levels with d-values of 25 or 30% would lead to unacceptable imprecision, otherwise $d\text{-values} \leq 10\%$ would result in very high sample sizes which are not practical (Figure 6). If other precision levels are necessary in future trials or other CV will be taken as a basis for the calculation, Figure 6 allows to deduce the appropriate sample size.

To finally evaluate the improved procedure the homogenization quality for soil sampling, suitable sample sizes (twelve for the auger samples; six for the monolith samples) and the risk for nitrogen carryover along the soil core were implemented into the sampling strategy for the validation trial. Furthermore, soil sampling (Figure 1) was done in both middle maize-rows per plot to reduce the influence of a possible heterogeneity of the slurry application for a single slurry injection share.

The results of the validation trial concerning SMN distribution agree with the basic results described for the test trial, although SMN level at the Hollage site was somewhat lower compared to the Wehnen site. Due to the implemented measures the spread in values between the replications was considerably reduced. This is evident by comparing the distribution of the coefficients of variation from the test trial with the validation trial (Figure 7), revealing that this improved sampling strategy is considerably more representative for field situations with localized fertilizer application.

Comparing Figs. 3 and 5 it becomes clear that especially the CV of the auger samples were reduced. However, looking at the samples in the direct range of the slurry injection zone (SM 2) a certain spread in values has to be accepted. For example, the rather high coefficient of variation of soil monolith 1 at the first sampling date ($\text{CV} = 58\%$, Figure 3) most probably was caused by splashing of slurry in the injection slot during application. That is a basic problem of the slurry-injection technique. At the second sampling date the CV of this grid was only 7%. Overall, the CV of the soil monolith samples were also reduced in the validation trial compared to the test trial. This is due to the clearly improved homogenization process (comparing Figure 5 to Figure 3). Compared to the CV presented by ZebARTH et al. (1999) regarding soil nitrate concentrations using different sampling strategies after mineral nitrogen side-dressing at the 6-leaf stage of maize, the values of the present sampling strategy are markedly lower.

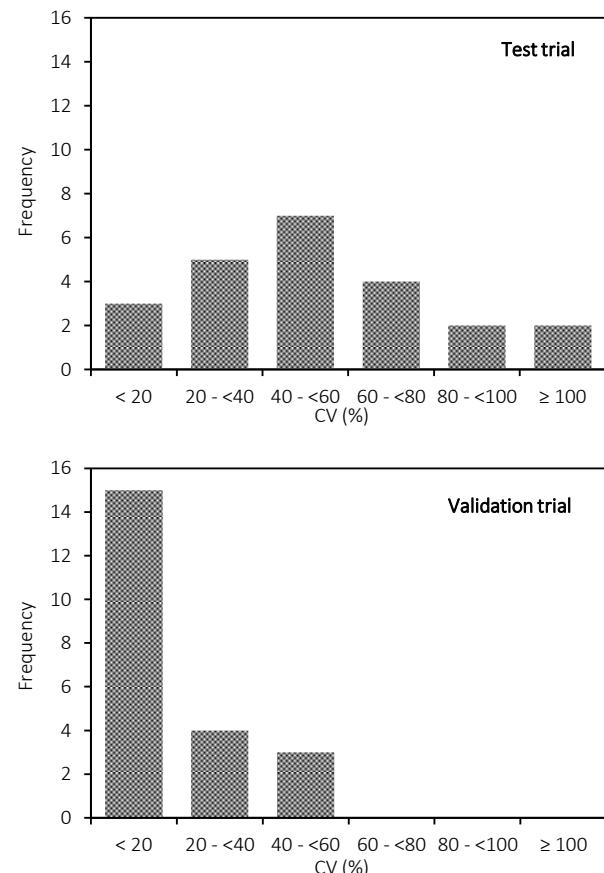


Figure 7: Distribution of the coefficients of variance (CV) of the test trial (Table 3) compared to the validation trial (Table 5).

CONCLUSION

The developed soil sampling strategy results in representative soil samples to allow a reliable characterization of the spatial and temporal SMN distribution in soils where slurry has been band-injected. The soil shovel allows a precise sampling of the soil zone with the slurry band and the auger is suitable to sample the interrow space. Furthermore, it is important to intensively homogenize the soil monolith sample including the slurry band and to take a suitable amount of samples per pooled sample. Drilling an auger through the slurry band has to be avoided. The basic methodology and the developed measures to obtain reliable SMN values can simply be transferred to various row crops and slurry injection spacings.

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2.2 Nitrogen dynamics following slurry injection in maize: Soil mineral nitrogen

Author Contributions

Matthias Westerschulte developed the experimental design, conducted the field trials, sampled and analyzed all data, performed the statistical evaluation, and wrote the manuscript

Carl-Philipp Federolf contributed to the development of the experimental design, conduction of the field trials and samplings as well as the evaluation

Dieter Trautz supervised Matthias Westerschulte

Gabriele Broll supervised Matthias Westerschulte

Hans-Werner Olfs supervised Matthias Westerschulte

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Nitrogen dynamics following slurry injection in maize: Soil mineral nitrogen

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ABSTRACT

In northwestern Germany slurry injection below maize (*Zea mays L.*) seeds is gaining increasing interest of farmers, because of the expected enhanced nitrogen (N) and phosphorus (P) use efficiencies compared to the usual fertilizing practice. The present study aims to compare the spatial and temporal soil mineral nitrogen (SMN) dynamics for these fertilizing strategies. Field trials with four treatments (unfertilized control, broadcast application + N P mineral starter fertilizer (MSF), injection and injection + nitrification inhibitor (NI)) were conducted using pig slurry on sandy soil in 2014 and 2015. Soil samples were taken from three soil layers at 30 cm intervals down to 90 cm and at three positions (below the maize row, 15 and 30 cm distance to the row) at several dates over the growing season. Soil monoliths (15 x 15 x 10 cm) were sampled around the injection zone and for all other soil zones an auger was used. In 2014 due to heavy rainfall all fertilized N was displaced from the top soil layer of the broadcast treatment until 6-leaf stage, while N displacement was significantly smaller after slurry injection (about 20 kg SMN ha⁻¹ more in top layer). The lateral movement of injected slurry N was negligible. In 2015 almost no displacement of fertilized N out of the top soil layer occurred independently of treatments, because of lower rainfall. The release of slurry N was delayed following broadcast application and large SMN concentrations were detected in the injection zones until 10-leaf stage. The addition of a NI resulted in significantly increased ammonium N concentrations in the injection zone throughout the early growth stages (+ 46% (2014) and + 12% (2015) at 6-leaf stage). Thus, N displacement was delayed in 2014 and in 2015 at 6-leaf stage increased SMN concentrations (+ 1/3 with NI) were found around the slurry band. Due to slurry injection, especially when combined with a nitrification inhibitor, the applied nitrogen is located in a soil zone with better spatial availability for plant roots compared to broadcast application and the risk of nitrate leaching is significantly reduced.

KEY WORDS

Spatial nitrogen distribution; nitrate leaching; nitrification inhibitor; nitrogen displacement; liquid manure

INTRODUCTION

In northwestern Germany, livestock husbandry and biogas production represent a major share of agricultural production. The dominant crop is maize (*Zea mays L.*) used as fodder or as substrate for biogas production (Warnecke et al. 2011; Kayser et al. 2011). The accruing liquid manure is predominantly used for fertilization of the next maize crop covering the nitrogen (N) and phosphorus (P) demand. Mainly these slurries are applied broadcast using a splash-plate or nowadays more usual trailing hose applicator followed by incorporation into topsoil (e.g. disc harrow, cultivator). In addition a mineral starter N P fertilizer (MSF) is supplemented side-banded at planting in order to overcome the limited nutrient availability during the early growth stages (Federolf et al. 2016). This fertilizing practice causes problems such as ammonia volatilization (Bacon 1995; Cameron et al. 2013; Carozzi et al. 2013; Misselbrook et al. 2002), surface runoff (Ceretta et al. 2010; Smith et al. 2001a,b; Webb et al. 2013), and nitrate leaching on the typically sandy soils of the region (Kayser et al. 2011; Sticksel et al. 1999).

In recent years, slurry injection below the maize row before planting is gaining increasing interest by farmers. Several studies show enhanced nitrogen use efficiencies (Ahmed et al. 2013; Schmitt et al. 1995; Schröder et al. 2015). The improved nutrient availability obviates the need for a mineral starter (Federolf et al. 2016). Addition of a nitrification inhibitor (NI) might be an option to further reduce N losses via denitrification or leaching finally resulting in a higher crop N uptake (Amberger 1986; Cameron et al. 2013; Dell et al. 2011; Federolf et al. 2016; Ruser and Schulz 2015; Singh and Verma 2007; Subbarao et al. 2006). For a better understanding of interrelationships within the soil-plant system after slurry injection characterization of the soil mineral nitrogen (SMN) dynamics is essential (Dell et al. 2011).

In the early 1980s Sutton et al. (1982) characterized SMN dynamics of pig slurry broadcast application compared to injection for

maize fertilization and found slightly increased N leaching within the injection treatment, however, they had applied slurry N amounts in distinct excess of plant requirements. Schmitt et al. (1995) examined inorganic N concentrations for cattle and pig slurry broadcast application versus injection within the top soil layers (0 – 30 cm; 30 – 60 cm). On silty and clayey loams they found larger SMN concentrations after slurry injection. This was confirmed by Sørensen and Jensen (1998) for sheep slurry injection in spring barley on a sandy soil. They concluded that an increased N immobilization after broadcast application had occurred, however, these studies disregarded the lateral movement of band-applied N. Van Dijk and Brouwer (1998) considered this when comparing broadcast and banded application of mineral N for maize on several sandy soils in The Netherlands. They determined a homogeneous SMN distribution for broadcast and just a small lateral and more downwards SMN displacement after banded application. For injected cattle or pig slurry Sawyer et al. (1990b), Cameron et al. (1996), and Chen et al. (2010) confirmed just a slight lateral SMN movement. The distribution of SMN and roots comparing standard injection (25 cm between injection slots) and banded injection (75 cm between injection slots) of cattle slurry for maize on a sandy soil was observed by Schröder et al. (1997). In the first 5 – 7 weeks after planting, nutrients were mostly supplied by the soil volume close to the plant row and thus banded injection led to a better availability. Up to now just a few studies examined the effect of NI addition to injected slurry. While McCormick et al. (1983) and Schmitt et al. (1995) found significantly enhanced ammonium N concentrations due to addition of Nitrapyrin (2-Chloro-6-(trichlormethyl)pyridin)) for some weeks after slurry injection, Comfort et al. (1988) could not confirm this effect for the same agent. Dittert et al. (2001) verified that due to combining DMPP (3,4-dimethylpyrazol phosphate) with injected cattle slurry N₂O emissions can be reduced by 32%, however, they did not focus on SMN dynamics.

The objective of our study is the characterization of the spatial and temporal SMN dynamics after

slurry broadcast application compared to slurry injection. Additionally the effect of a NI (DMPP) is verified for slurry injection. Our study is based on the hypotheses that after slurry injection the mineral N remains directly below the maize row and hence is located in a soil zone, which is better accessible for maize roots during the growing season. Slurry N remains in the top soil layer for a longer time period and lateral movement of slurry N into the interrow space is reduced. Furthermore, we assume that the addition of DMPP within the slurry band delays the nitrification of ammonium N, especially effective due to the small soil-slurry interaction, resulting in a significant reduced risk of N leaching losses. Federolf et al. (2017) cover the corresponding effects on crop development.

MATERIAL AND METHODS

Experimental sites, soil characteristics, and weather conditions

The trial was conducted at two adjacent fields in Hollage, Lower Saxony, northwestern Germany in 2014 and 2015. The altitude is \approx 65 m a.s.l. and coordinates are 52°20'N, 07°58'E. According to IUSS Working Group WRB (2014) the soil type is a Plaggic Podzol and the textural class of the topsoil is sand at both sites. Preplant SMN

contents (0 – 60 cm) were 35 kg ha⁻¹ in 2014 and 45 kg ha⁻¹ in 2015 (Table 1). The region is characterized by maritime climate. Long-term (1994 – 2014) mean annual precipitation was 799 mm and mean annual air temperature 10.0 °C.

Weather conditions during the vegetation season were rather different in 2014 and 2015. In 2014 precipitation events occurred regularly from April until the middle of July (Figure 1). The cumulative precipitation was much higher compared to the long-term mean until this time. From the end of July the amount of precipitation decreased and was very low in September. From April to October 2014 the cumulative precipitation amounted to 544 mm. Precipitation was comparable to the previous year in 2015 until the end of April.

Table 1: Soil properties (0 – 30 cm).

		2014	2015
Sand	(%)	91	87
Silt	(%)	8	9
Clay	(%)	1	4
pH	(CaCl ₂)	5.3	5.5
C _{org}	(%)	1.14	1.66
C/N		13.0	16.5
Total N	(%)	0.09	0.10
P	(CAL; mg 100 g ⁻¹)	8.0	7.8
SMN	(kg ha ⁻¹)*	35	45

SMN = soil mineral nitrogen (NH₄-N + NO₃-N); P (CAL) = phosphorus extracted with calcium-acetate-lactate solution;

* = soil layer 0 – 60 cm

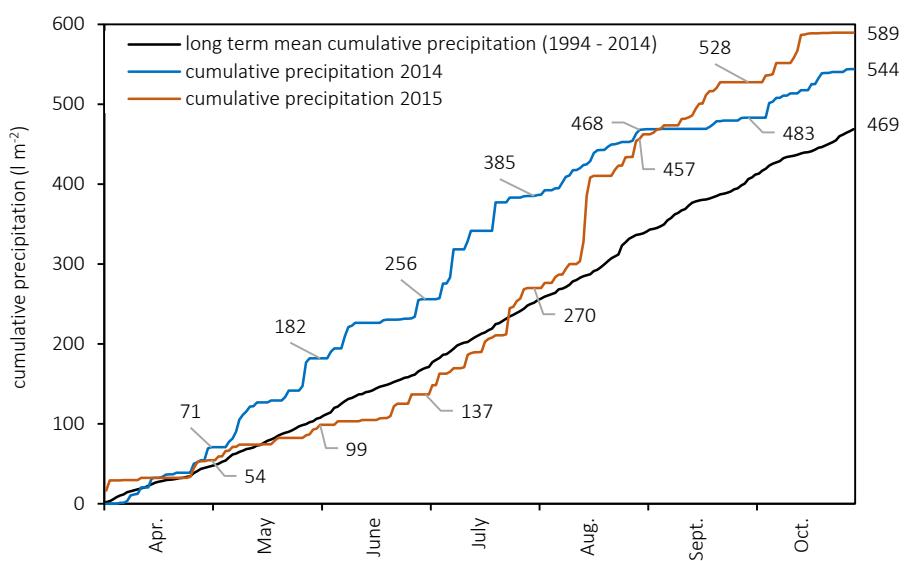


Figure 1: Cumulative precipitation ($l m^{-2}$) from April to October in 2014 and 2015 compared to the long term mean (1994 – 2014).

In the second half of May until the end of June a dry period occurred. Between April and the end of June 2015 the cumulative precipitation was 119 mm less than in 2014. Later in the 2015 growing season the amounts of precipitation increased. A very heavy precipitation event (110 mm in three days) took place in the middle of August (Figure 1). In September and October precipitation occurred frequently. The cumulative precipitation amounted to 589 mm from April to October 2015 and thus 45 mm more than in 2014.

The two trial years differed distinctly with respect to air temperature (Table 2). In 2014 compared to 2015 the monthly mean temperatures were higher over almost the whole maize growing season (exception: August). Mean temperature (for the period April – October) was 15.3 °C in 2014 and 14.0 °C in 2015. The thermal time (according to McMaster and Wilhelm 1997, Method 1) between planting and harvest date amounted 1,450 °C in 2014 and only 1,272 °C in 2015 (Federolf et al. 2017). Thus wet and warm conditions were given during early growth stages of maize in 2014 compared to dry and colder conditions in 2015.

Experimental design and treatments

The field trial had a randomized complete block design with four replicates and four treatments in both years. One experimental unit consisted of a plot 3 m wide by 25 m long with four maize rows at a spacing of 75 cm. The following treatments were conducted:

- Control (C): without any fertilization,
- Broadcast (B): slurry application by trailing hose applicator followed by immediate incorporation using a disc harrow and

additional application of a mineral, side-banded starter fertilizer (MSF) containing 23 kg N ha⁻¹ (9.4 kg nitrate N ha⁻¹, 13.6 kg ammonium N ha⁻¹) and 10 kg P ha⁻¹ (5.6 kg ha⁻¹ water-soluble P ha⁻¹) at planting,

- Injection (I): slurry injection without MSF,
- Injection + NI (I(N)): slurry injection with addition of the NI DMPP (3,4-dimethylpyrazol phosphate; ENTEC® FL, EuroChem Agro GmbH, Mannheim, Germany) at a rate of 10 l ha⁻¹ (1.21 kg DMPP ha⁻¹) and without MSF.

This field experiment was designed to enable a system comparison between current agricultural practice (broadcast; Section 1) and the new, innovative fertilizing system (slurry injection with and without NI). The injection treatments (I, I(N)) were conducted 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 12 cm (2014) and 10 cm (2015) below the soil surface. Maize was later planted 4.5 cm deep directly above the slurry bands at a rate of 9.2 grains m⁻². The N fertilization rate was calculated according to local standards (i.e. Chamber of Agriculture Lower Saxony; Baumgärtel et al. 2010). The recommended N fertilization rate is 180 kg N ha⁻¹ minus N applied as MSF and preplant soil mineral N (SMN; 0 – 60 cm). Furthermore, site-specific conditions like recent organic fertilizer application and catch cropping are considered. This led to a slurry application rate in treatments B, I, and N of 23 m³ ha⁻¹ (127 kg ammonium N ha⁻¹, 42 kg P ha⁻¹) in 2014 and 24 m³ (84 kg ammonium N ha⁻¹, 34 kg P ha⁻¹) in 2015. The omission of MSF in the injection treatments resulted in a smaller nutrient input by 23 kg N ha⁻¹ and 10 kg P ha⁻¹.

Table 2: Monthly mean temperature (°C) at the experimental sites in 2014 and 2015 compared to the long term average (1994 – 2014); Mean = mean over growing season (April – October).

	April	May	June	July	August	September	October	Mean
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

Crop management practices

In spring 2014 a disc harrow was used two times (March 5 and 27, 2014) for stubble cultivation (previous crop maize) and seedbed preparation (working depth \approx 10 cm). The pig slurry from a regional pig fattening farm (Table 3) was applied on April 11, 2014 and maize was planted on April 25, 2014 (variety: Ricardinio, KWS SAAT AG, Einbeck, Germany). Two herbicide applications were performed according to local standards on May 16 and June 07, 2014. For harvesting, a special plot forage harvester was used on October 08, 2014. Previous crop for the maize grown in 2015 was spring barley (*Hordeum vulgare* L.), followed by a catch crop [blend of mustard (*Sinapis alba* L.) and oil radish (*Raphanus sativus* L.)]. Stubble cultivation was carried out with a disc harrow on March 04, 2015 (working depth \approx 10 cm) and immediately prior to slurry application on April 14 (working depth \approx 5 cm). The pig slurry was from the same pig fattening farm as in 2014 (Table 3) and the same maize variety was planted on April 22, 2015. Two herbicide applications were applied according to local standards on April 23 and May 22, 2015. Harvesting was carried out on September 28, 2015.

Table 3: Slurry properties.

	2014	2015
DM (%)	9.3	6.5
Total N (g kg^{-1})	7.2	5.4
$\text{NH}_4\text{-N}$ (g kg^{-1})	5.5	3.5
P (g kg^{-1})	1.8	1.4
C/N	4.7	4.7
pH (CaCl_2)	7.7	7.6

DM = dry matter content

Soil sampling

The soil sampling was performed according to Westerschulte et al. (2015), because a standardized auger sampling procedure is not suitable to characterize the spatial and temporal SMN dynamics when slurry has been band injected. In brief the soil profile was sampled grid-like by combining an auger sampling procedure with a purpose-built metal shovel (15 cm wide, 15 cm high, and 10 cm deep), which

yielded rectangular soil monoliths (SMs). To characterize the lateral displacement of fertilized N after slurry injection samples were taken in the interrow space (IRS) in a distance of 15 and 30 cm to the maize row using an auger (IRS 15 and 30; Figure 2). The soil of twelve augers, distributed evenly on the left and right side of the two middle maize rows, was combined into one pooled sample per IRS and per plot. Each sample was divided into three soil layers: top (0 – 30 cm), middle (30 – 60 cm), and bottom (60 – 90 cm). Directly below the maize row (BMR; Figure 2) a small pit was dug (about 35 cm deep) and soil monoliths (SM 1 – 3) were sampled in three depths from the soil profile using the metal shovel (Figure 2). Because of the slightly different depths of the top of the slurry bands (Section ‘Experimental design and treatments’ 2.2), the monoliths were taken in 0 – 8 cm (SM 1), 8 – 23 (SM 2), and 23 – 30 cm in 2014 and in 0 – 6 (SM 1), 6 – 21 (SM 2), and 21 – 30 cm (SM 3) in 2015, thereby ensuring that the slurry band is completely sampled in SM 2.

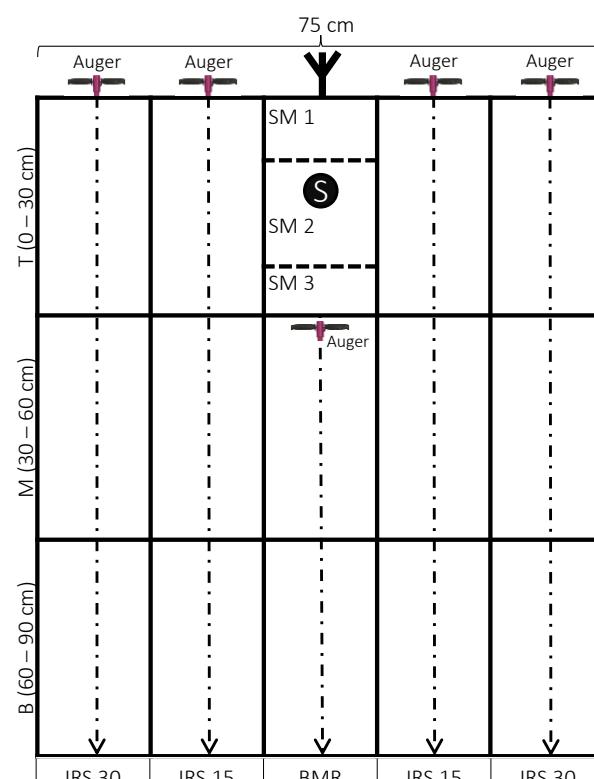


Figure 2: Scheme of the soil sampling method. M = maize row; T = top; M = middle; B = bottom; IRS 15 / 30 = interrow space with 15 or 30 cm distance to the maize row; BMR = below maize row; S = slurry band; SM = soil monolith, sampled with a purpose built metal shovel (Westerschulte et al. 2015, modified).

Six monolith samples were taken per depth and per plot (three from each middle maize row) to obtain a representative pooled sample. Below SM 3 an auger was used for sampling the middle and bottom soil layers (Figure 2; Westerschulte et al. 2015).

Each of the six monolith samples per plot was manually homogenized thereby removing all visible roots. For further homogenization a typical household electric hand mixer was used (using the whisks). Afterwards subsamples of about 300 mL per monolith were pooled and again intensively homogenized. The auger samples were also homogenized by using the electric hand mixer. Finally, the samples were passed through a 5 mm sieve and about 300 – 400 g of soil per sample were packed into plastic bags. Then they were immediately frozen until analyzed.

The soil samples were taken at defined developmental stages (Table 4). These were adjusted to the expected plant N uptake. In 2014 no samples were taken post application, so just five sampling dates were completed compared to six sampling dates in 2015. Federolf et al. (2017) describe the corresponding plant N uptake during the vegetation season of both trials.

Table 4: Soil sampling dates.

Growth stage	2014		2015	
	Date	DAA	Date	DAA
Post application	not sampled		April 15	1
VE	May 05	24	May 06	22
V6	June 11	61	June 09	56
V10	July 01	81	June 30	77
VT	July 22	102	July 28	105
Post harvest	Oct. 13	185	Sept. 30	169

DAA = days after application; VE = vegetative emergence stage; V6 or 10 = vegetative leaf stage 6 or 10; VT = tasseling

Soil and slurry analysis

The soil samples were thawed at 4 °C. Afterwards the field moist samples were extracted with a calcium chloride solution ($c(\text{CaCl}_2) = 0.0125 \text{ mol l}^{-1}$) at a ratio of soil to solution of 1:4 (mass:volume) (DIN 19746 2005). Then the concentrations of ammonium and nitrate were determined spectrophotometrically. Total N concentration of pig slurries

was analyzed using the Kjeldahl method after nitrate reduction with Devarda's alloy (DIN EN 15476 2009). The ammonium concentration was determined by direct distillation of the slurry with magnesium oxide followed by titration (according to Bremner and Keeney 1966). After oven drying at 105 °C to constant weight the dry matter content of the slurries was determined gravimetrically.

Data analysis

Concentrations of ammonium N and nitrate N smaller than the detection limit of 0.5 mg kg⁻¹ were replaced by the half detection limit (0.25 mg kg⁻¹; Kanisch et al. 1998). The SMN concentrations (mg kg⁻¹) were calculated by adding up ammonium N (mg kg⁻¹) and nitrate N (mg kg⁻¹) and subsequently outliers were defined according to Grubbs (1950) at 5% level of significance. Detected outliers ($\approx 1\%$ of all data) were excluded from further calculations. Results presented in tables and figures are arithmetic means. For calculating the SMN contents in kg ha⁻¹ the bulk density was set to 1.4 g cm⁻³ for top, 1.5 g cm⁻³ for middle, and 1.6 g cm⁻³ for bottom soil layers. Normal distributions were tested based on Kolmogorov-Smirnov-Test and the variance homogeneity was verified using the Levene-Test. If the variance homogeneity of the original data was not given, a log or arctan transformation was done, however, in all tables and figures the original values are shown. Differences in soil mineral N contents (kg ha⁻¹) between all treatments (Figure 4; Table 5) were tested by univariate analysis of variance (ANOVA). 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. For one dataset (Table 5; 2014, V6, BMR), the Games-Howell-Test was used, because no transformation could establish variance homogeneity. Differences between treatments I and I(N) concerning SMN, nitrate N and ammonium N concentrations (mg kg⁻¹) in the direct range of the slurry band (Table 6) were tested by an independent samples t-test (5% level of significance).

RESULTS

Spatial distribution and temporal changes in soil mineral nitrogen concentrations

Spatial distribution within the soil profile and temporal changes during the vegetation seasons of SMN for both years are shown in Figure 3. In 2014 only minor changes in SMN were detected for the unfertilized control at a low level during the whole growing season (about $1 - 6 \text{ mg kg}^{-1}$). At VE sampling date (24 DAA) in the broadcast treatment the applied fertilizer N (by slurry and MSF) was still located in the top layer, however, until V6 the SMN concentrations in this layer decreased nearly down to values close to unfertilized control level. SMN values markedly increased in the subsoil (30 – 90 cm). During the following weeks the concentrations decreased in the whole soil profile until tasseling (102 DAA), however, the largest concentrations were found in the bottom layer (especially in the interrow space; IRS 30: 7.9 mg kg^{-1}). Spatial and temporal SMN dynamics of both injection treatments were similar during the whole growing season. At VE sampling date they were characterized by extremely large SMN concentrations in the grid around the slurry band (SM 2: 170 and 213 mg kg^{-1}). These values declined distinctly until V6, somewhat stronger without using a NI, but the level was still clearly higher compared to broadcast treatment. Beneath the injection zones the SMN values increased. At V10 and tasseling the greatest SMN values were given in the bottom soil layers, as already shown for the broadcast treatment. During the entire growing season just a very small lateral slurry N displacement into the interrow space 15 could be observed. The smallest SMN concentrations were detected in the middle layers below the maize row and in the soil grids IRS 15 at tasseling, regardless of treatments. At post-harvest no notable differences in SMN distribution could be found.

In 2015 during almost the whole growing season changes in SMN occurred primarily in the top layer. In the control treatment SMN concentrations increased until V10 (IRS: 5.0 to

16 mg kg^{-1}) and decreased afterwards, while in SM 2 values declined already from V6 onwards. SMN dynamics in the broadcast treatment developed rather similar, although starting on a markedly increased level (IRS: $14 - 16 \text{ mg kg}^{-1}$). From VE onwards larger concentrations were given in the soil zones below the maize row due to MSF application. Also in 2015 the spatial distribution and temporal changes in SMN were similar for both injection treatments. The injection zones were characterized by extremely large values at the first (1 DAA) and second (22 DAA) sampling dates ($274 - 298 \text{ mg kg}^{-1}$). Thereafter, SMN concentrations declined markedly in these soil grids. A more pronounced drop in SMN occurred without using the NI. In the interrow space SMN dynamics were similar to the control treatment at the beginning, but larger values were determined in IRS 15 from V6 (I and I(N): 22 mg kg^{-1} versus C: 14 mg kg^{-1}) and in IRS 30 from tasseling (I: 7.3 mg kg^{-1} and I(N): 7.7 mg kg^{-1} versus C: 4.2 mg kg^{-1}) onwards. Post-harvest the spatial SMN distribution for the fertilized treatments (B, I, I(N)) was comparable. Similarly small concentrations were observed in the soil zones below the maize row and larger values in the IRS. As in 2014, the smallest SMN concentrations were detected directly below the maize row, independently of treatments, at the last two sampling dates (105 and 169 DAA).

Soil mineral nitrogen contents

SMN contents per soil layer

The SMN contents for each layer (top, middle, and bottom) and the total SMN contents (0 – 90 cm) are shown in Figure 4. At VE sampling date in 2014 the total SMN contents ranged from 45 (C) to 150 kg ha^{-1} (I(N)). The broadcast treatment had a significantly smaller level with 115 kg ha^{-1} compared to injection + NI, while injection without NI was in between. These significant differences in total SMN were based on the contents given in the top soil layer. At V6 the total SMN content after broadcast application (142 kg ha^{-1}) was significantly larger compared to injection (122 kg ha^{-1}) and injection + NI (116 kg ha^{-1}). In the top soil layer, however, there was no longer a significant difference between control and broadcast.

2014

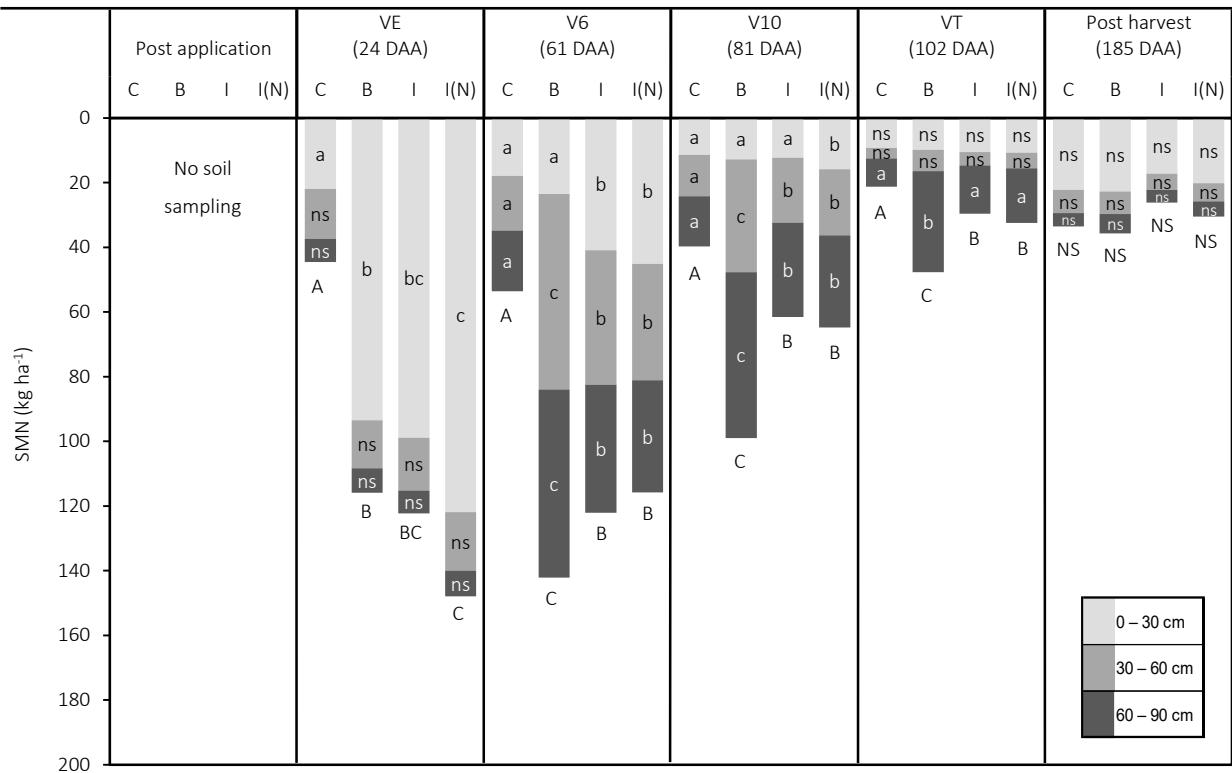
		P. a.					VE (24 DAA)					V6 (61 DAA)					V10 (81 DAA)					VT (102 DAA)					P. h. (185 DAA)									
C	No soil sampling						5.3	5.7	3.3 3.9	5.7	5.3	4.4	4.5	4.3 3.2	4.5	4.4	2.5	2.8	5.6 2.2	2.8	2.5	2.0	2.2	4.2 2.2	2.2	2.0	5.7	5.9	3.2 3.4	5.9	5.7					
		3.4	3.1	4.1	3.1	3.4	3.7	4.1	3.2	4.1	3.7	3.0	3.3	1.8 1.4	3.3	3.0	3.1	3.5	3.0 3.5	3.3	3.0	1.0	0.8	0.9 0.8	0.8	1.0	1.5	1.7	1.5 1.7	1.7	1.5					
		1.4	1.4	1.8	1.4	1.4	3.9	4.1	3.4	4.1	3.9	3.1	3.5	3.0 3.5	3.0	3.1	2.5	1.8	0.5 1.8	2.5	2.5	1.0	0.9	0.6 0.9	0.9	1.0	1.0	1.7	1.5 1.6	1.5	1.5					
B	No soil sampling						19 19	19 45	35 9.4	19	19	4.6	7.4	4.8 2.8	7.4	4.6	3.0	3.1	7.2 1.8	3.1	3.0	2.2	2.3	4.9 1.9	2.3	2.2	5.9	6.2	2.8 2.9	6.2	5.9					
		3.1	3.1	4.1	3.1	3.1	11	15	15	15	11	7.0	9.6	5.7 1.5	9.6	7.0	2.5	0.9	0.5 0.9	2.5	2.5	2.3	2.3	1.9 1.5	2.3	2.2	1.6	1.6	1.3 1.3	1.6	1.6	1.6 1.6	1.6	1.6		
		1.7	1.5	1.6	1.5	1.7	11	12	15	12	11	8.9	11	10 11	11	8.9	7.9	6.5	3.8 6.5	6.5	7.9	7.9	6.5	3.8 6.5	7.9	7.9	1.6	1.0	1.0 1.0	1.0	1.6	1.6 1.6	1.6	1.6		
I	No soil sampling						4.9 6.6	6.8 34	170 34	6.6	4.9	4.0	4.9	4.8 34	4.9	4.0	2.6	3.1	6.1 2.2	3.1	2.6	4.5	4.0	4.1 2.5	4.0	4.5	2.6	2.2	4.0 2.7	2.2	2.6	1.3	0.7	0.6 0.7	1.3	1.3
		3.0	3.2	5.6	3.2	3.0	3.6	5.7	28 54	5.7	3.6	2.6	4.6	9.3 5.5	4.6	2.6	1.0	1.1	1.3 1.1	1.1	1.0	1.3	1.0	1.1 1.1	1.3	1.0	1.3	1.3	0.7	0.6 0.7	1.3	1.3	1.3			
		1.4	1.4	1.8	1.4	1.4	4.1	6.5	20 20	6.5	4.1	3.1	5.3	13 5.3	5.3	3.1	0.9	0.8	0.7 0.8	0.8	0.9	3.0	4.1	2.1 2.1	4.1	3.0	2.6	2.2	4.0 3.7	2.7	5.0	5.0 5.0	5.0	5.0		
I(N)	No soil sampling						5.2 7.4	13 43	213 43	7.4	5.2	3.7	4.9	5.4 49	4.9	3.7	3.0	3.5	7.8 5.5	3.5	3.0	2.6	2.3	4.7 2.8	2.3	2.6	5.0	5.0	4.7 4.7	5.0	5.0	5.0 5.0	5.0	5.0		
		3.6	3.7	5.6	3.7	3.6	3.4	5.0	25 46	5.0	3.4	2.7	4.5	8.2 4.5	4.5	2.7	1.3	1.0	0.8 1.0	1.0	1.3	1.3	1.0	0.8 0.8	1.4	1.3	1.4 1.4	1.3	1.2	1.2 1.2	1.2	1.3	1.2	1.2	1.2	
		1.6	1.6	1.9	1.6	1.6	3.8	5.4	18 18	5.4	3.8	3.2	4.8	14 4.8	4.8	3.2	2.9	4.0	3.8 4.0	4.0	2.9	2.9	1.0	0.7 0.7	1.0	1.0	1.1	1.1	1.0	0.7 0.7	1.0	1.1	1.1	1.1	1.1	

2015

		P. a. (1 DAA)					VE (22 DAA)					V6 (56 DAA)					V10 (77 DAA)					VT (105 DAA)					P. h. (169 DAA)													
C	No soil sampling	4.9 4.6	5.4 2.8	4.6 2.8	4.9 2.8	4.9	10 11	11 13	22 6.2	11	10	14 14	14 12	26 9.3	14 14	14	15 16	24 7.3	16 12	15 16	15	4.2 4.1	6.9 3.2	4.1 3.0	4.2	3.5 3.6	4.1 2.8	3.6 3.6	3.5 2.0	3.5 3.5										
		1.9	1.6	1.8	1.6	1.9	3.2	2.6	2.3 54	2.6	3.2	2.7	2.7	28 49	2.7	2.7	3.0	3.1	3.5 23	3.1	3.0	1.9	1.7	1.5 1.7	1.7	1.9	1.2	1.1	0.6 1.1	1.1	1.2									
		1.9	1.7	1.9	1.7	1.9	2.3	2.3	2.0 27	2.3	2.3	2.2	2.2	22 27	2.2	2.2	2.3	2.3	2.1 23	2.1	2.1	2.2	1.1	1.2	1.2 1.2	1.2	1.4	0.6	0.5 0.6	1.4	1.4									
B	No soil sampling	14 16	6.1 3.4	16 14	16 14	14	25 27	27 54	22 6.5	27	25	30 29	29 58	94 12.8	29	30	31 38	23 19	84 38	31 31	31 31	13 6.7	10 3.2	6.7 2.5	13	15 14	13 7.5	14 5.1	15 15	14 14	15 15									
		1.8	2.6	2.2	2.6	1.8	2.7	2.8	3.0 54	2.8	2.7	2.9	2.9	2.8 12.8	2.9	2.8	3.2	4.1	3.5 4.1	4.1	3.2	6.2	1.8	0.8 1.8	1.8 6.2	6.2	2.4	2.2	1.7 2.2	2.2	2.4									
		2.6	2.1	2.8	2.1	2.6	2.4	2.4	2.3 2.4	2.4	2.4	2.1	2.2	2.1 36	2.1	2.2	2.1	2.4	2.1 43	2.4	2.1	2.1	2.7	2.7	1.0 1.0	0.7 0.7	1.0 1.6	1.6	1.6	1.6 1.6	1.6	1.6	1.6	1.6	1.6					
I	No soil sampling	4.6 4.8	23 4.8	4.8 4.8	4.8 4.8	4.6	9.9 12	12 15	36 298	12	9.9	15 22	22 123	67 36	22	15	18 31	73 77	31 31	18 18	7.3 20	6.9 11	20 18	7.3 7.3	8.8	7.6	6.2 3.9	9.2 7.6	7.6	8.8 8.8	8.8	6.3	3.0 3.0	1.8 3.0	3.0 6.3					
		1.8	1.9	2.5	1.9	1.8	2.4	2.6	3.1 15	2.6	2.4	2.7	2.7	2.6 34	2.7	2.6	3.0	3.2	4.5 4.5	3.2	3.0	2.6	2.6	3.3 3.3	2.3 2.3	3.3 3.3	2.6 2.6	6.3	3.0	1.8 3.0	3.0 3.0	3.0 6.3	3.0	3.2	1.5 1.5	1.4 1.5	3.2 3.2			
		1.9	1.9	2.4	1.9	1.9	2.3	2.5	2.6 2.5	2.5	2.3	2.0	2.0	2.0 36	2.0	2.0	2.3	2.5	2.2 41	2.3	2.5	2.3	1.9	2.0 2.0	1.3 1.3	2.0 2.0	1.9 1.9	3.2	1.5	1.4 1.4	1.5 1.5	3.2 3.2	3.2	3.2						
I(N)	No soil sampling	4.0 4.3	17 282	4.3 3.3	4.0 4.0	4.0	11 12	12 11	35 279	12	11	15 22	22 182	72 36	22	15	17 32	67 89	32 41	17 17	7.7 18	9.1 25	18 11	7.7 7.7	11	12	6.6 3.7	10 11	12 11	11	7.0	3.8 3.8	1.6 1.6	3.8 3.8	7.0 7.0	7.0	2.0	1.9 2.0	1.5 1.5	1.9 2.0
		1.8	1.9	2.5	1.9	1.8	2.5	2.4	2.2 2.1	2.4	2.5	2.5	2.8	3.6 3.6	2.8	2.8	2.5	2.8	4.0 4.0	4.0	4.0	2.8	2.9	2.6 2.6	2.6 2.6	2.9 2.9	2.6 2.6	7.0	3.8	1.6 1.6	3.8 3.8	7.0 7.0	7.0	2.0	1.9 1.9	1.5 1.5	1.9 2.0			
		1.7	2.1	1.8	2.1	1.7	2.4	2.1	1.9 2.1	2.1	2.4	2.0	2.2	2.3 2.3	2.2	2.0	2.1	2.3	2.0 2.1	2.0	2.3	2.1	1.4	1.6	1.5 1.5	1.6 1.6	1.4 1.4	2.0	1.9	1.5 1.5	1.9 2.0	2.0 2.0	2.0	2.0						

Figure 3: Spatial distribution and temporal changes in soil mineral nitrogen [SMN (mg kg^{-1}) = $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$] according to Figure 2. Red indicates large and green small SMN concentrations; P. a. = post application; P. h. post harvest; VE = vegetative emergence stage; V6 or 10 = vegetative leaf stage 6 or 10; VT = tasseling; C = control; B = broadcast; I = injection; I(N) = injection + nitrification inhibitor; DAA = days after application.

2014



2015

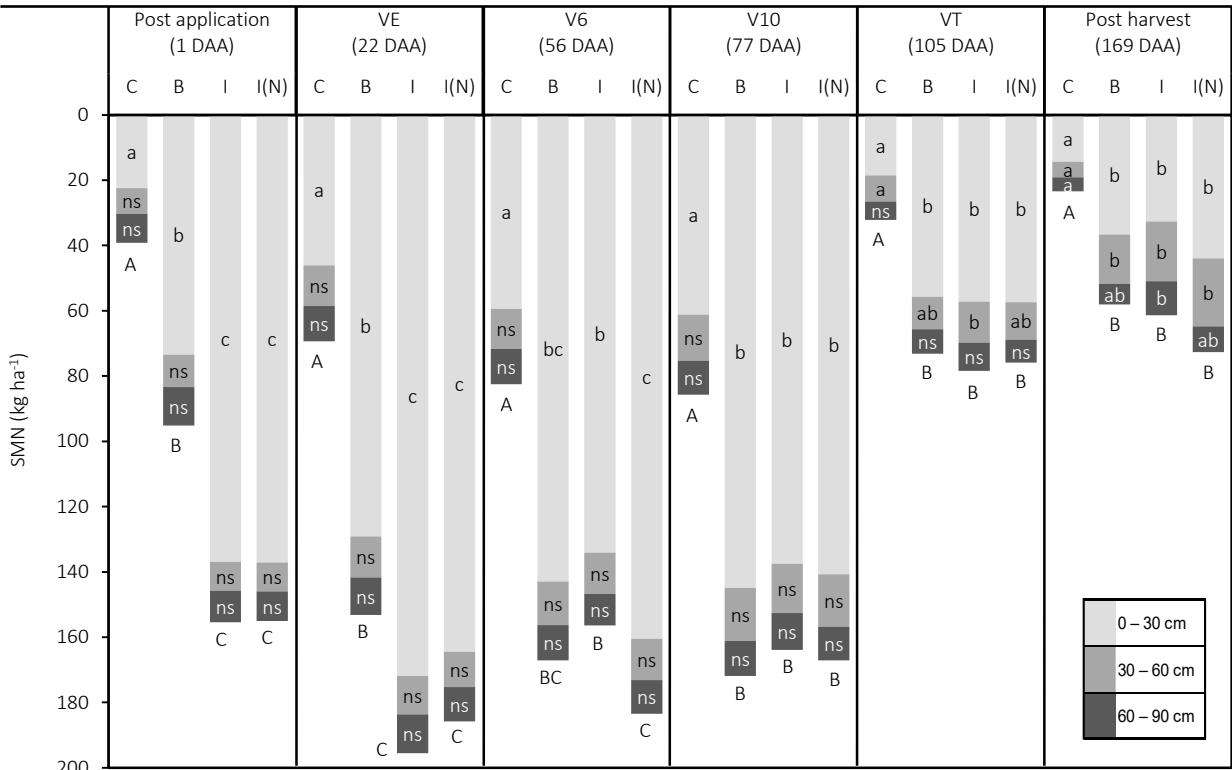


Figure 4: Soil mineral nitrogen contents (kg SMN ha⁻¹) per layer in 2014 and 2015; C = control; B = broadcast; I = injection; I(N) = injection + nitrification inhibitor; DAA = days after application; lowercase letters = significant differences between treatments for SMN in each layer (0–30 cm; 30–60 cm; 60–90 cm) for each sampling date, uppercase letters = significant differences between treatments for SMN in 0–90 cm for each sampling date; ns = not significant; VE = vegetative emergence stage; V6 or 10 = vegetative leaf stage 6 or 10; VT = tasseling.

Both injection treatments had significantly greater levels ($+ \approx 20 \text{ kg ha}^{-1}$). By contrast, the broadcast treatment showed the significantly largest SMN contents in subsoil ($\approx 60 \text{ kg ha}^{-1}$ per layer) followed by I, I(N), and C treatments (≈ 40 , 35, and 18 kg ha^{-1} per subsoil layer). Until V10 the significant differences regarding total SMN contents and both subsoil layers remained, although on a smaller level. In the top soil layer Injection + NI showed a significantly larger content (16 kg ha^{-1}) compared to the other treatments ($11 - 13 \text{ kg ha}^{-1}$). At tasseling greater contents of SMN in the bottom layer ($+ 15 - 20 \text{ kg ha}^{-1}$) resulted in significantly enhanced total SMN of the broadcast treatment. Post-harvest the total SMN content ranged between 25 and 35 kg ha^{-1} without any significant differences between treatments.

In 2015 no significant differences were found according to SMN contents in the subsoil layers until V10. During this period, the SMN contents varied from 20 to 30 kg ha^{-1} in 30 – 90 cm depth. Thus, significant differences between treatments for total SMN contents (0 – 90 cm) corresponded to the differences in the top layer. One day after slurry application the total SMN content for the injection treatments (155 kg ha^{-1}) was significantly greater compared to broadcast (95 kg ha^{-1}). Until VE SMN contents increased independently of treatments and the statistically significant differences remained. At V6 the injection treatment had a significantly smaller SMN content than injection + NI (155 versus 185 kg ha^{-1}), while the broadcast treatment was in between. At the V10 sampling date the fertilized treatments showed no longer significant differences for total SMN contents, ranging from 164 to 172 kg ha^{-1} . Until tasseling the values decreased clearly by more than 50% and about three quarters of the total SMN content ($66 - 78 \text{ kg ha}^{-1}$) was still located in the top layer. Between tasseling and post-harvest sampling date total SMN contents decreased only marginally, with decreasing in top and increasing levels in the middle layer. Post-harvest no significant differences between fertilized treatments were detected, while the unfertilized control showed a significantly smaller SMN content.

Lateral displacement of fertilized N

To describe the lateral displacement of fertilized N into the interrow space after slurry injection, SMN contents were calculated for the soil zones 'below the maize row' (BMR) and both 'interrow spaces' (IRS 15 and 30; Table 5, for more details see Figure 2). In this respect, during the whole growing season in 2014 no significant differences were detected between the injection treatments. No significant differences between these and the unfertilized control were observed in the IRS 30. At VE sampling date the SMN content was, as expected, significantly larger in the soil zones below the maize row in the injection treatments (86 (I) and $108 \text{ kg ha}^{-1} \text{ I(N)}$) compared to broadcast (34 kg ha^{-1}) and control (8.9 kg ha^{-1}) treatments. In both interrow spaces broadcast application of slurry resulted in significantly larger contents ($+ \approx 20 \text{ kg ha}^{-1}$) than in treatments I, I(N), and C. Until V6 statistical differences in the IRS remained, albeit on an increased level. Below the maize row the injection treatments had still the significantly largest contents and even in the broadcast treatment significantly greater values compared to the control treatment were determined. At V10 there was no longer a difference between the fertilized treatments at the BMR location ($20 - 26 \text{ kg ha}^{-1}$). The injection treatments showed first lateral displacement of slurry N indicated by greater SMN values in a distance of 15 cm to the maize row compared to control treatment ($+ 6 - 7 \text{ kg ha}^{-1}$), while after broadcast application larger contents ($+ 20 \text{ kg ha}^{-1}$ per IRS) were given compared to the injection treatments. Comparing the fertilized treatments at tasseling, solely the broadcast treatment had significantly larger SMN contents in the soil zone IRS 30. Post-harvest the treatments did not differ significantly, however, greater SMN contents were observed in the interrow space ($10 - 15 \text{ kg ha}^{-1}$ per IRS) compared to BMR ($\approx 5.0 \text{ kg ha}^{-1}$).

During the whole growing season 2015 the injection treatments had significantly greater SMN contents below the maize row compared to broadcast application, although the differences decreased over time.

Table 5: Soil mineral nitrogen contents (kg SMN ha^{-1}) in different soil sections in 2014 and 2015.

		Post application			VE			V6			V10			VT			Post harvest							
		BMR	IRS 15	IRS 30		BMR	IRS 15	IRS 30		BMR	IRS 15	IRS 30		BMR	IRS 15	IRS 30		BMR	IRS 15	IRS 30				
C		8.9 a	18 a	18 a		9.2 a	23 a	22 a		6.9 a	17 a	15 a		3.5 a	8.5 a	10 a		4.7 ns	15 ns	14 ns				
B		34 a	41 b	41 b		31 b	64 b	48 b		20 b	44 c	34 b		6.2 ab	18 b	23 b		4.5 ns	15 ns	16 ns				
I		86 b	20 a	16 a		70 c	31 a	21 a		24 b	24 b	15 a		4.9 ab	13 ab	13 a		4.7 ns	10 ns	11 ns				
I(N)		108 b	22 a	18 a		70 c	28 a	20 a		26 b	23 b	16 a		6.9 b	13 ab	12 a		5.2 ns	13 ns	13 ns				
		2015			1 DAA			22 DAA			56 DAA			77 DAA			105 DAA			169 DAA				
C		10 a	14 a	15 a		15 a	27 a	27 a		17 a	32 a	34 a		15 a	36 a	35 a		5.7 a	12 a	13 a		3.4 a	9.1 a	11 a
B		28 a	35 b	32 b		48 b	54 b	51 b		49 b	58 c	61 b		34 b	76 b	62 b		10 b	31 ab	30 b		5.1 b	16 b	38 b
I		126 b	15 a	15 a		140 c	30 a	25 a		76 c	46 b	34 a		62 c	63 b	39 a		15 c	43 b	20 a		8.1 c	21 bc	32 b
I(N)		127 b	15 a	13 a		130 c	29 a	27 a		103 d	47 b	34 a		65 c	66 b	37 a		18 c	38 ab	21 a		8.4 c	30 c	31 b

DAA = days after application; BMR = below maize row; IRS 15 / 30 = interrow space with 15 or 30 cm distance to the maize row; VE = vegetative emergence stage; V6 or 10 = vegetative leaf stage 6 or 10; VT = tasseling; C = control; I = injection; I(N) = injection + nitrification inhibitor; lowercase letters = significant differences between treatments for each section (BMR, IRS 15, IRS 30); ns = not significant

No significant differences were given between the injection treatments and the unfertilized control in IRS 30 until tasseling. Post application, as expected, by far most of the SMN in the injection treatments was located in the BMR soil zone (126 (I) and 127 kg ha⁻¹ (I(NI)), while broadcast application resulted in significantly larger SMN contents in the interrow space (\approx 32 – 35 kg ha⁻¹ per IRS). Until VE only slight changes in SMN distribution occurred. The statistical significant differences remained. One exception was the greater SMN content in BMR soil zone of treatment B compared to the unfertilized control treatment because of MSF application at planting. At V6 sampling date all treatments differed significantly at BMR location as follows: C (17 kg ha⁻¹) < B (49 kg ha⁻¹) < I (76 kg ha⁻¹) < I(N) (103 kg ha⁻¹). In the interrow space broadcast application had still the significantly largest SMN contents (\approx 60 kg ha⁻¹ per IRS), while both injection treatments showed a significant lateral slurry N displacement by \approx 15 kg ha⁻¹ into the IRS 15 compared to the control treatment. At V10 no longer significant differences were given between both injection treatments in the soil zone below the maize row (62 (I) and 65 kg ha⁻¹ I(N)). In addition, treatments B, I, and I(N) did no longer differ in IRS 15, but broadcast application showed larger values in the IRS 30 (+ \approx 25 kg ha⁻¹). Until tasseling the SMN contents distinctly decreased in the BMR soil zone, especially for the injection treatments. In the IRS 15 zone the SMN contents for the fertilized treatments were larger compared to the control (+ 19 – 31 kg ha⁻¹), but this was only significant for injection treatment without NI. In the IRS 30 soil zone the significantly greatest content was still found for the broadcast treatment (30 kg SMN ha⁻¹). The injection treatments were equal (20 and 21 kg ha⁻¹) and no significant lateral displacement of slurry N into the IRS 30 was detected compared to control treatment (13 kg ha⁻¹). Until post-harvest below the maize row and in soil zone IRS 15 SMN values decreased further, while they increased in IRS 30. At this location the fertilized treatments showed no significant differences (31 – 38 kg ha⁻¹), while for the control treatment significantly smaller contents occurred (11 kg ha⁻¹).

Effect of the nitrification inhibitor

The nitrification inhibitor DMPP was added to the slurry in treatment I(N) to protect the slurry ammonium N against nitrification and thus to keep the mineral N longer in the injection zone, which should result in a better plant availability.

At VE sampling date in 2014 no significant differences in nitrate N, ammonium N, and SMN concentrations between both injection treatments were identified, but the percentage of ammonium N in SMN was significantly higher by 8% when using the NI (Table 6). Until V6 all concentrations decreased distinctly. A significantly larger ammonium N concentration (+ 25.5 mg kg⁻¹) was detected in the injection + NI treatment, resulting in a 46% higher percentage of ammonium N. At V10 the N concentrations around the injection zones were on a very low level, nevertheless a significant difference in ammonium N was determined [1.3 mg kg⁻¹ (I) versus 3.7 mg kg⁻¹ (I(N))]. Furthermore, the nitrate N and SMN concentrations were significantly larger when using the NI.

In 2015 the first soil sampling was performed one day after slurry application. At that date similar SMN concentrations with 274 mg kg⁻¹ (I) and 282 (I(N)) were determined. In both treatments 99% of the SMN was ammonium N. Until VE sampling date the SMN concentrations remained on a similar level, but the nitrate N concentration was significantly lower when using the NI [38 mg kg⁻¹ (I(N)) compared to 60 mg kg⁻¹ (I)]. This resulted in a significantly greater percentage of ammonium N. Between VE and V6 SMN and ammonium N concentrations decreased, while the nitrate N concentrations increased, regardless of treatments. At V6 a significantly smaller SMN concentration was found in treatment I. Additionally, the nitrate and ammonium N concentrations and the percentage of ammonium N in SMN were significantly smaller without using the NI. Until the V10 sampling date the ammonium N concentrations decreased remarkably in both treatments, but with NI it was still about three times larger (4.9 versus 16 mg kg⁻¹).

Table 6: Nitrate N, ammonium N, and soil mineral nitrogen (SMN = nitrate N + ammonium N) concentrations (mg kg^{-1}) in the direct range of the slurry band (SM 2; Figure 2).

Stage	DAA	Nitrate N (mg kg^{-1})		Ammonium N (mg kg^{-1})		SMN (mg kg^{-1})		Ammonium- percentage (%)	
		I	I(N)	I	I(N)	I	I(N)	I	I(N)
2014									
Post application				No soil sampling					
VE	24	54	52	116	161	170	213	68 *	76 *
V6	61	27	16	7.5 *	33 *	34	49	22 *	68 *
V10	81	0.9 *	1.8 *	1.3 *	3.7 *	2.2 *	5.5 *	58 *	68 *
2015									
Post application	1	1.6	1.7	272	281	274	282	99	99
VE	22	60 *	38 *	238	242	298	279	80 *	87 *
V6	56	81 *	100 *	40 *	82 *	121 *	182 *	33 *	45 *
V10	77	73	73	4.9 *	16 *	77	89	6 *	18 *

VE = vegetative emergence stage; V6 or 10 = vegetative leaf stage 6 or 10; I = injection; I(N) = injection + nitrification inhibitor; DAA = days after application; * = significant differences between treatments

DISCUSSION

Spatial and temporal soil mineral nitrogen dynamics comparing fertilizing systems

Distinct differences in SMN dynamics were observed between both trial years (Figure 3). Taking into account that the experimental sites and slurries were rather similar (Table 1 and 3), these differences are most of all due to varying weather conditions (Section 2.1). Higher temperature and precipitation from April to June 2014 compared to 2015 stimulated mineralization, nitrification, and displacement processes of fertilized N (Bacon 1995; Cameron et al. 2013; Haynes 1986; Webb et al. 2013).

Until V6 (61 DAA) in 2014 nearly the entire fertilized N (slurry and MSF N, Section 2.2) in the broadcast treatment was displaced out of the top soil layer due to heavy rainfall (Figure 1, 3, 4). The greater total SMN content ($\approx 20 \text{ kg ha}^{-1}$) compared to the injection treatments can be explained by the additionally applied MSF (Section 2.2). Movement of fertilized N into the middle and bottom soil layers also occurred when slurry had been injected (Figure 3). Significantly larger SMN contents ($\approx 20 \text{ kg ha}^{-1}$) were determined in the top soil layer of both injection treatments (Figure 4) and thus N displacement was distinctly smaller compared to broadcast application. No lateral displacement of slurry N could be detected for the injection treatments until V6 (Table 5). Similar results

were found by van Dijk and Brouwer (1998), who compared mineral N broadcast with band application. SMN showed a better plant availability after slurry injection until V6, resulting in higher plant N uptake by 5 to 8 kg ha^{-1} compared to the broadcast treatment (Federolf et al. 2017). This displacement and uptake effects continued in the following weeks. At V10 only in treatment I(N) significantly larger SMN contents were present in the top soil layer. At this date about $25 - 35 \text{ kg ha}^{-1}$ more N was found in above ground biomass when slurry had been injected (Federolf et al. 2017). Also just a slight lateral slurry N displacement into the 15 cm interrow space occurred until this sampling date (Table 5). This matches to findings by Comfort et al. (1988) and Sawyer et al. (1990b), who characterized SMN dynamics after injection of cattle slurry. Chen et al. (2010) confirm these trends for pig slurry injection. In the broadcast treatment significantly larger SMN contents were detected in the 60 – 90 cm soil layer at V10 (81 DAA) and VT (102 DAA), especially in the interrow space. In this soil zone the SMN is obviously less available for maize roots and thus the risk of N leaching below the rooting zone is increased (Schröder et al. 1997; Sticksel et al. 1999). At post-harvest sampling date very small SMN contents were detected in the soil layer from 30 to 90 cm, regardless of treatments (Figure 4). This can be explained by the aforementioned displacement and plant uptake processes (Federolf et al. 2017). The slightly larger SMN contents in the top soil layer

are caused by mineralization of soil organic matter (SOM) during the warm summer months between tasseling and harvest (in particular in September 2014; Section 2.1). Considering the additionally applied 23 kg ha⁻¹ N by MSF at planting and the smaller N uptake by plants (-16 kg⁻¹; Federolf et al. 2017) the increased N leaching losses within the broadcast treatment becomes obvious. In addition, the priming effect (i.e. extra mineralization of N from SOM) might be stronger for slurry broadcast application resulting in unpredictable SMN contents, which increase the risk of N leaching losses (Cameron et al. 1996).

In 2015 SMN dynamics after fertilizer application only occurred in the top soil layer until tasseling (Figure 3 and 4) due to very low precipitation (Figure 1). The smaller SMN content (\approx 50 kg ha⁻¹) of broadcast compared to injection treatments 1 DAA (Figure 4) can be attributed to different reasons: At first the increased ammonia volatilization after broadcast application compared to slurry injection has to be taken into account (Haynes 1986; Webb et al. 2013). According to Webb et al. (2010) N losses of 10 – 31% are possible, even when slurry had been incorporated using a disc harrow. Thompson and Meisinger (2002) detected losses of 8% in a field trial with a similar experimental design. On the other hand slurry injection can reduce ammonia volatilization to 0 – 2% (Schmitt et al. 1995; Webb et al. 2010). Another aspect is the larger N immobilization in soil, which can occur rapidly after slurry broadcast application compared to slurry injection (Burger and Venterea 2008; Cameron et al. 2013; Sørensen and Amato 2002; Webb et al. 2013). Kirchmann and Lundvall (1993) determined an N immobilization of 20% of the applied ammonium N one day after pig slurry broadcast application. Fixation of ammonium N at clay-humus-complexes most probably play a minor role on this sandy site (Table 1). A greater impact may result from the plant residues of the catch crop, which were very dry and brittle and thus certainly absorbed a lot of the liquid phase of the slurry. Since the soil was sieved prior to analysis (Section 2.4) this portion of the applied slurry N was not part of the analyzed sample. The final

aspect is the assumed bulk density of 1.4 g cm⁻³ within the top soil layer (Section 2.6). In the injection zone, in which it is nearly impossible to characterize the bulk density exactly, it may have been a bit smaller. This possible overestimation could explain up to a maximum of 10 – 15 kg ha⁻¹ of the difference. Until V6 (56 DAA) the difference in SMN between broadcast application and the injection treatments was counterbalanced by remineralization of immobilized slurry N (Sørensen and Amato 2002; Webb et al. 2013), release of the absorbed N and partly by an increased priming effect (Kuzyakov et al. 2000). In this respect the slightly larger organic matter content of the site in 2015 compared to 2014 has to be taken into account (Table 1).

Due to the lower temperature in 2015 compared to 2014 a relatively slow SMN release was observed in the interrow space of the top soil layer until V10, regardless of treatments. In addition, plant N uptake was delayed (Federolf et al. 2017). For the broadcast treatment a decrease of SMN concentration below the maize row (SM 2) was first determined between V6 and V10 due to N uptake by plants and a slight lateral displacement of MSF N. A strong decline of the SMN concentration occurred in the main period of plant N uptake until tasseling (Federolf et al. 2017). At that growing stage the smallest SMN concentration was given below the row (Figure 3 and Table 5). The injection treatments showed larger SMN concentrations in the injection zone (SM 2) for a longer time due to the different weather conditions compared to 2014. The first slight displacement into grid SM 3 and into IRS 15 was determined at V6 (Figure 3 and Table 5), caused by a precipitation event (\approx 25 l m⁻²) in the end of May (Figure 1). Further lateral movement of slurry N into IRS 15 occurred until V10, but as in 2014 no significant displacement into the IRS 30 was detected until tasseling. Similar SMN dynamics under dry conditions after injection of cattle slurry into pasture were described by Cameron et al. (1996). At tasseling (105 DAA) the injection treatments had significantly greater SMN contents (5 – 8 kg ha⁻¹) below the maize row and smaller values (9 – 10 kg ha⁻¹) in the IRS 30

compared to broadcast (Table 5). Precipitation, which occurs at this growth stage, is caught by the plants and the water flows along the stems into the soil zone directly below the maize row. Thus, SMN in the injection treatments is more plant available, resulting in higher N uptake between tasseling and harvest compared to broadcast (Federolf et al. 2017). In August 2015 a very heavy precipitation event occurred ($\approx 110 \text{ l m}^{-2}$ within three days; Figure 1), resulting in lateral N displacement into the IRS 30 of the top and middle soil layer (post-harvest sampling date; Figure 3), because the slurry ammonium N had been nitrified up to this date.

Smallest SMN concentrations are given in soil grids where most of the N uptake occurred. At tasseling and post-harvest these were below the maize row up to the IRS 15 in the middle (VT) and bottom (post-harvest) soil layer in both trial years (Figure 3). That coincides with the main rooting zone of maize described by Lichtenegger et al. (2009). Because the fertilized N mostly remains below the maize row when slurry had been band injected, a better availability for plants becomes obvious even under very different weather conditions in 2014 versus 2015. This matches the findings of Schröder et al. (1997), who characterized the root development of maize, when slurry has been injected. Also van Dijk and Brouwer (1998) confirmed that the main SMN depletion soil grids are directly below the row.

Effect of the nitrification inhibitor

Due to addition of the nitrification inhibitor DMPP to the slurry significantly increased ammonium N concentrations were determined within the injection zone (SM 2) at V6 and V10 in both trial years (Table 6). This complies with results of Zerulla et al. (2001), who stated that DMPP leads to stabilization of ammonium N in an incubation experiment using pig slurry on loamy sand. The significantly larger SMN values of the treatment with DMPP (at V10 in 2014 and V6 in 2015; Table 6) suggest a longer plant N availability. The overall greater SMN concentrations in the injection zones during the

growing season 2015 compared to 2014 can be explained by a delayed plant N uptake in 2015 (Federolf et al. 2017) and especially by the N displacement effects in 2014 (Section 4.1). Comparable SMN concentrations around the slurry band were determined by McCormick et al. (1983) after injection of pig slurry and by Comfort et al. (1988) as well as Sawyer and Hoeft (1990a) for injected cattle slurry.

In 2014 for both injection treatments N displacement was detected (Figure 4). By addition of DMPP the dislocation of slurry N into in the middle and bottom soil layer (30 – 90 cm) was reduced by 11 kg ha^{-1} at V6. Because of the large total range between the four treatments ($< 60 \text{ kg ha}^{-1}$ (C) and $> 140 \text{ kg (B)}$), the ANOVA output does not show a statistical significance for this result. It has to be taken into account that the plant N uptake was significantly higher at V6, when DMPP had been added ($+ 3 \text{ kg ha}^{-1}$; Federolf et al. 2017). Furthermore, we only detected the status at one point in time. Possibly increased leaching losses into depths below 90 cm occurred between the VE and V6 sampling date. The basic potential of DMPP to reduce N leaching losses has been described by Ruser and Schulz (2015), Subbarao et al. (2006), and Zerulla et al. (2001). Results of Yu et al. (2007) show a significantly smaller SMN displacement due to combining urea with DMPP in multi-layer soil columns. At V10 a significantly larger SMN content by 4 kg ha^{-1} was detected in the top soil layer of the treatment with DMPP. By summing up the higher plant N uptake ($+ 12 \text{ kg ha}^{-1}$, Federolf et al. 2017) a plus of 16 kg N ha^{-1} was protected in the top soil layer and the above ground biomass due to DMPP at that time.

At the first sampling date (1 DAA) in 2015 no significant differences regarding nitrate N, ammonium N, and SMN between both injection treatments were found and 99% of the total SMN was detected as ammonium N (Table 6). The significantly greater nitrate N concentration without using DMPP at 22 DAA (VE) is remarkable (difference of 22 mg kg^{-1} , Table 6). At the following sampling date (61 DAA, V6) this treatment is characterized by significantly smaller SMN concentrations by 61 mg kg^{-1} . This

leads to the greater SMN contents in the top soil layer (26 kg ha^{-1} , Figure 4) or below the maize row (27 kg ha^{-1} , Table 5), respectively. It can be excluded that the missing SMN has been taken up by plants (Federolf et al. 2017) or displaced into other soil areas (Figure 3). In addition, N immobilization is improbable, because soil microorganisms prefer ammonium N (Paul and Clark 1989, Guiraud et al. 1992), which concentration is increased after using DMPP (Table 6). Furthermore, DMPP acts specifically on Nitrosomonas bacteria, while other soil microorganisms, which have the greatest impact on N immobilization, are not affected (Dittert et al. 2001). Thus, it seems that the earlier released nitrate has already been denitrified. A high risk of denitrification losses after slurry injection has been described in several studies (Dell et al. 2011; Dosch and Gutser 1996; Misselbrook et al. 2002; Ruser and Schulz 2015; Vallejo et al. 2005). Due to addition of a NI to the slurry, associated with a delayed nitrate release, N losses caused by denitrification can be significantly reduced. Own, unpublished measurements in the same field trial resulted in a reduction of $\text{N}_2\text{O-N}$ losses by about 40 – 50%. This order of magnitude is confirmed by Dittert et al. (2001), who measured $\text{N}_2\text{O-N}$ losses after injection of dairy slurry with and without DMPP. Apart from $\text{N}_2\text{O-N}$ a reduction of N_2 and NO-N losses is possible due to reducing denitrification by addition of NI. Thompson et al. (1987) and Thompson (1989) determined a decrease of total gaseous N losses by 22 – 90% after addition of dicyandiamide (DCD) to injected cattle slurry. Apart from V6 (56 DAA) no significant differences in SMN contents (Table 6 and Figure 4) were noticed due to the addition of DMPP in 2015. As well as in 2014 no effect on lateral slurry N displacement was detected (Table 5). The fact that the addition of DMPP did not reduce the displacement processes into the interrow space between tasseling (105 DAA)

and post-harvest (169 DAA) in 2015 is caused by the nearly complete breakdown of ammonium N due to nitrification until this late growth stages (Table 6).

The effectiveness of DMPP as nitrification inhibitor when slurry has been band injected was shown in both trial years, which were completely different with respect to the weather conditions. In 2014 the NI addition resulted in significantly larger SMN contents in the top soil layer at 61 DAA (V6). In 2015 markedly increased SMN concentrations were detected within the injection zone at 56 DAA (V6), most likely due to decreased gaseous N losses caused by denitrification. Further on, in both trial years an increased plant N uptake was observed in early growth stages due to addition of DMPP (Federolf et al. 2017).

CONCLUSION

Due to slurry injection below the maize row applied nitrogen is located in a soil zone with a better spatial availability for plant roots compared to broadcast application. The risk of nitrate displacement is significantly reduced. As revealed by Federolf et al. (2017) this leads to an increased N use efficiency for the maize crop after liquid manure injection. Addition of a nitrification inhibitor increases ammonium N concentrations in the injection zone during early maize development until the 10-leaf stage. Thus, nitrate displacement is delayed and reduced emissions due to denitrification are likely. In addition, the plants will take up more N as ammonium followed by a pH decrease in the soil surrounding the roots. Further studies are required to investigate whether this leads to an improved availability of P and micronutrients (e.g. zinc) resulting in better crop growth.

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

Author Contributions

Matthias Westerschulte developed the experimental design, conducted the field trials, sampled and analyzed all data, performed the statistical evaluation, and wrote the manuscript

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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 N P starter fertilizer (MSF) and thus reduces nutrient surpluses in regions with intensive livestock husbandry. We investigated the plant phosphorus (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 (+/- 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 stage in 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- (19%) leaf 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⁻¹, 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.

KEY WORDS

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 mainly 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). P 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. 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. 2001b).

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. (1990b), 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 NH₄-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 NH₄⁺ uptake by roots enhances the P availability due to reduction of the rhizosphere pH compared to uptake of NO₃⁻ (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.

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

	Texture			pH	Corg	C/N	Total N	P	Zn	Mn	SMN ¹
	Sand %	Silt %	Clay %	CaCl ₂	%		%	(CAL) mg 100 g ⁻¹	(CAT) mg kg ⁻¹	(CAT) mg kg ⁻¹	kg ha ⁻¹
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 ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$); CAL = extracted with calcium-acetate-lactate solution; CAT = extracted with calcium chloride/DTPA solution; ¹ = soil layer 0 – 60 cm

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 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 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, northwest Germany ($52^\circ 20'N$, $07^\circ 58'E$). The altitude is ≈ 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 minutes after application) and additional application of a mineral, side-banded fertilizer (MSF) containing 10 kg P ha⁻¹ and 23 kg N ha⁻¹ (9.4 kg nitrate N ha⁻¹, 13.6 kg ammonium N ha⁻¹) 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® FL, EuroChem Agro GmbH, Mannheim, Germany) at a rate of 10 l ha⁻¹.

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⁻¹ 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 m³ ha⁻¹ in 2014 and 24 m³ ha⁻¹ 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 two years (Table 3). The omission of the MSF in the injection treatments resulted in a smaller nutrient input by 10 kg P ha⁻¹ and 23 kg N ha⁻¹, without affecting the amount of Zn and Mn.

The pig slurry from a regional pig fattening farm was applied on April 25 in 2014 and on April 22 in 2015 (Table 3 and 4). About one week later maize (*Zea mays* L. cv. Ricardinio, KWS SAAT AG, Einbeck, Germany) was planted at a rate of 9.2 grains m⁻² (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: V_n stage was reached when collar of nth leaf was visible after broadcast application; Table 4). In 2014 samples were taken at V6 and V10, tasseling (VT) and harvest.

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).

	April	May	June	July	August	September	October	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
Sum								
Precipitation (mm)								
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

Table 3: Pig slurry properties and application rates.

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

DM = dry matter content

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 minutes and held for 10 minutes, followed by a cool down period of 15 minutes. 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 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.

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

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).

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 onwards. At harvest, the DM yield of the broadcast treatment (16.2 t DM ha⁻¹) was lower by 11 – 14% compared to the injection treatments [18.1 (I) and 18.9 t DM ha⁻¹ (I(N))].

In 2015 no significant differences could be detected at the first sampling date (V3). From V4 onwards 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 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⁻¹ were determined, without significant differences.

Phosphorus

The P concentrations decreased from 4.5 – 5.3 (V6) to 2.0 – 2.3 g P kg⁻¹ (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⁻¹) > B > I (4.5 g P kg⁻¹). 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⁻¹) without using a NI there was no longer a significant difference compared to I(N) from V10 onwards. For broadcast application a significantly lower P uptake compared to I(N) occurred until VT (-7 kg P ha⁻¹ at VT). At harvest there was no significant difference between all fertilized treatments.

Table 5: Dry matter, nutrient concentration, and nutrient uptake in the field trials 2014 and 2015.

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

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 onwards. The broadcast treatment with additional mineral N P starter, showed the significantly highest P concentrations until V6 (+ 0.7 – 1.9 g P kg⁻¹ compared to I(N)). However, the values were significantly lower (\approx 1.3 g P kg⁻¹) 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⁻¹). Regarding the P uptakes in 2015 no significant differences between the unfertilized control and the injection treatment without NI were detected until V6. Further onwards, 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⁻¹).

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⁻¹. 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))], 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⁻¹), before they decreased until harvest (17 – 21 mg Zn kg⁻¹) (Table 5). Significant differences

between the fertilized treatments were only present at V6: C = B (27 and 29 mg Zn kg⁻¹) < I (34 mg Zn kg⁻¹) < I(N) (37 mg Zn kg⁻¹). 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 g Zn ha⁻¹ (I) and 341 g Zn ha⁻¹ I(N) were determined, while broadcast application had just a Zn uptake of 280 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 mg Zn kg⁻¹ were found compared to 71 mg Zn kg⁻¹ for the injection treatment and 87 mg Zn kg⁻¹ for injection with NI and broadcast.

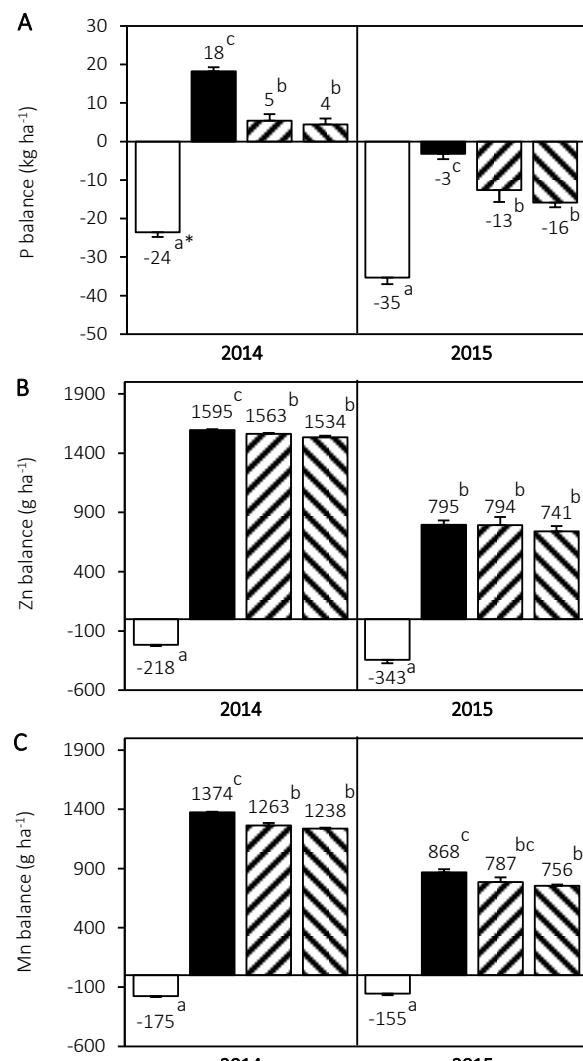


Figure 1: Phosphorus (A), zinc (B), and manganese (C) balances in 2014 and 2015; □ = control; ■ = broadcast; ▨ = injection without NI; ▨▨ = injection with NI; * values for each trial year followed by different lowercase letters are different according to the Tukey test ($P < 0.05$); whisker = standard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

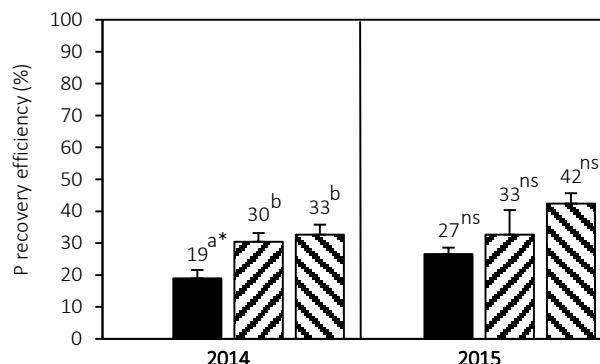


Figure 2: Phosphorus recovery efficiency in 2014 and 2015; □ = control; ■ = broadcast; ▨ = injection without NI; ▨▨ = injection with NI; * values for each trial year followed by different letters are different according to the Tukey test ($P < 0.05$); whisker = standard error.

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 mg Zn kg^{-1} during this period. The injection treatment without NI remained at nearly 70 mg Zn kg^{-1} until V6 and increased until V8 to 80 mg Zn kg^{-1} . From V8 onwards the Zn concentration declined for all fertilized treatments and were on the same level at harvest date ($29 - 31 \text{ mg Zn kg}^{-1}$). 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: $600 - 653 \text{ g Zn ha}^{-1}$ for fertilized treatments, 343 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: $1.5 - 1.6 \text{ kg Zn ha}^{-1}$; 2015: $0.74 - 0.80 \text{ kg Zn ha}^{-1}$). Injection treatments had a significantly lower surplus compared to broadcast application in 2014.

Manganese

In 2014 the Mn concentrations decreased from $65 - 69 \text{ mg Mn kg}^{-1}$ at V6 to $16 - 21 \text{ mg Mn kg}^{-1}$ at harvest (Table 5). Significant differences between the treatments occurred at V10 and harvest date. At V10 injection with NI (52 mg Mn kg^{-1}) was significantly higher compared to injection without NI (45 mg Mn kg^{-1}), while after broadcast application a significantly lower Mn concentration was determined compared to

both injection treatments (41 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 onwards 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 ($9 - 20 \text{ mg Mn kg}^{-1}$; Table 5). Comparing the fertilized treatments no significant differences occurred at V3, V6, V8, and V10 (range: 67 and 97 mg Mn kg^{-1}). At V4 a significantly lower Mn concentration was detected for the injection treatment without NI (72 mg Mn kg^{-1}) compared to injection with NI ($100 \text{ mg Mn kg}^{-1}$) and broadcast application (97 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 g Mn ha^{-1}) \leq I (483 g Mn ha^{-1}) \leq I(N) (514 g Mn ha^{-1}).

Looking at the Mn balances very high surpluses for the three fertilized treatments occurred in both trial years (2014: $1.2 - 1.4 \text{ kg Mn ha}^{-1}$; 2015: $0.76 - 0.87 \text{ kg Mn ha}^{-1}$), 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 to V4 (Cooper and MacDonald 1970). However, Figure 3 shows a distinctly better early growth development (V3 to 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

$4.0 - 6.0 \text{ g P kg}^{-1}$, when the maize plants are $< 10 \text{ cm}$ ($\approx \text{V3 and V4}$) and $3.0 - 5.0 \text{ g P kg}^{-1}$ 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^{-1}) and V6 (2.7 g P kg^{-1}). 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 approximately 1 cm^2 , while the extent of the slurry band is at least 25 cm^2 ($\approx 5 \times 5 \text{ cm}$), the different spatial expansion of this placement zones in combination with the P amounts applied (10 kg (B) versus $34 \text{ kg P ha}^{-1} (\text{I})$) 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 have also 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.

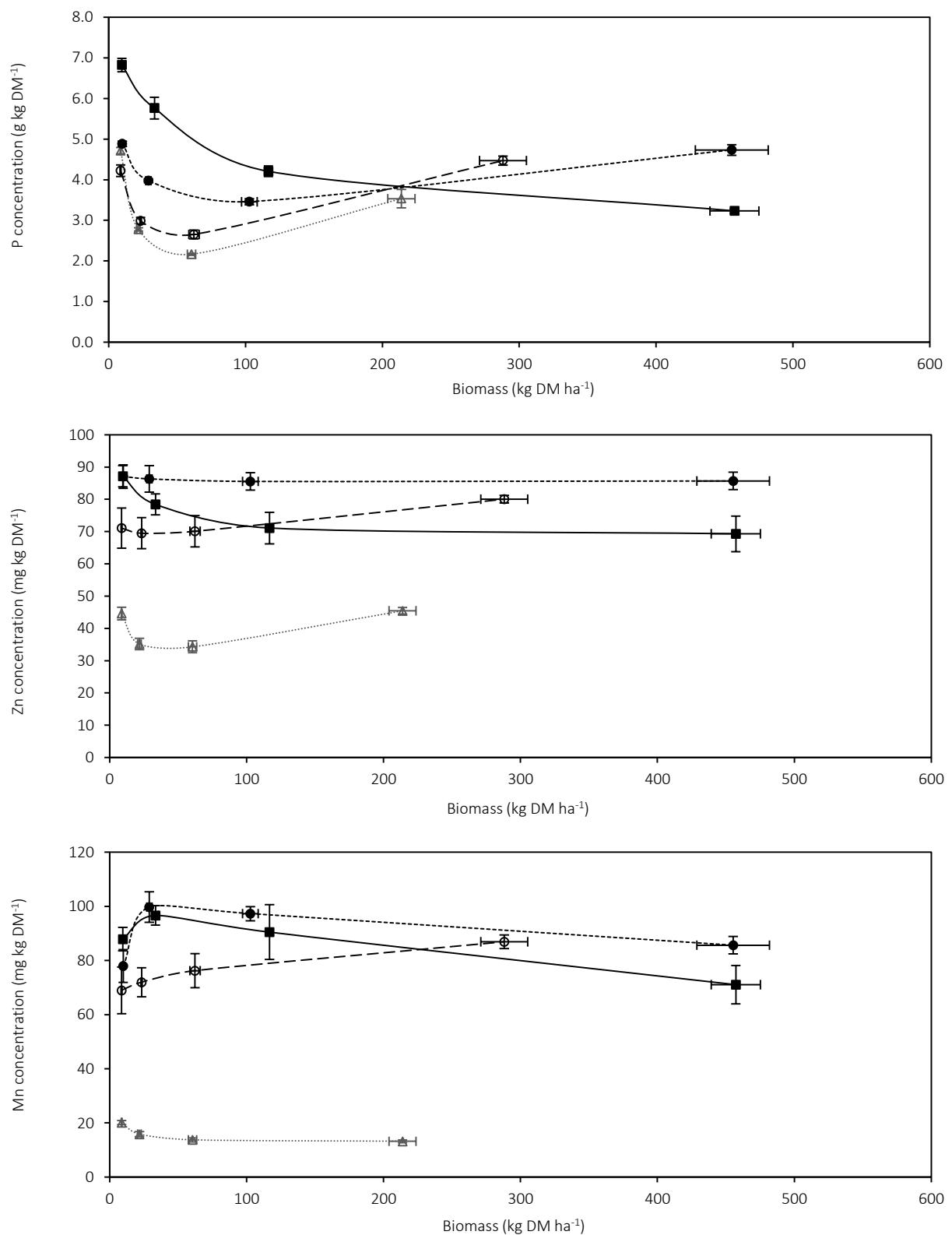


Figure 3: Phosphorus (A), zinc (B), and manganese (C) concentrations depending on biomass accumulation from V3 to V8 in 2015; $\cdots\triangle\cdots$ = control; $\blacksquare\blacksquare\blacksquare$ = broadcast; $-\diamond-\cdots$ = injection without NI; $-\bullet-\cdots$ = injection with NI; whisker = 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 (Section "Results").

Additionally, lower availability of P, Zn, and Mn could have been induced by a higher soil pH (Fageria 2009; Neumann and Römhild 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. (1990b) 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 NH₄-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 Römhild 2003).

Furthermore, the enhanced uptake of NH₄⁺ compared to NO₃⁻, results in a lowering of the rhizosphere pH (Hinsinger et al. 2003; Neumann and Römhild 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 Römhild 2012). The enhanced uptake of N as NH₄⁺ 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 Römhild (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⁻¹, 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⁻¹) 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.

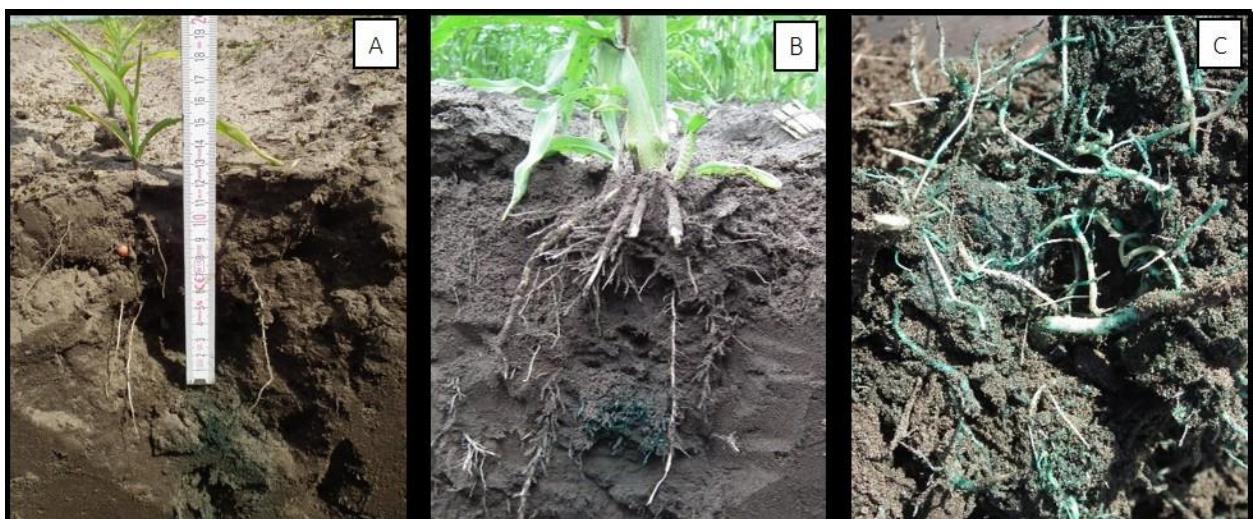


Figure 4: Root proliferation within a slurry band with nitrification inhibitor in 2015. A = 3-leaf stage; B and C = 10-leaf stage; the slurry was colored using food colouring (brilliant blue, E 133).

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^{-1}) and V6 (3.5 g P kg^{-1}) P concentrations were close to the critical range according to Campbell and Plank (2000) ($< 4.0 \text{ g P kg}^{-1}$ (V4) or $< 3.0 \text{ g P kg}^{-1}$ (V6)). But from V6 onwards, 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^{-1}) 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_4^+) 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 (+/- 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 ($\approx 5 \text{ cm}$) 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 Römhild 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^{-1}) 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^{-1} (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 ($756 - 1,374 \text{ g Mn ha}^{-1}$) and Zn ($741 - 1,595 \text{ g Mn ha}^{-1}$) 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.

ACKNOWLEDGEMENTS

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Chapter 3

General Discussion

3.1 Soil sampling after slurry injection

The characterization of the spatial and temporal SMN dynamics was based on a new soil sampling strategy (Section 2.1). Samples are taken from three soil layers at 30 cm intervals down to a depth of 90 cm, and at three positions (below the maize row, 15 and 30 cm distance to the row). Thereby, soil monoliths (SM; 15 x 15 x 10 cm) are sampled above, in, and below the slurry band down to 30 cm below the maize row using a purpose-built soil shovel. For all other soil zones an auger is used (Figure 1 and 2 in Section 2.1). Through this approach, detailed information concerning transformation processes in the direct range of the slurry band and the spatial SMN distribution into the interrow space can be determined. The results of the SMN dynamics of the different fertilizing strategies (Section 2.2) proved the excellent suitability of this sampling approach. Although it was demonstrated that SMN values can widely scatter, differences between the fertilizing schemes could mostly be statistically proven (Figure 4 and Table 5 in Section 2.2). The SM sampling approach resulted in detailed information about the effectiveness of NI within a slurry band (Table 6 in Section 2.2). Based on the data, which were collected using this sampling strategy, differences in crop development between the slurry application treatments were explained (Section 2.3; Federolf et al. 2017). Furthermore, the insight into SMN transformation processes around the slurry band were extremely useful for the interpretation of different N₂O emissions measured between the treatments (for more details see Zurheide et al. 2016).

Due to its high variability in respect to fertilizer placement depths or row distances, the sampling approach can simply be adapted to other fertilizer placement strategies as well as various row crops. In addition to SMN dynamics, using the sampling procedure to determine the dynamics of further nutrients also seems possible. For example, more detailed knowledge on the P transformation processes in the direct range of the slurry band might be of interest to

understand the interactions of slurry P with soil P pools (Damon et al. 2014).

However, there are also a few disadvantages of the developed procedure. Digging the necessary small pit to take the SMs seems to be inappropriate for highly skeletal soils. Additionally, sampling would be more difficult at sites with heavier soils, especially following severe dry periods, compared to the sandy soils examined in context of this thesis. But, to a certain content, this also applies to standardized auger sampling strategies. Furthermore, it must be considered that the new sampling strategy requires extensive manpower, e.g. to sample the soil from the 16 plots of our field experiment about 80 man-hours per sampling date were needed. This procedure is suitable to obtain detailed information in field trials, but to collect SMN samples from many fields to determine the SMN status in the soil layers 0 – 30, 30 – 60, and 60 – 90 cm this strategy is rather unfeasible. However, controlling the SMN status after harvest of maize on farmers' fields is an important measure to evaluate the fertilizing practice especially in NVZ.

It has to be expected that until harvest date the SMN concentration around the slurry band is more or less on the same level as in the surrounding soil (Section 2.2). Thus, changes in SMN along the soil core due to drilling through the slurry band are negligible. Furthermore, SMN transformations in the direct range of the slurry band are no longer relevant for monitoring the SMN status post-harvest. Thus, a simplified strategy seems to be suitable. Tewolde et al. (2013) developed a soil sampling strategy to detect the mineral nutrient status post-harvest after subsurface band-application of solid manure. They recommended taking auger samples in a defined ratio from directly above the fertilizer band and perpendicular to the band towards the unfertilized interspace. The number of soil cores can be calculated by dividing the spacing between two slurry bands by the width of the band. This approach could

also be implemented in detecting the SMN status post-harvest, following slurry injection to maize. The row spacing of 75 cm and a width of the slurry band of approximately 5 cm (Figure 5 in

Section 1.5) would lead to 15 auger samplings perpendicular to the slurry band (37.5 cm left and right of the maize row). However, further research is needed to evaluate this approach.

3.2 Slurry injection instead of broadcast application plus mineral starter fertilizer

In addition to the presented trials (Chapter 2), a series of field experiments was conducted in context of our research project at seven sites. These were distributed all over northwestern Germany in 2013 and 2014 (Federolf et al. 2016; abstract in the annex). It could be shown that the MSF, which is usually applied by farmers in that region (Section 1.5), can be substituted by slurry injection below the maize row. Equal yields and higher nitrogen use efficiencies were determined. Comparable findings were also published by Schröder et al. (2015), who evaluated data of 14 field trials on sandy soils in The Netherlands from several years. Besides similar yields and higher nitrogen use efficiencies, an improved PRE was calculated. However, the main focus of both studies was on final yield and nutrient uptake at harvest. Due to the results of the present study combined with the publication of Federolf et al. (2017) these findings can be explained in more detail with respect to SMN dynamics and plant development.

The illustrated SMN dynamics (Figure 3 in Section 2) show that the main N uptake by maize roots is located directly below the maize row (+/- 15 cm distance) regardless of the fertilizing strategy. This could be shown for very contrasting trial years concerning the weather conditions and clearly explains why slurry N is less available for maize roots from the interrow space after broadcast application. After slurry injection, the applied nitrogen is located directly below the maize row and thus in the soil zone with better spatial accessibility. This is especially beneficial when heavy rainfall occurs during the period of early growth development, because the applied slurry N remains in the top soil layer for a longer period. As a result of these findings, the higher NUEs, which were calculated for slurry

injection in the extensive field trial series by Federolf et al. (2016) and Schröder et al. (2015), can be explained. Furthermore, a consequence of these findings might also be a potential new research direction, addressing the question of whether NUEs may also be enhanced due to smaller row spacings after slurry broadcast application.

In addition, Federolf et al. (2016) and Schröder et al. (2015) observed a retarded growth development of maize after slurry injection without NI addition compared to broadcast application plus MSF at 8-leaf developmental stage. In the context of this thesis, the early growth development was characterized in detail from 3-leaf stage onwards (Section 2.3). The results showed that the findings of Federolf et al. (2016) and Schröder et al. (2015) are most probably due to lower chemical and spatial availability of P and micronutrients from the slurry band (without NI) compared to a MSF combined with broadcast slurry application.

In the field trial series in different regions of northwestern Germany the addition of a NI into the slurry band was also checked (Federolf et al. 2016). An increased N uptake and biomass accumulation was determined compared to injection without NI at 8-leaf developmental stage. The present study shows that the NI addition results in significantly enlarged NH₄-N concentrations in the direct range of the slurry band (Section 2.2). The lower N displacement and/or reduced losses via denitrification finally lead to increased SMN contents in the top soil layer. Based on this, Federolf et al. (2017) found enhanced N uptake and biomass accumulation during the entire early growth phase compared to slurry injection without NI. Additionally, the higher NH₄-N concentration increased the

availability of P, Zn, and Mn (Section 2.3). This is most possibly caused by rhizosphere acidification due to uptake of NH_4^+ (Jing et al. 2012) and enhanced root proliferation within the slurry band (Bloom 1997). Thus, an equal early growth development compared to broadcast application plus MSF, most probably

can be assured when combining slurry injection with the application of a NI. Overall, slurry injection with or without NI addition led to an at least equal yield of silage maize in northwestern Germany and increased N and P use efficiencies compared to the current farm practice.

3.3 Environmental impact and consequences relating to the legal framework

Based on the data presented in the general introduction it became obvious that high N and P surpluses occur in northwestern Germany (Section 1.3). In respect to N, surpluses of $> 100 \text{ kg N per ha AUA}$ were calculated for 21 administrative districts (Figure 3). P surpluses of $> 20 \text{ kg P}_2\text{O}_5 \text{ per ha AUA}$ (and surpluses as high as $> 40 \text{ kg P}_2\text{O}_5 \text{ per ha AUA}$) were found (Figure 4), especially in regions with intensive pig husbandry. Maize is the dominant crop with a share of partially $> 40\%$ of the AUA in these regions. Using slurry injection instead of the current fertilizing practice (i.e. slurry broadcast application plus N P MSF), the nutrient balances could be distinctly decreased. In relation to N, a reduction potential of about $20 - 40 \text{ kg N ha}^{-1}$ (Federolf et al. 2017) and concerning P a decrease of ca. $10 - 14 \text{ kg P ha}^{-1}$ is possible for maize cropping (Section 2.3 and Schröder et al. 2015). This fact is of particular importance when considering that the revised Fertilizer Application Ordinance will set maximum surplus values of 50 kg N ha^{-1} and $10 \text{ kg P}_2\text{O}_5$ as multi-annual farm average. Thus, slurry injection for maize fertilization is an effective instrument for the farmers in the region to reach these aims.

Due to the large accumulation of organic manures it has become necessary to export these products from regional hotspots (LWK Niedersachsen 2015; Warnecke et al. 2011). The transport is very cost-intensive and could be distinctly decreased due to higher NUEs from organic manures when using slurry injection.

Additionally, the long-term use of too high P rates led to P accumulation in the soils of the region (Section 1.3; Leinweber 1996; Osterburg

2012). Due to lowering the P balances by substitution of the MSF, the accumulation could be decreased. This would lead to a more sustainable use of P, taking the scarcity in quantity and quality of rock P reserves into account (Cordell et al. 2009).

The N balances for maize in the field trials published by Federolf et al. (2016, 2017) were, in part, strongly negative (up to $-110 \text{ kg N ha}^{-1}$). In the long-term this practice would lead to depletion of the soil organic N accompanied by reduced soil fertility (Lal 2009). In this case, soil organic N has to be recovered based on an appropriate crop rotation. However, this fact also offers an increased flexibility for farmers regarding to the crop management of other crops within the rotation.

When heavy rainfall occurs during the early growth development of maize, the displacement of fertilized N is distinctly lower after slurry injection, especially when combined with a NI, in comparison with the current farm practice (Section 2.2). As a consequence, the risk of nitrate leaching can be lowered until the period of main N uptake by maize. Thereafter, nitrate leaching is mostly of minor relevance. As mentioned in the introduction chapter (Section 1.3), many groundwater bodies are above the limit of $50 \text{ mg NO}_3^- \text{ l}^{-1}$ in northwestern Germany. Hence, the EU commission is considering taking legal action at the European Court of Justice, due to the failure of the German government to act appropriately (Bach et al. 2016). Using farm-ready slurry injection techniques, instead of current farm practice, could be a method of

reducing nitrate leaching into groundwater bodies.

The pollution of German surface waters can be attributed to different influences of agricultural land ($\approx 70 - 80\%$ for N, $\approx 50\%$ for P; BMUB and BMEL 2016). A large share is caused by runoff or erosion, especially with respect to P (BMUB and BMEL 2016; Taube et al. 2013). The risk of nutrient losses via erosion is distinctly higher for slurry broadcast application compared to slurry injection, because the upper rim of the slurry band is $\approx 10 - 12$ cm below the soil surface (Figure 5 in Section 1.5; Smith et al. 2001a,b; Ceretta et al. 2010). Nutrient losses due to runoff and erosion could be further decreased when combining the new fertilizing approach 'slurry injection' with 'strip-tillage' (Prasuhn 2012). In this case, the slurry will be injected directly into the previous cover crop, leading to increased ground coverage with plant residues during maize growing.

The new EU Directive 2016/2284/EU enhances the pressure on German agriculture to reduce emissions of NH₃ to the atmosphere, because of its negative environmental impact (Section 1.4). 95% of the German NH₃ emissions can be attributed to agricultural activities (Taube et al. 2013). According to Misselbrook et al. (2002) spreading of slurries on fields accounts for nearly one-third of the total NH₃ emissions from agriculture. Slurry injection can decrease NH₃ losses compared to broadcast application without incorporation by up to > 90% (Dell et al. 2011). Even if the broadcast applied slurry is immediately incorporated into the soil, losses of 10 – 30% of the total N may occur (Webb et al. 2010). Especially if direct incorporation of liquid manures becomes mandatory in course of the revision of the Fertilizer Application Ordinance, slurry injection may further gain in importance.

Diminishing the emission of NH₃ from slurry application can have adverse effects on denitrification losses, leading to increased N₂O emissions (Misselbrook et al. 2002). Through SM sampling, very high SMN concentrations (up to 298 mg SMN kg⁻¹) with temporary high shares of NO₃-N (up to 100 mg NO₃-N kg⁻¹) were detected

in the direct range of the slurry band (Table 6 in Section 2.2). Additionally, exceptionally large amounts of readily oxidizable or water-soluble carbon occur in the slurry band (Ruser and Schulz 2015). Furthermore, high rates of microbial activity combined with intensive root proliferation (Figure 4 in Section 2.3) depletes oxygen in this soil region, resulting in anaerobic conditions, which are prerequisite for denitrification (Dell et al. 2011). Thompson et al. (1987) found 76 – 293% larger N losses from denitrification after slurry injection compared to broadcast application. In the context of our research project (Section 1.1) N₂O measurements were also recorded, which showed 40 – 500% larger emission of N₂O after slurry injection (without NI) compared to broadcast plus MSF (Zurheide et al. 2016). The differences are strongly dependent on site and weather conditions and higher N₂O emissions cannot always be measured (Misselbrook et al. 2002). As mentioned earlier, N₂O is a climate relevant trace gas and has a high global warming potential (Ruser and Schulz 2015). Like for NH₃, the EU Directive 2016/2284/EU sets ambitious goals for reducing nitrous oxide emissions (Section 1.4). Due to addition of NI into the slurry band the emissions can be markedly lowered. This is most probably due to the delayed nitrate release as shown in Table 6 of Section 2.2. According to Ruser and Schulz (2015) DMPP has a N₂O reduction potential of about 30 – 50%. This order of magnitude was confirmed by Dittert et al. (2001) and this study's field measurements (Zurheide et al. 2016). Our unpublished data also reveal that, due to NI addition, a reduction down to the same level as detected for the slurry broadcast application is possible. However, the potentially increased N₂O emissions are a disadvantage of slurry injection. In this respect, further research is needed, especially concerning the reduction potential of NI addition.

Based on the field trials of this study several advantages of NI usage combined with slurry injection were obvious. However, possible negative environmental impacts should also be considered. DMPP has been checked by extensive toxicological and ecotoxicology tests

according to European legislation and no negative side effects could be found to date (Zerulla et al. 2001). However, according to Scheurer et al. (2016) a possible discharge of NI compounds and their metabolites into aquatic environments have to be considered. Besides DMPP, 1*H*-1,2,4-Triazole, and DCD were investigated in their study. 1*H*-1,2,4-Triazole could only occasional be detected in concentrations above the limit of quantitation. The highest concentrations occurred in the River Rhine near Basel (max. 5.4 µg l⁻¹) and the values decreased further downstream. DCD was ubiquitously found in the samples from the Rhine River. Thereby the main inflow originated from the Jagst River and was most possibly caused by a point source. Samples of other surface waters did not show unexpectedly high values. All in all, the discharges were varied and

the findings were most likely caused by a combination of wastewater treatment plant effluence as well as from industrial discharges, and only to a minor extent related to agriculture. DMPP could not be detected in any of the analyzed surface waters. However, further research is required, to ensure that negative environmental impacts of NI application are not relevant.

Slurry injection is an adequate method for farmers to lower N and P balances, NH₃ emissions and nutrient losses via surface runoff. On the other hand, this fertilizing system has an increased potential for N₂O losses, which contributes to climate change. Thus, a life cycle assessment could be a useful tool to achieve a holistic view of potential environmental impacts (Brentrup et al. 2004).

Chapter 4

Conclusion

Overall Conclusion

Slurry injection for maize fertilization leads to equal yields and higher N and P use efficiencies compared to the current farm practice in northwestern Germany.

The spatial and temporal SMN dynamics in soils where slurry has been injected can be reliably characterized using the new soil sampling strategy. The soil shovel allows a precise sampling of the injection zone and the interrow space is sampled using a conventional auger. It is important to intensively homogenize the SM sample, including the slurry band, and to take a suitable amount of samples per pooled sample. This basic sampling methodology can simply be adapted to various fertilizer placement strategies as well as other row crops.

Based on this strategy, it can be demonstrated that after slurry injection below the maize row the applied N is located in a soil zone with a better spatial availability for plant roots compared to broadcast application. Furthermore the risk of nitrate displacement is significantly reduced. Both these aspects principally explain the increased NRE. However, slurry injection without NI addition can lead to a critical plant P, Zn, and Mn status accompanied with retarded

early growth under cold weather conditions. Mixing a NI into the slurry significantly increases the NH_4^+ concentration in the injection zone until 10-leaf developmental stage of maize. This triggers a better P, Zn, and Mn availability resulting in an equal early growth compared to broadcast application plus MSF. Additionally, due to NI addition the nitrate release and displacement from the injection zone is delayed accompanied by an increased N uptake during early growth.

Overall, slurry injection, especially when combined with a NI, is an appropriate approach for many farmers in northwestern Germany to decrease N and P surpluses. Implementation of this slurry application technique would support several goals concerning sustainable land use: Decrease pollution rates of ground and surface waters, reduced emission of NH_3 , more efficient use of the limited rock P reserves, and less need of exporting organic manures. On the other hand, slurry injection enhances the risk of N_2O emissions, which contributes to climate change. Thus, for a final evaluation of the environmental impact a life cycle assessment would be worthwhile.

Summary

Maize is the dominant crop in northwestern Germany and is mostly cultivated on sandy soils. Additionally, due to intensive livestock husbandry and biogas production, large amounts of liquid manures are produced. The current farm practice leads to high N and P surpluses at field level accompanied by environmental pollution, like nitrate leaching, eutrophication of non-agricultural ecosystems, and N₂O emissions. The accruing liquid manures are often used for maize fertilization. Thereby, slurries are mainly broadcast applied using trailing hose applicators followed by incorporation into the topsoil. In addition, a mineral N P starter fertilizer (MSF) is band-applied below the seed-corn at planting to overcome the limited nutrient availability during the early growth stages. Using a slurry injection technique below the maize row before planting might serve a substitute for MSF. Addition of a nitrification inhibitor (NI) into the slurry before injection seems to be an option to further decrease N losses. The objectives of this thesis were to compare the current and novel fertilizing strategies with a special focus on soil mineral nitrogen (SMN) dynamics and plant P, zinc (Zn), and manganese (Mn) status. For both issues the effect of adding a NI into the slurry was investigated.

To characterize the SMN dynamics after slurry injection an appropriate soil sampling strategy had to be developed. Therefore, three consecutive field trials were conducted. The first testing of the new soil sampling approach was implemented in an existing experiment where the slurry was injected at a depth of 12 cm (upper rim) below the soil surface. The soil profile (75 cm wide) centered below the maize row was sampled using a grid-like approach to a depth of 90 cm. Around the injection zone, soil monoliths (SM) were sampled using a purpose-built soil shovel. Below the SMs and in the interrow space (15 and 30 cm distance to the row) a standardized auger procedure was used. The second experiment aimed to improve the sampling strategy with focus on sample

homogenization quality and necessary sample sizes per pooled sample. In the third experiment this improved sampling strategy was validated. Results from the first testing of the sampling procedure showed that the strategy is suitable, although some problems occurred. Especially the high spread in values among the replications caused high coefficients of variation (CV; mostly 40 – 60%). The improvement trial revealed that for the SM, which contains the slurry band, an intensive homogenization is required. In addition, suitable sample sizes (twelve auger samples and six soil monolith samples per pooled sample) have to be collected to obtain reliable SMN values. Following this enhanced sampling strategy in the final validation trial, the spread in values was considerably reduced and resulted in CV values of mostly < 20%. The method can be adapted to other fertilizer placement strategies and further row crops.

To compare both fertilizing strategies with respect to the spatial and temporal SMN dynamics as well as to the plant nutrient status two field trials were conducted using pig slurry on sandy soils in 2014 and 2015. Four treatments were tested: unfertilized control, broadcast application + MSF, injection, and injection + NI. Soil samples were taken using the new sampling strategy at several dates during the growing season. Plant samples were simultaneously collected to evaluate the plant P, Zn, and Mn status at different growth stages. In 2014, all fertilized N was displaced from the top soil layer of the broadcast treatment until the 6-leaf stage due to heavy rainfall, while N displacement was significantly smaller after slurry injection. The lateral movement of injected slurry N was negligible. In 2015, almost no displacement of fertilized N out of the top soil layer occurred independently of treatments, due to distinctly lower rainfall. The release of slurry N was delayed following broadcast application and large SMN concentrations were detected in the injection zones until the 10-leaf stage. The addition of a NI resulted in significantly increased NH₄-N shares in the injection zone

throughout the early growth stages (+ 46% in 2014 and + 12% in 2015 at 6-leaf stage). Thus, in 2014 SMN displacement was delayed and in 2015 increased SMN concentrations were found around the slurry band, most probably due to lower N losses via denitrification. Furthermore, NI addition significantly increased the nutrient uptake by maize during early growth in both years. With P deficiency due to cold weather conditions in 2015, broadcast application showed higher P uptake until the 6-leaf stage (36 – 58%), while it was lower at the 8- (32%) and 10- (19%) leaf stages compared to slurry injection (+ NI). Zn availability was enhanced during early growth after slurry injection (+ NI) and Zn as well as Mn uptake were higher at harvest. Furthermore, dry matter yields were higher (2014) or equal (2015) compared to broadcast application. The P balances were decreased by 10 – 14 kg P ha⁻¹, while Zn and Mn balances were excessive independent of treatments.

The field trials showed that after slurry injection, especially when combined with a NI, the applied nitrogen is located in a soil zone with better spatial availability for plant roots compared to broadcast application. Furthermore, the MSF can be substituted without affecting early growth of maize.

In conclusion, slurry injection leads to equal (or even higher) yields and enables farmers in northwestern Germany to reduce the P and N surpluses. This would support several goals concerning sustainable land use: Lower pollution of ground and surface waters, reduced emission of NH₃, more efficient use of the limited rock P reserves, and less need of transporting organic manures out of regions with intensive animal husbandry and/or biogas production. However, slurry injection enhances the risk of N₂O emissions, which contributes to climate change. Thus, for a final evaluation of the environmental impact a life cycle assessment would be worthwhile.

Zusammenfassung

Auf den überwiegend sandigen Böden im Nordwesten Deutschlands wird in großem Umfang Mais angebaut. Weiterhin ist die Region durch intensive Tierhaltung und Biogasproduktion geprägt, wodurch große Mengen an Wirtschaftsdüngern anfallen. Die aktuelle Landnutzung führt zu starken Überschüssen in den Stickstoff- und Phosphorbilanzen. Die Folge sind Umweltbelastungen, wie z. B. Nitratauswaschung, Eutrophierung nicht agrarischer Ökosysteme oder Lachgasemissionen. Zur Maisdüngung wird die anfallende Gülle aktuell meist breitflächig mittels Schleppschlauchverteiler ausgebracht und anschließend eingearbeitet. Zusätzlich wird zur Förderung der Jugendentwicklung ein mineralischer N P Unterfußdünger (UFD) zur Saat appliziert. Mittels Gölleinjektion unter die später folgende Maisreihe („Depotapplikation“) könnte dieser UFD ersetzt werden. Zusätzlich könnte ein Nitrifikationshemmstoff (NI) die Stickstoffverluste weiter reduzieren. Das Ziel der vorliegenden Arbeit war es, die beiden Düngungssysteme mit Blick auf die Bodenstickstoffdynamik (N_{min}) und die Pflanzenversorgung mit Phosphor (P), Zink (Zn) und Mangan (Mn) zu vergleichen. Dabei wurden auch die Effekte einer NI-Zugabe in das Gülleband überprüft.

Um die räumliche und zeitliche N_{min} -Dynamik bei Depotapplikation beschreiben zu können, musste eine neue Bodenbeprobungsmethode entwickelt werden. Dazu dienten drei aufeinander aufbauende Feldversuche. Der Entwicklungsversuch wurde in einen bestehenden Feldversuch, in dem das Gülleband ca. 12 cm (Oberkante) unter der Maisreihe lag, integriert. Mittig unter einer Maisreihe wurde das Bodenprofil rasterförmig 75 cm breit (= Reihenabstand) und 90 cm tief beprobt. Im Injektionsbereich wurden mit Hilfe einer speziellen Metallschaufel Bodenmonolithen (BM) entnommen. Darunter und im Zwischenreihenbereich (15 und 30 cm Abstand zur Reihe) kam das Standardbohrstockverfahren zum Einsatz. Der zweite Feldversuch diente der Optimierung dieses Ansatzes, wobei insbesondere die Probenhomogenisierung und der

notwendige Stichprobenumfang überprüft wurden. Im dritten Versuch wurde die optimierte Methode validiert. Der Entwicklungsversuch bestätigte den methodischen Ansatz, allerdings ergaben sich Probleme mit sehr hohen Variationskoeffizienten (VK; überwiegend 40 – 60%). Diesbezüglich ergab sich im Optimierungsversuch, dass der BM, der das Gülleband enthält, besonders intensiv homogenisiert werden muss. Außerdem ist zur Erhebung verlässlicher Daten ein notwendiger Stichprobenumfang einzuhalten (zwölf Bohrstöcke und sechs BM pro Mischprobe). Diese Maßnahmen führten zu einer deutlich gesteigerten Datenqualität (VK überwiegend < 20%) im Validierungsversuch. Aufgrund ihrer Flexibilität kann diese Methode auch auf weitere Verfahren mit platzierte Düngung und andere Reihenkulturen übertragen werden.

Für den Vergleich der Düngungsstrategien hinsichtlich der N_{min} -Dynamik und der Pflanzennährstoffversorgung wurden zwei Feldversuche auf einem sandigen Standort in 2014 und 2015 durchgeführt. Dabei kam Mastschweinegülle zum Einsatz und es wurden vier Varianten geprüft: ungedüngte Kontrolle, Schleppschlauch + UFD (Standard), Depot und Depot + NI. Mit Hilfe der neuen Bodenbeprobungsmethode wurden N_{min} -Proben zu mehreren Terminen über die gesamte Vegetationsperiode gezogen. Parallel wurden Pflanzenproben entnommen, um die Nährstoffversorgung bewerten zu können. 2014 war aufgrund starker Niederschläge und damit einhergehender N_{min} -Verlagerung beim Standardverfahren zum 6-Blattstadium kaum noch N_{min} im Oberboden vorzufinden. In den Depot-Varianten war die N_{min} -Verlagerung hingegen signifikant geringer. Weiterhin war nach Depotapplikation kaum eine Verteilung des gedüngten N in die Zwischenreihenbereiche festzustellen. Aufgrund wesentlich geringerer Niederschläge verblieb der N_{min} 2015 unabhängig vom Verfahren über die gesamte Vegetationsperiode im Oberboden. Dabei wurde eine verzögerte N_{min} -Freisetzung nach breitflächiger Gülleapplikation festgestellt. Außerdem kam es zu

extrem hohen N_{min}-Konzentrationen im Gülleband bis zum 10-Blattstadium. In beiden Jahren führte die Zugabe des NI zu signifikant höheren NH₄-N-Konzentrationen im Gülleband (+ 46% in 2014 und + 12% in 2015 zum 6-Blattstadium). Dadurch konnte die N_{min}-Verlagerung in 2014 verzögert werden; in 2015 wurden signifikant höhere N_{min}-Konzentrationen im Bereich des Güllebandes bis zum 10-Blattstadium festgestellt. Diese sind höchst wahrscheinlich auf geringere Denitrifikationsverluste zurückzuführen. Darüber hinaus wurden durch NI-Zugabe höhere Nährstoffentzüge während der Jugendentwicklung in beiden Versuchsjahren erzielt. Unter den kalten Bedingungen im Frühjahr 2015 führte der mineralische UFD des Standardverfahrens zunächst zu höheren P-Entzügen bis zum 6-Blattstadium (36 – 58%), während sie zum 8- (32%) und 10-Blattstadium (19%) geringer waren im Vergleich zu Depot + NI. Weiterhin profitierte die Variante Depot + NI von einer verbesserten Zn-Verfügbarkeit und es ergaben sich höhere Zn- und Mn-Entzüge zur Ernte. Die Trockenmasseerträge waren in 2014 bei beiden Depotvarianten signifikant um 2.0 – 2.6 t/ha höher als beim Standardverfahren, während in 2015 keine signifikanten Unterschiede festgestellt werden konnten. Folglich waren nach Depotapplikation die P

Bilanzen um 10 – 14 kg/ha geringer, während die Zn- und Mn-Bilanzen verfahrensunabhängig extrem hoch waren.

Durch die Feldversuche konnte gezeigt werden, dass der Güllestickstoff nach Depotapplikation (v. a. mit NI), räumlich besser verfügbar ist als nach breitflächiger Applikation. Dadurch ergibt sich eine verbesserte Stickstoffnutzungseffizienz. Außerdem konnte der mineralische UFD ersetzt werden ohne die Jugendentwicklung negativ zu beeinflussen.

Letztlich führt Gülle-Depotapplikation zu Mais zu mindestens gleichwertigen Erträgen und bietet den Landwirten im Nordwesten Deutschlands die Möglichkeit die N- und P-Bilanzen zu senken. Damit könnte auch eine Reihe von Umweltzielen leichter erreicht werden: Geringere Belastung von Grund- und Oberflächen Gewässern, geringere NH₃-Emissionen, sowie eine nachhaltigere Nutzung der knappen Rohphosphatreserven. Allerdings gilt es zu berücksichtigen, dass durch Depotapplikation das Potential für Lachgasverluste erhöht wird. Deshalb könnte für eine abschließende Bewertung der vielfältigen Auswirkungen auf die Umwelt eine Ökobilanzierung sinnvoll sein.

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ANNEX

Abstracts of co-authored papers

List of publications

- PEER REVIEWED PUBLICATIONS
- OTHER PUBLICATIONS

Conference contributions

- TALKS
- POSTER

Curriculum vitae

Declaration of originality

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Enhanced nutrient use efficiencies from liquid manure by positioned injection in maize cropping in northwest Germany

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ABSTRACT

Maize (*Zea mays L.*), the dominating crop in northwestern Germany usually receives mineral nitrogen (N) and phosphorous (P) fertilizer side dressed (MSD) at planting as a starter to ensure proper early-growth development, on top on a usually nutrient demand covering manure application. Recently developed injection techniques, along with auto-guidance systems allow liquid manure injection below the maize seeds in a separate operation. Thus, the need for starter fertilizer might be obviated. Field trials were conducted on seven sites in northwestern Germany to compare liquid manure broad-cast application versus injection at recommended rate with and without addition of a nitrification inhibitor in 2013. Several treatments were tested with and without MSD (23–10 kg N-P ha⁻¹). In 2014, the trials were adapted to a proper two-factorial setup with additional reduced manure application rate treatments. Biomass accumulation and nitrogen uptake were assessed at V8 growing stage and at harvest. Compared to broadcast application with MSD, liquid manure injection without MSD showed retarded early-growth, but equal yield and N uptake at harvest in both years. Adding a nitrification inhibitor to injected liquid manure led to equal early-growth and yield, but significantly increased N uptake by 7% in 2013 and 6% in 2014, respectively. Regarding the proper performance of reduced rate injection treatments, the increase in N use efficiency is even more noticeable. The reduction of P input did not influence early growth and yield. P use efficiency from manure is higher when manure is injected prior to planting. These results indicate that liquid manure injection might reduce N and P surpluses in maize growing and therefore benefit farmers and environment.

KEY WORDS

Starter fertilizer; nitrogen uptake; nitrification inhibitor; manure injection

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Nitrogen dynamics following slurry injection in maize: Crop development

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ABSTRACT

Using pig slurry as starter fertilizer for maize (*Zea mays L.*), injected below the row prior to planting is a reasonable way to omit application of additional mineral fertilizer in areas with intensive animal farming. However, delayed early growth and a lack of knowledge on nutrient availability limit the interest of farmers. To extenuate farmers concerns a field trial was conducted in 2014 and 2015 to get detailed information on nitrogen (N) uptake, the subsequent influences on crop growth at different vegetative growth stages and final yield of silage maize. Besides an unfertilized control, two liquid manure injection treatments (without and with nitrification inhibitor [NI]) were compared to slurry broadcast application + mineral N and phosphorus (P) starter fertilizer at planting (MSF). In 2014, NI treatment yields increased (+ 16.5%) and N uptake increased (+ 9.6%) compared to broadcast treatment. In 2015, cold and dry conditions during early growth limited P plant availability and reduced crop growth in treatments without MSF. However, when a NI was added to the slurry prior to application, plants showed less P deficiency symptoms and better growth. At harvest no differences between the fertilized treatments were observed. In both years apparent N recovery was increased when manure was injected (48% without, and 56% with NI, respectively) compared to broadcast application of manure (43%) indicating that N losses were lower. However, further knowledge on soil N transformation and N loss pathways in systems with slurry injection is needed.

KEY WORDS

Starter fertilizer; nitrogen uptake; nitrification inhibitor; liquid manure injection; phosphorus; pig slurry

List of publications

- PEER REVIEWED PUBLICATIONS -

Federolf C-P, **Westerschulte M**, Olfs H-W, Broll G, Trautz D (2017) Nitrogen dynamics following slurry injection in maize: Crop development. *Nutr Cycl Agroecosyst* 107:19–31. doi: 10.1007/s10705-016-9813-y

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Westerschulte M, Federolf C-P, Trautz D, Broll G, Olfs H-W (2017b) Slurry injection with nitrification inhibitor in maize: Plant phosphorus, zinc and manganese status. (Accepted by *J Plant Nutr*)

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Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016d) Potential of manure injection to increase N and P use efficiencies in maize. *Berichte aus dem Julius Kühn-Institut* 184:7

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016c) Gülleunterfußdüngung zu Silomais im Nordwesten – Die Nährstoffbilanz entlasten. *LOP* 5:23–27

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Technik (insbesondere mobile Datenerfassung), Pflanzenbau, Pflanzenschutz und Qualität von Feldversuchen. Vorträge der Fachtagung vom 26. und 27. Januar 2016 in Hannover, 127–136

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Conference contributions

- TALKS -

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016c) Gülleunterfußdüngung zur Optimierung der Stickstoffnutzungseffizienz im Maisanbau. SKW-Fachtagung „Düngung 2016“ – Stickstoff in der Pflanze. 13.12.2016, Leipzig, Butzbach, Rieste, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016b) Gülleunterfußdüngung zu Mais – Nährstoffaufnahme in der Jugendentwicklung. 59. Tagung der Gesellschaft für Pflanzenbauwissenschaften e.V. – Klimawandel und Qualität, 27.-29.09.2016, Gießen, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016a) Gülledepot zu Mais – Gülleunterfußdüngung und Strip Till. Abschlussworkshop „Gülledepot zu Mais“ der Hochschule Osnabrück, 20.05.2016, Osnabrück, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015e) Applikation von Wirtschaftsdüngern im Depot als Beitrag zur nachhaltigen Landbewirtschaftung. Projektpräsentation für Rainer Spiering (MDB), 20.10.2015, Osnabrück, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015d) Using manure injection to decrease nutrient surpluses in northwestern Germany. VI International Scientific Agriculture Symposium ‘Agrosym 2015’, 15.–18.10.2015, Jahorina, Bosnia and Herzegovina

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015c) Gülleunterfußdüngung zu Silomais in Nordwestdeutschland. 58. Tagung der Gesellschaft für Pflanzenbauwissenschaften e.V. – Multifunktionale Agrarlandschaften: Pflanzenbaulicher Anspruch, Biodiversität, Ökosystemdienstleistungen, 22.–24.09.2015, Braunschweig, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015b) Potential of manure injection to increase N and P use efficiencies in maize. 23rd International Symposium of the International Scientific Centre for Fertilizers (CIEC) – Plant nutrition and fertilizer issues for the cold climates, 08.–10.09.2015, Son, Norway

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015a) Applikation von Wirtschaftsdüngern im Depot als Beitrag zur nachhaltigen Landbewirtschaftung 1. Pflanzenbauliche Aspekte. Osnabrücker Geographisches Kolloquium, University of Osnabrück, 27.05.2015, Osnabrück, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2014) Applikation von Wirtschaftsdüngern im Depot als Beitrag zur nachhaltigen Landbewirtschaftung. 3. Doktoranden-Symposium 2014, University of Applied Sciences of Osnabrück and Münster, 26.09.2014, Osnabrück, Germany

Neddermann N, Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016) Gülle Strip Till ohne Bodenbearbeitung? Arbeitskreis Gülle-Strip-Till, LWK NRW, 19.01.2016, Saerbeck, Germany

Olfs H-W, Federolf C-P, **Westerschulte M**, Trautz D (2015) Effects of the nitrification inhibitor DMPP on soil N turnover and N use efficiency using a new slurry injection technique in maize. Top Science – Symposium 2015 on Nitrification inhibitor usage in organic and mineral fertilizers, BASF SE, 25.02.2015, Dortmund, Germany

Olfs H-W, Federolf C-P, **Westerschulte M**, Trautz D (2013) Platzierte Düngung: Auswirkung auf Umsetzungsprozesse im Boden, Nährstoffaufnahme und Pflanzenwachstum. Konzentrationstagung, Agravis and EuroChem Agro, 07.11.2013, Lüneburg, Germany

- Trautz D, Federolf C-P, **Westerschulte M**, Olfs H-W (2016) Effiziente Nährstoffnutzung aus Wirtschaftsdüngern – Gülleunterfußdüngung und Strip Till. KTBL Tage 2016 – Ressourcen effizienter nutzen, 18.-20.04.2016, Kassel, Germany
- Trautz D, Federolf C-P, **Westerschulte M**, Olfs H-W (2014) Gülleunterfußdüngung von Mais – aktuelle Forschungsergebnisse. Kotte Lohnunternehmertagung, Garant Kotte, Rieste, Germany
- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2016f) Gülleunterfußdüngung zur Optimierung der Stickstoffnutzungseffizienz im Maisanbau. SKW-Fachtagung „Düngung 2016“ – Stickstoff in der Pflanze. 13.12.2016, Hasbergen; 14.12.2016, Neumünster; 15.12.2016, Soltau; Germany
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- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2016d) Wirkung unterschiedlicher Nitrifikationshemmstoffe zur Stabilisierung des Ammoniumstickstoff bei Gülledepot-Applikation. 128. VDLUFA-Kongress: Anforderungen an die Verwertung von Reststoffen in der Landwirtschaft, 13. – 16.09.2016, Rostock, Germany
- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2016c) Ergebnisse eines mehrjährigen Feldversuchs zur Unterfuß-Gülleapplikation in Mais. Sommertagung AG „Landwirtschaftliches Versuchswesen“, 23.-24.06.2016, Osnabrück, Germany
- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2016b) Gülledepot zu Mais – Stickstoffdynamik bei Gülledepotdüngung. Abschlussworkshop „Gülledepot zu Mais“ der Hochschule Osnabrück, 20.05.2016, Osnabrück, Germany
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- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2015d) Bodenstickstoffdynamik unter Mais bei Gülle-Unterfußdüngung im Vergleich zur breitflächigen Applikation. Jahrestagung der Deutschen Gesellschaft für Pflanzenernährung e.V.: Boden, Nährstoffe, Wasser – Forschung für die nachhaltige und effiziente Nutzung von Ressourcen, 17.-18.09.2015, Göttingen, Germany
- Westerschulte M**, Federolf C-P, Pralle H, Trautz D, Olfs H-W (2015c) Entwicklung einer Beprobungsmethode zur Beschreibung der Bodenstickstoffdynamik nach Gülleinjektion in Maisfeldversuchen. 127. VDLUFA-Kongress: Böden – Lebensgrundlage für Pflanze und Tier, 15.-18.09.2015, Göttingen, Germany
- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2015b) Soil nitrogen dynamics in maize field trials after slurry injection compared to broadcast application using a new sampling strategy. 23rd International Symposium of the International Scientific Centre for Fertilizers (CIEC) – Plant nutrition and fertilizer issues for the cold climates, 08.-10.09.2015, Son, Norway
- Westerschulte M**, Federolf C-P, Trautz D, Olfs H-W (2015a) Applikation von Wirtschaftsdüngern im Depot als Beitrag zur nachhaltigen Landbewirtschaftung – Teil 2: Stickstoffdynamik in Boden und Pflanze. Osnabrücker Geographisches Kolloquium, University of Osnabrück, 27.05.2015, Osnabrück, Germany

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2014b) „Depot-Applikation“ von Wirtschaftsdüngern zur Optimierung der N- und P-Effizienz. Arbeitskreis Gülle-Strip-Till, LWK NRW, 11.12.2014, Saerbeck, Germany

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2014a) Nmin-Bodenbeprobung bei platziertter Gülle-Applikation. Treffen der Wasserschutzberater der LWK NS, LWK NS, 29.07.2014, Oldenburg, Germany

- POSTERS -

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016c) Using liquid manure as side dress fertilizer in maize. 14th ESA Congress, 05.-09.09.2016, Edinburgh, Scotland

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016b) Güttelepot zu Mais II. Ertragsleistung, Landwirtschaft und Wasserschutz – Feldtag für Kooperationslandwirte und Wasserschutzberatung, LWK NS, 15.06.2016, Oldenburg, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2016a) Güttelepot zu Mais I. Stickstoffdynamik. Landwirtschaft und Wasserschutz – Feldtag für Kooperationslandwirte und Wasserschutzberatung, LWK NS, 15.06.2016, Oldenburg, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015c) Optimierung der Stickstoff- und Phosphat-Effizienz aus flüssigen organischen Wirtschaftsdüngern durch Depot-Applikation zur Verminderung der Umweltbelastung. Verbraucherschutzministerkonferenz, University of Applied Sciences of Osnabrück, 06.05.2015, Wallenhorst, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015b) Using manure injection to decrease nutrient surpluses in northwestern Germany. Introductory Seminar for the International Climate Protection Fellows, German Federal Environmental Foundation [Deutsche Bundesstiftung Deutschland (DBU)], 16.03.2015, Osnabrück, Germany

Federolf C-P, **Westerschulte M**, Olfs H-W, Trautz D (2015a) Optimizing nitrogen and phosphorus use efficiencies from liquid manure by slurry injection to reduce environmental pollution. Agriculture and Climate Change – Adapting Crops to Increased Uncertainty (AGRI 2015), 15.-17.02.2015, Amsterdam, The Netherlands

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2016c) Nitrogen dynamics following slurry injection in maize. 24th Annual Conference of the International Fertilizer Society (IFS), 08.-09.12.2016, Cambridge, United Kingdom (Runner Up of the “Brian Chambers Award”)

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2016b) Soil mineral nitrogen dynamics in maize after slurry injection compared to broadcast application. International Conference of the German Society of Plant Nutrition, 28. – 30.09.2016, Hohenheim, Germany (received a poster price: one of the three best posters)

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2016a) Soil mineral nitrogen dynamics in maize after slurry injection compared to broadcast application. 14th ESA Congress, 05.-09.09.2016, Edinburgh, Scotland

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2015) Optimizing the nitrogen and phosphate use efficiencies from liquid manure by slurry injection to reduce environmental pollution. Agritechnica 2015, DLG e.V., 09.-14.11.2015, Hannover, Germany

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2014) Optimierung der Stickstoff- und Phosphateffizienz aus flüssigen organischen Wirtschaftsdüngern durch „Depot-Applikation“ zur Verminderung der Umweltbelastung. Informationsveranstaltung Wasserschutz, LWK NS, 23.-24.09.2014, Oldenburg, Germany

Curriculum Vitae

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WORK EXPERIENCE

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