Slurry injection to optimize nutrient use efficiency in maize: Regional performance of manure based fertilizer strategies

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I. Index

II. List of abbreviations

AMO	Ammonium-monooxygenase
B+ / B-	broadcast treatment with / without mineral starter fertilizer
BNF	biological nitrogen fixation
C+ / C-	control treatment with / without mineral starter fertilizer
CAN	Calcium-Ammonium-Nitrate
Cm	Cambic
DAP	days after planting
DBU	Deutsche Bundesstiftung Umwelt
DCD	Dicyandiamide
DM	dry matter biomass
DMPP	3,4-dimethylpyrazol phosphate
EU / EU27	European Union / 27 nations of the European Union
FOV	field of view
gl	Gleyic
ha	Haplic
I(N)+ / I(N)-	injection treatment with nitrification inhibitor with / without mineral starter fertilizer
I+ / I-	injection treatment with / without mineral starter fertilizer
Ir(N)+ / Ir(N)-	reduced rate injection treatment with nitrification inhibitor with / without mineral starter fertilizer
Ir+ / Ir-	reduced rate injection treatment with / without mineral starter fertilizer
LAI	leaf area index
LM	liquid manure
LMA	liquid manure application treatment
LS	Lower Saxony
LV	Luvisol
MP	mouldboard plough
MSD / MSF	mineral side dress fertilizer = mineral starter fertilizer
N_2O	nitrous oxide
N_c / N_{conc}	Nitrogen concentration
NDVI	naturalized differentiated vegetation index
NH_3	Ammonia
NH_4^+	Ammonium
NI	nitrification inhibitor
NIR	near infrared
NO_2^-	Nitrite
NO_3^-	Nitrate
NRE	apparent nitrogen recovery efficiency
NRW	North Rhine-Westphalia
N_{upt}	crop nitrogen uptake
PZ	Podzol
r^2	coefficient of determination
REIP	red edge inflection point
RT	reduced tillage
SH	Schleswig-Holstein
SMN	soil mineral nitrogen
Trt	Treatment
VI	vegetation index
Vn	vegetative growth stage nth leaf
VT	vegetative growth stage tasseling
W	Dry matter biomass

Chapter 1

General introduction

1.1 Background and objective

The increase of livestock husbandry and the extension of biogas production in northwestern Germany has led to an increase in the production of organic manure. Nitrogen (N) and phosphorous (P) from manures outbalance crop demand by far, and large amounts of manure must be exported from areas of intensive livestock farming (Warnecke et al. 2011).

Cultivated on up to 60% of the arable land in the region, maize (*Zea mays* L.) is the dominant crop (Keckl 2015). Although the nutrient demand of maize is already covered by broadcast application of organic manure, usually a mineral N and P fertilizer is side-banded ("starter fertilizer") to ensure proper early growth development when low soil temperatures limit P bioavailability and root growth. This fertilization practice often leads to nutrient surpluses that might be lost to non-agricultural ecosystems like surface and groundwater resources (Withers et al. 2000).

Recently developed techniques for slurry injection allow the banded application of liquid manures below the maize seeds (Figure 1). Due to high nutrient concentrations in the fertilizer band, N immobilization and nitrification is reduced and roots have a better spatial access to the applied N and P (Schröder et al. 2015). This might lead to an increased nutrient use efficiency from organic manures and the substitution of starter fertilizer without impairing maize yields and quality. Furthermore, the addition of nitrification inhibitors might be able to help synchronizing nitrification of the applied ammonium to the crop N demand, which predominantly happens several weeks after application of manure (Ruser and Schulz 2015).

The aim of this thesis was to investigate consequences following the injection of liquid manures on the growth performance of maize. Based on these results, different agronomic options to improve crop development are discussed particularly in regard of a sustainable intensification in northwestern German maize production.

The objectives were to

a) compare broadcast application of slurry with different injection treatments (without and with mineral starter fertilizer, without and with a nitrification inhibitor, and at two rates) under different soil and climate conditions in northwestern Germany;

b) gather deeper insight into the effects of different soil N dynamics following slurry injection show on crop nutritional status and yield performance;

c) show how differences in crop growth can be easily detected by using high throughput hyperspectral sensors to improve the acquisition of information in agronomic field trials.

Figure 1: Maize plant at 3 leaves stage. The roots have already started intensive branching into the manure band (orange circle).

1.2 Methodology and structure

This thesis is based on the project "Optimierung der Stickstoff- und Phosphat-Effizienz aus flüssigen organischen Wirtschaftsdüngern durch "Depot-Applikation" zur Verminderung der Umweltbelastung" ("Optimizing nitrogen and phosphorus use efficiencies from liquid manure by slurry injection to reduce environmental pollution") funded by the German Federal Environmental Foundation ("Deutsche Bundesstiftung Umwelt"; Grant 30364/01). While injection of liquid manure has been primarily used to mitigate ammonia volatilization in recent years, the objective of this project was to develop slurry injection into an integrated starter fertilizer strategy for northwestern German maize production. In this respect, generating knowledge for using band-injected slurry to obviate mineral N and P starter fertilizers is a necessity for reducing N and P inputs to maize fields. The addition of nitrification inhibitors to the slurry prior to application delays the nitrification of ammonium, which might reduce nitrate leaching and enhance the availability of nutrients like P and zinc.

In close cooperation with the extension services in the federal states of North-Rhine Westphalia, Lower Saxony and Schleswig-Holstein field experiments in typical maize production areas are conducted. In a uniform trial setup, slurry injection treatments are compared to broadcast application of liquid manure with mineral starter fertilizer. The field trials mainly focused on nitrogen dynamics in the soil-plant-system and their respective consequences on maize-nutrient uptake. Additionally, methodological solutions to increase the validity of soil mineral N investigations, as well as to ease in-season crop performance investigations via spectral sensors were evaluated. This inclusive research approach with regional uniform trials in combination with in-depth field and laboratory trials at the research station of the Osnabrück University of Applied Sciences is expected to extensively add to existing knowledge of the subject. The cooperation with the extension services and local companies in the regions of interest led to a rapid transfer of knowledge and further research questions from and to the participating scientists, advisors, companies and farmers.

The present thesis begins with a general introduction, including a literature review, which illustrates issues in the N and P cycles in areas of intensive livestock farming. The introduction then explores the resultant consequences on northwestern German maize production.

The subsequent chapter is comprised of three articles, which are published in international peer-reviewed journals. The chapter begins with a study in which the performance of liquid manure injection on several sites within the region is tested and discussed using two rates of application (recommended and reduced) and the presence or absence of a nitrification inhibitor. In the second article, the effects of slurry injection on nitrogen dynamics during crop growth were examined in detail. The third article is a methodological approach to using sensor technologies in agronomical field trials with maize. Thereby high throughput of plots with low labor input should increase the expressiveness of trial series.

The results of the project are then discussed on a broader scale followed by a general conclusion, which summarizes the potentials of liquid manure injection to increase nutrient use efficiencies in northwestern Germany and limitations in terms of sustainability.

1.3 Nutrient cycling in areas of intensive livestock farming

To meet the demand for food, feed and fiber of an ever-expanding global population, overall agricultural production is constantly increasing and is a driver for long-term negative changes of ecosystems (Foley et al. 2005). In recent decades the increase of production was mainly driven by intensification of a constant production area through increasing inputs like fertilizers or irrigation in crop production (Foley et al. 2011). Additionally, global trade increased regional specialization and thus, intensification (Schipanski and Bennett 2012). With the possibility to trade globally, decoupling livestock production from fodder production led to so called "secondary nutrient flows" (e.g. N, see Figure 2) into areas with intensive livestock operations, which leads to nutrient surpluses (MacDonald et al. 2011; Schipanski and Bennett 2012). Major imports into the EU27 are feedstuff like soybeans and soybean cake from South America, maize from North America, and palm oils from Southeast Asia. Major exports to Russia and Japan mainly consist of meat products, whereas the Maghreb and Middle East countries are major importers of European feed grains (Lassaletta et al. 2014).

On a global scale, intensive production of mainly monogastric species tends to be concentrated in regions with good market opportunities, which are in close proximity to densely populated areas (Gerber et al. 2005).

While in most parts of the northern hemisphere these intensive production systems already exist and production quantities are relatively stable, they are rapidly growing in the global south (Gerber et al. 2005). Several areas of intensive livestock farming "clustered" in the south (Cataluña and Galicia in Spain, Lombardy in Italy), and the north (Brittany in France, Belgium, the Netherlands, Denmark, and the federal states of Nordrhein-Westfalen, Niedersachsen and Schleswig-Holstein in Germany) of Europe (Bäuerle and Tamásy 2012; Melse and Timmerman 2009) and show major inflow of feed grain and soymeal (Steinfeld 2006). This inflow results in N and P surpluses in several municipalities in Germany (Osterburg and Techen 2012). The emergent biogas production based on energy crops like maize diminished the share of feed production on arable land in northwestern Germany, leading to a further rise of feed imports (Wüstholz et al. 2014). The subsequent nutrient surpluses necessitate transport of manures into regions where they can be used in arable farming (Warnecke et al. 2011).

In high-density livestock operations however, excess fertilization (Carpenter et al. 1998; Steinfeld 2006) leads to P accumulation in soils (Leinweber et al. 1994; MacDonald et al. 2011), and air and water pollution (Jongbloed et al. 1999; Builtjes et al. 2011) which hinders efforts to meet sustainability targets (Tilman et al. 2002; Sutton et al. 2013). In order to overcome the aforementioned concerns, European countries introduced legislations to mitigate nutrient losses to the environment (Jongbloed et al. 1999). Directives dealing with N emissions to water bodies (91/676/EEC Nitrates directive; 2000/60/EC Water Framework Directive; 2006/118/EC Groundwater Directive) and the atmosphere [2001/81/EC National Emission Ceilings; 2008/1/EC Integrated Pollution, Prevention and Control (Oenema et al. 2011)] therefore evolved in the EU during the last three decades.

Although the optimization of feeding strategies in animal production, which directly decreases nutrient imports and, thus, all following emission factors (Webb et al. 2006; Webb et al. 2010) has already been implemented in northwestern Europe (Poulsen et al. 1999), to reduce nutrient surpluses further measures are in demand.

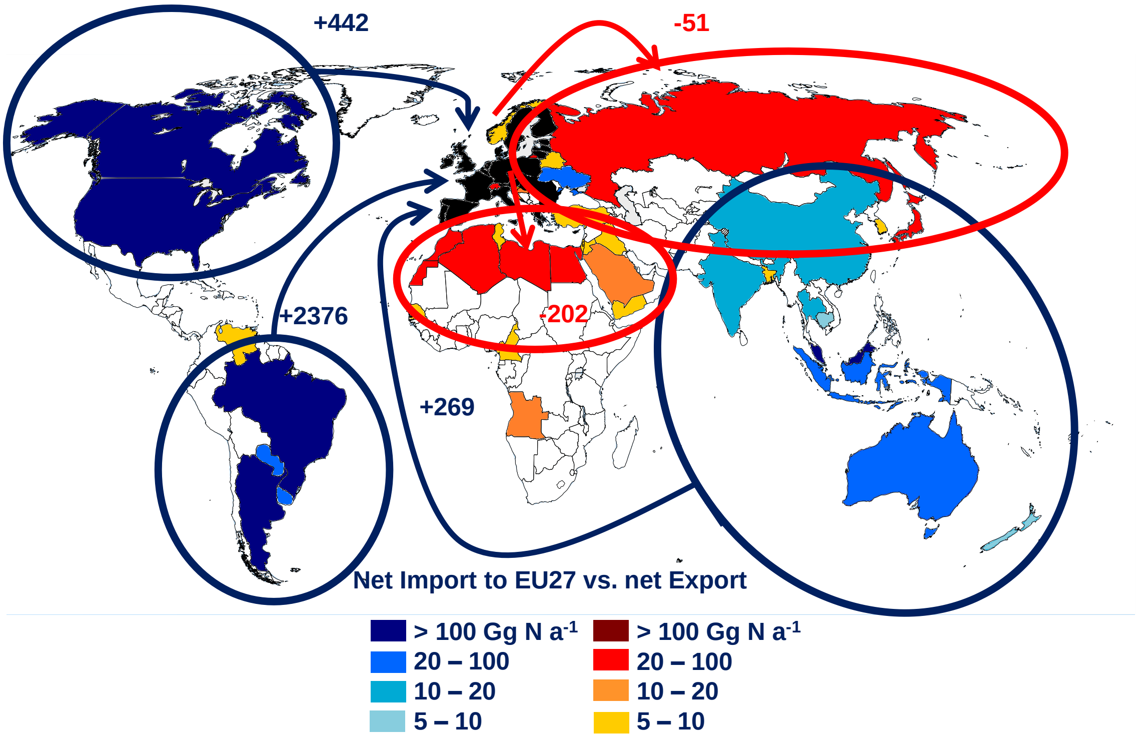

Figure 2: N flows from EU27 (filled black) to other countries for the year 2004. Net exporting countries in blue. Yellow, orange, red countries are those, which are net importers from Europe. Arrows show flows between different regions and EU27. Map by weltkarte.com (2017), data from Leip et al. (2015).

1.4 Nitrogen and phosphorus in the environment

Global N and P fertilizer use

Nitrogen (N) and phosphorus (P) are crucial nutrients for crop growth and their plant availability is usually a limiting factor in crop production (Bouwman et al. 2017). To overcome these limitations, farmers apply mineral fertilizers, which account for 70-75% of total P and 40-55% of total N inputs throughout the globe (Bouwman et al. 2017). N and P fertilization rates in excess of crop nutrient demand lead to low nutrient use efficiencies, high amounts of reactive N lost to the environment, and P accumulations in soils (Bouwman et al. 2017; Carpenter et al. 1998; MacDonald et al. 2011; Sutton et al. 2011). On the other hand, N_2 is amply present in the ambient air, but not plant available. Different pathways convert inert N_2 to reactive N, totaling 413 Tg y^{-1} globally (Fowler et al. 2013). Biological N fixation (BNF) converts N_2 into ammonium compounds which are then transformed to amino acids, or oxidized (Fowler et al. 2013). Marine BNF and terrestrial BNF account for 120 Tg y^{-1} and 58 Tg y^{-1}, respectively. The contribution of N_2 transformed by lightning and anthropogenic combustions (5 Tg y^{-1} and 30 Tg y^{-1}, respectively) to crop production is limited (Fowler et al. 2013). Agricultural BNF, mainly based on symbiotic processes between plants and rhizobia adds 50-70 Tg N y^{-1} (Herridge et al. 2008). The major contribution to crop nutrition since the beginning of the 20th century is the Haber-Bosch process with an annual N production of 120 Tg y^{-1} (Fowler et al. 2013). Direct carbon dioxide emissions contributing to global warming are a negative aspect of N fertilizer production, as a lot of energy is needed to convert N_2 into reactive forms (Sutton et al. 2013).

Reactive N as nitrate pollutes ground- and surface waters and nitrous oxide (N_2O) significantly contributes to global warming (Leip et al. 2015).

Ammonia emissions and abatement strategies

Livestock-related ammonia emissions are the major source of agricultural ammonia emissions, which account for 80% of the total European ammonia emissions (van der Hoek 1998; Velthof et al. 2012). On-farm measures to reduce ammonia emissions include exhaust air treatment systems, when active ventilation systems are used for animal housing (Melse and Timmerman 2009) and manure storage cover (Sommer et al. 1993). Ammonia emissions from land application of manure account for ~40% of livestock ammonia emissions (van der Hoek 1998; Velthof et al. 2012). When spreading liquid manure on the soil surface, depending on several factors (e.g. soil pH, plant cover, temperature, and wind speed) up to 100% of the applied ammonia volatilizes (Huijsmans et al. 2003; Misselbrook et al. 2002). Reducing the exposure area to air via band spreading, or injection (Frost 1994), as well as slurry dilution to increase infiltration rate into the soil are measures to reduce ammonia emissions (Sommer and Hutchings 2001). The potential to decrease ammonia emissions has been widely confirmed for liquid manure surface banding (-48%), shallow incorporation (-68%), and deep injection (-97%) (Webb et al. 2010). As these improvements come at a relatively low cost (Webb et al. 2006), they are increasingly being adopted by European farmers. Reducing ammonia emissions from land application of liquid manure however, leads to high amounts of ammonium in the soil, which is then nitrified and increases not only the potential for N_2O losses on the nitrification and denitrification pathways (Dosch and Gutser 1996; Webb et al. 2010), but also contributes to nitrate (NO_3^-) leaching (Köhler et al. 2006).

Nitrous oxide emissions

Nitrous oxide (N_2O) is the third most significant greenhouse gas contributing to climate change and is today predominantly emitted from soils treated with N fertilizers (Hartmann et al. 2013; van Groenigen et al. 2010). During the application of ammonium fertilizers, substantial amounts of N_2O are emitted during the process of nitrification via nitrifier denitrification (Figure 3). The quantitative relevance of N_2O emissions from these processes are currently discussed (Baggs and Philippot 2010; Ruser and Schulz 2015; Wrage et al. 2001). The major source of N_2O in cropping systems however, is denitrification of NO_3^-, which depends on available NO_3^- and carbon (C), soil moisture and oxygen contents, as well as soil structure and soil respiration (Müller and Clough 2014). Denitrification rates can be altered by cropping and fertilizer systems (Bouwman et al. 2002; Ma et al. 2010). A reduction of N_2O emissions can be achieved by keeping soil NO_3^- concentrations low, as it directly leads to reduced denitrification potential and to a higher N_2/N_2O-ratio in the denitrified product (Ruser et al. 2006; Weiske et al. 2001). Consequently, linking N fertilizer applications and nitrification to crop N demand is advised (van Groenigen et al. 2010).

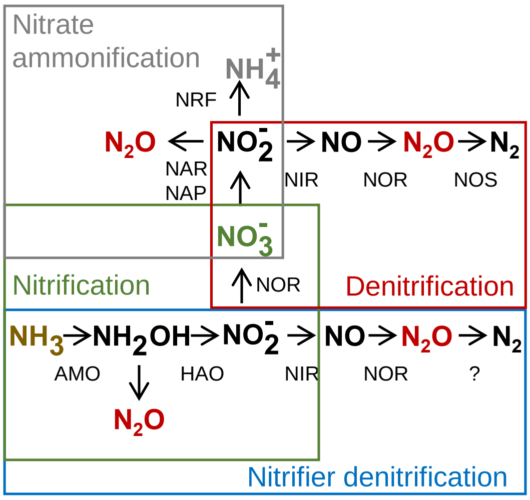

Figure 3: Pathways of nitrification in ammonium oxidizing bacteria (green box; Arp and Stein (2003)), nitrifier denitrification (blue box) and denitrification (red box). Adapted from Baggs and Philippot (2010).

Nitrate leaching

As NO_3^- is typically not bound to soil particles, it is the dominant N form in seepage (Di and Cameron 2002). Leaching of NO_3^- is an inevitable implication with crop production in humid regions (Köhler et al. 2006). Leached NO_3^- contaminates ground- and surface waters (through drainage systems) and poses a threat to human and animal health (Di and Cameron 2002). NO_3^- leaching furthermore results in eutrophication of aquatic ecosystems, as N is, besides P, the most limiting factor for marine life in temperate regions (Howarth 1988). As for N_2O, the major driver for NO_3^- leaching is a surplus of NO_3^- above plant requirements (originating from mineralization of organic matter, organic and mineral fertilizers), paired with precipitation events that lead to drainage (Kayser et al. 2011). This is often the case during vegetation-free periods in cropping systems and particularly critical after soil cultivation (Francis et al. 1995). Possible measures to avoid NO_3^- leaching are reducing N surpluses and coupling fertilizer applications with crop N demand, as well as permanent soil coverage by catch crops or other cropping system adaptions (Köhler et al. 2006; Strebel et al. 1989). Deep ploughing and drainage of peatlands in northwestern Germany provided large areas of sand-mix culture soils available for agriculture (Hageman 1978). These soils show an increased potential for N mineralization and the coarse texture favors leaching of NO_3^- (Kayser et al. 2011).

Other N loss pathways

Total N losses increase with increased N input (Liu et al. 2016). Further N losses from agricultural systems can occur when N bound to organic matter or soil particles is eroded from the site and when N (particularly NO_3^-) is dissolved in surface runoff water (Figure 4). Compared to the gaseous and leaching, the magnitudes of these losses are low. While some significant N losses through erosion are reported in the literature (e.g. Quinton et al. 2010), losses through runoff and erosion usually account for less than 5% of the applied input (Carpenter et al. 1998; Keppner et al. 2017). These differences, however, largely depend on interactions between soil, slope, tillage, crop management, and precipitation (Le Bissonnais et al. 2002).

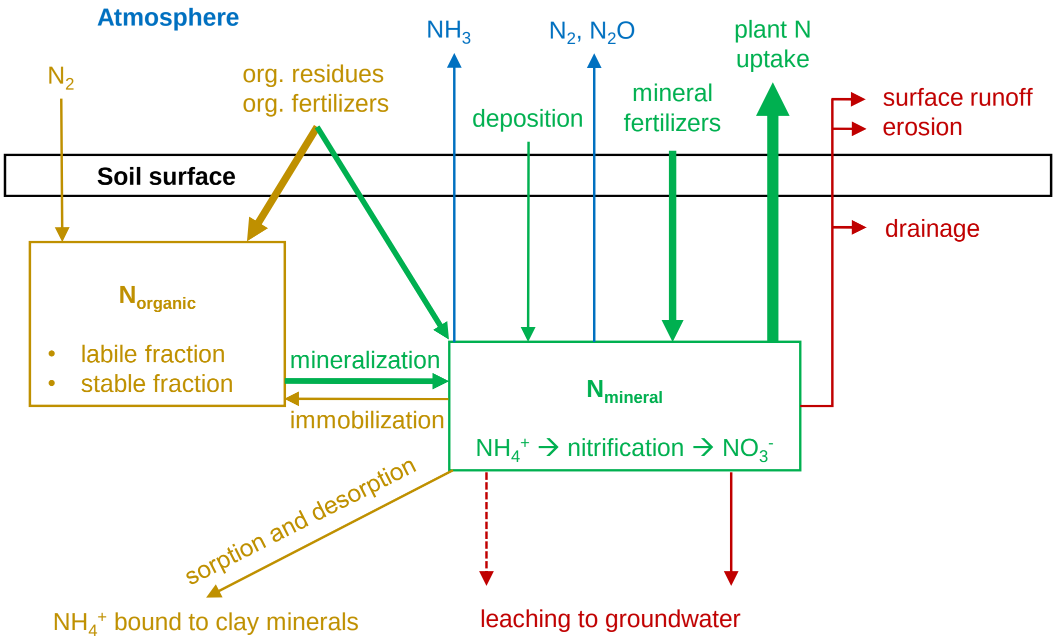

Figure 4: Nitrogen cycle in the soil with major fractions, flows and turnover processes. Adapted from (Blume et al. 2010).

Phosphorus

P is a crucial nutrient for plants. Unlike N, P fertilizers are produced from mined rock phosphate since the mid of the 19th century at a current rate of 13-16 Tg P y^{-1} (Smil 2000). Sutton et al. (2013) estimate a depletion of rock phosphate reserves in ~370 years. Although P reserves are expected to last longer than e.g. fossil fuels, most P mines are located in politically unstable regions, or the P produced is of poor quality due to high concentrations of heavy metals.

Manures from livestock systems are a substantial source of P used in agriculture. Smil (2000) and Bouwman et al. (2009) estimate that 16-20 Tg P y^{-1} and 14-17 Tg P y^{-1}, respectively, are recycled from organic manures. A total input of 23.8 Tg P y^{-1} to global croplands exceeded total removal by harvested crops (12.3 Tg P y^{-1}) according to a study by MacDonald et al. (2011). Consequently, P input to agricultural systems is not balanced, especially in areas of intensive livestock farming with surplus fertilizer use (organic plus mineral), which accumulate P in soils (Carpenter et al. 1998; MacDonald et al. 2011; Schipanski and Bennett 2012).

These P accumulations in soils are prone to runoff and a major driver for eutrophication of aquatic systems (MacDonald et al. 2011). Global annual P erosion from cropland accounts for 15 Mt P y^{-1}, from permanent pastures 13 Mt P y^{-1} and 2 Mt P y^{-1} from non-agricultural land, respectively. Smil (2000) estimates a total discharge of 20 Mt P y^{-1} to oceans, the other 10 Mt P y^{-1} are deposited in other alluvia, e.g. in riverbeds. Soil P accumulations can lead to leaching of P, especially on soils with low sorption capacities and low retention potential for water (Behrendt and Boekhold 1993; King et al. 2015). On the coarse textured soils in northwestern Germany with intensive livestock farming, arable fields are typically drained, and show high P concentrations. In these conditions, leaching of P has been identified as a relevant route for P losses (King et al. 2015; Leinweber et al. 1997; Pihl and Werner 1995).

1.5 Fertilization strategies for maize in northwestern Germany

Low suitability to produce high-income crops on the sandy soils in major parts of northwestern Germany led to a traditionally high concentration of animal farming. In the last decade a further incease in stocking densities and the emergent biogas production led to high nutrient surpluses (Bach and Frede 1998; Osterburg and Techen 2012).

The estimates for N and P excretions from animal husbandry in certain areas like the Weser-Ems region (177 kg N ha^{-1} and 42 kg P ha^{-1}, respectively), with a focus on the districts Vechta (310 kg N ha^{-1}, 85 kg P ha^{-1}, respectively) and Cloppenburg (252 kg N ha^{-1}, and 69 kg P ha^{-1}, respectively; (Landwirtschaftskammer Niedersachsen 2017)) already outbalance potential crop nutrient uptake. Consequently, huge amounts of manure have to be exported to other regions (Warnecke et al. 2011). Furthermore, leaching is a common problem and nitrate concentrations in the groundwater are elevated (Keppner et al. 2017; Wachendorf et al. 2004). Maize (*Zea mays* L.) is the dominant crop in the region (Kayser et al. 2011; Keckl 2015). As ample amounts of manure are available, farmers commonly apply liquid manure prior to planting with splash plates, or trailing hoses followed by immediate incorporation. Large amounts of N are required by many plants and N represents a major constituent of proteins, chlorophyll and other compounds (Hawkesford et al. 2012). N limitation typically leads to reduced growth of plants, senescence of older leaves and reduced formation of reproductive organs (Hawkesford et al. 2012). At early growth stages of maize however, Muchow (1988) showed only minor effects of N deficiency on biomass production. Accordingly, on a wide range of N fertilization levels, Vos et al. (2005) observed only limited response of maize in terms of leaf appearance, elongation, and final area. However, leaf N concentrations and, consequently, photosynthetic capacity were significantly reduced.

According to Plénet and Lemaire (1999) N concentrations in maize plants should not fall below a critical value of 3.4% N if aboveground biomass is below 1 Mg ha^{-1}, in order to prevent yield loss. For further growth, they suggest a function for critical N ($\%N_C = 3.4 * (W)^{-0.37}$ where W is the dry matter biomass (DM) in Mg DM ha^{-1}). A similar approach developed by Herrmann and Taube (2004) leads to slightly lower target values for N_C.

To assure optimum N concentrations on farms with animals or biogas, usually a combination of manure and mineral fertilizers is used. While farmers apply manure and N fertilizers prior to planting, or top-dress at 4 to 10 leaves stage, mineral N-P fertilizers are commonly used as starter fertilizer close to the maize row at planting to overcome early-growth nutrient deficiency stress (Schröder et al. 2015; Withers et al. 2000).

In recent fertilizer recommendations, manure application rates were prevailingly adapted to N demand (Baumgärtel et al. 2010), leading to P surpluses on farms with pigs or poultry. Nevertheless, at early growth stages when soil temperatures are low, incorporated manure P and soil P are not fully available to maize roots (Barber 1995; Mollier and Pellerin 1999). The low bioavailability of P to maize plants at low soil temperatures is a result of a restricted P diffusion speed as well as limited root growth, which limits spatial acquisition of P (Imran et al. 2013; Mollier and Pellerin 1999). Thus, P deficiency symptoms such as purpling of leaves are common in temperate maize production. Furthermore, suboptimal P concentrations impair crop growth and biomass production due to reductions in leaf appearance, leaf elongation and final leaf size (Assuero et al. 2004; Hawkesford et al. 2012). To acquire sufficient amounts of P from the soil, deficient plants increase their root growth and try to dissolve P with root exudates (Neumann and Römheld 2012). Starter fertilizers usually consist of ammonium N and P because lateral root proliferation as well as fine root proliferation are enhanced in zones where high concentrations of ammonium N and P occur (Drew 1975; Ma et al. 2013; Ohlrogge 1962). When plants take up the ammonium cation a H^+ is excreted by the root and, as a consequence, the pH in the rhizosphere is lowered, typically increasing the solubility of P in European growing conditions (Neumann and Römheld 2012). Thus, farmers commonly use starters containing both ammonium N and P (e.g. diammonium phosphate), which might lead to nutrient surpluses if not regarded in N and P fertilizer recommendations.

Fertilization recommendations

N fertilization recommendations for maize in northwestern Germany typically use target values for N in relation to yield expectation. These target values depend on soil mineral N (SMN) taking into account soil type, recent organic fertilization and previous crop (Baumgärtel et al. 2010; Fechner and Apel 2016; Landwirtschaftskammer Schleswig-Holstein 2013). As SMN shows very high in-field variations, large numbers of samples need to be obtained to get reliable SMN values (Aufhammer et al. 1989; Olfs et al. 2005; Westerschulte et al. 2015). SMN is used to calculate pre-plant N recommendation, as well as for pre-side dress N (Olfs et al. 2005). As the year-to-year variation of pre-plant SMN is rather negligible on sandy soils, the benefit of site-specific sampling does not outweigh the costs of sampling and analysis (Schröder et al. 1998). Thus, the extension services provide regionally average values for SMN based on large numbers of samples (Fechner and Apel 2016). The target value is a good indicator for N fertilization rates to sustain optimum yields on a regional basis, but the practice is not always able to reduce NO_3^- leaching potential (Bauer et al. 2014; Schiermann 2004). Due to the coarse soil texture of many maize fields in the region, leaching might occur between fertilization and plant N uptake (Westerschulte et al. 2016; Westerschulte et al. 2017). Furthermore, mineralization of N during the vegetation period varies substantially as a result of seasonal weather conditions, soil type, and soil organic matter (Aufhammer and Kübler 1997; Olfs et al. 2005; Schiermann 2004).

There are estimations based on recent observations available and already integrated into fertilizer recommendations. In-season samplings and analysis of SMN, although available, have not found their way into practical farming (Kitchen and Goulding 2001). To get a better indication of N mineralization, it might be wise to take samples during the vegetation. This practice, however, requires fertilization to be split into a moderate starter fertilizer application and a later side dressing, which can be based on soil and plant analysis (Olfs et al. 2005). Pre-side dress SMN sampling is a viable method to decide on N fertilization at later growth stages, when a relevant proportion of mineralization has already taken place (Bauer et al. 2014; Laurenz 2013; Richards et al. 1999; Stone 2000) and local recommendations are available. Different methods of plant analysis give insight into plant nutritional status. Tissue sampling can be used for N as well as for other nutrients, e.g. the aforementioned critical value by Plénet and Lemaire (2000). Plant-sap nitrate concentration in the stem-base of maize plants can indicate nutritional status (Bauer et al. 2014; Geyer and Marschner 1990), and chlorophyll meter readings also respond to N status (Rambo et al. 2010). In the last two decades, research to relate spectral measurements to plant nutritional status resulted in vegetation indices like the naturalized differentiated vegetation index (NDVI), and the red edge inflection point (REIP, for details see chapter 2.3; (Guyot and Baret 1988; Rouse et al. 1974). Farmers use these kind of measurements to optimize fertilization of maize in North America (Schmidt et al. 2011) and of oilseed rape and wheat in Europe (Samborski et al. 2016). Although plants seem to be the best indicator for their nutritional status, all in-season dressings for maize need to take recent weather conditions, as well as other possible growth factors, into account and are best combined with SMN sampling (Bauer et al. 2014; Rambo et al. 2010).

Liquid manure injection

To enhance nutrient use efficiency from liquid manure, reliable application systems with adequate longitudinal and lateral distribution became state of the art for most farmers and contractors in north-western Germany. Based on these technologies, different tools to directly inject liquid manure into the soil evolved. Shallow injection with disc openers is typically used for top-dressing in crops like cereals and grassland (Misselbrook et al. 2002). Deep injection with tines requires a significant movement of soil and is thus only applicable on bare soil, or between the rows of wide standing crops (Ball Coelho et al. 2005; Meisinger and Jokela 2000). Several authors quantify the ammonia emission reduction potential of the aforementioned injection techniques (e.g. Maguire et al. 2011; Misselbrook et al. 2002; Sommer and Hutchings 2001; Webb et al. 2012). Furthermore, when manure is band injected, the interaction of soil and manure and subsequently the transformation of NH_4^+ from manure into other N compounds is impeded (Sørensen and Amato 2002), leading to more plant-available N, and to a reduced N loss potential (Dosch and Gutser 1996). Although nitrification of NH_4^+ is slowed, still very high concentrations of NO_3^- are present in the manure band, increasing the risk for denitrification losses (Webb et al. 2010). Additionally, taking into account the substantial concentrations of other nutrients, injected liquid manure can be used as starter fertilizer for maize (Bittman et al. 2012; Petersen et al. 2010; Schmitt et al. 1995; Schröder et al. 2015). While several studies in north America were conducted on silt, loam, and clay soils, European studies were predominantly conducted on coarse-textured, sandy soils. Usually there is no possibility to do any further tillage operation after slurry injection. Thus, the injectors need to ensure a proper seedbed preparation. This is not possible on fine-textured soils, which typically show high soil moisture contents during maize seedbed preparation in Europe. For a quick root penetration of the manure band, slurry placement in close proximity to the seed row is recommended (Bittman et al. 2012; Schröder et al. 2015). However, in a study from Sawyer and Hoeft (1990) with injected beef manure, unfavorable chemical properties like NH_3 toxicity, low redox potential and high concentrations of nitrite (NO_2^-) did not allow roots to grow into the manure band. High concentrations of NO_2^- associated with high doses of NH_4^+ were also reported by Shaviv (1988), as NH_4^+ oxidation to NO_2^- is unaffected by NH_4^+ concentrations, whereas the oxidation of NO_2^- to NO_3^- was significantly reduced. Consequently, more NO_2^- is available for the nitrifier denitrification pathway, which can lead to increased N_2O emissions (see Figure 3).

Nitrification inhibitors

Further increase in nutrient use efficiency might be achieved when adding nitrification inhibitors to applied ammonium-based fertilizers (Subbarao et al. 2006). Nitrification inhibitors applied with substantial doses of liquid manure are able to decrease NO_3^- concentrations in the soils and, thus, the potential for leaching and denitrification (Di and Cameron 2007; Ruser and Schulz 2015). The process of nitrification is the oxidation of NH_3 via NO_2^- to NO_3^- by *Nitrosomonas* spp. and *Nitrobacter* spp. (Singh and Verma 2007). As the nitrification process requires oxygen, significant amounts of N_2O can be formed under oxygen limitation (Wrage et al. 2001). In the first step of nitrification, the enzyme ammonia monooxygenase (AMO) oxidizes NH_3 to NH_2OH, which poses no N loss pathway (McCarty 1999). Consequently, this is the relevant target enzyme for nitrification inhibitors.

To impede NH_3 oxidation by AMO, three specific modes of action are known:

a) direct interaction with the enzyme, either by competition for the target site, or by binding somewhere else to the enzyme changing its conformation [e.g. methane, carbon monoxide, nitrapyrin; Keener and Arp (1993)].
b) deprivation of co-factors. For AMO Cu is a relevant co-factor, thus Cu-chelating compounds like thiourea, dicyandiamide (DCD) and nitrapyrin can inhibit nitrification (Subbarao et al. 2006).
c) Mechanism-based inhibitors irreversibly inactivate the target enzyme through modification by the product of catalysis (McCarty 1999). However, compounds like trichloroethylene and allylsulfide (Juliette et al. 1993; McCarty 1999) did not find their way into practical farming.

The mode of action of heterocyclic compounds such as 1,2,4-triazole and dimethyl-pyrazol-phosphate is not yet fully understood (McCarty 1999). As any chemical compound for plant protection, the application of nitrification inhibitors to farmlands should also be target-oriented, with minimal environmental side effects (Hauck 1980). Furthermore, when using nitrification inhibitors special attention should be paid to avoiding potential discharge of active ingredients and their metabolites to waterbodies. Particularly as some nitrification inhibitor compounds were recently detected in German surface waters (Scheurer et al. 2016).

Chapter 2

Increasing nutrient use efficiency from liquid manure

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

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Matthias Westerschulte contributed to the development of the experimental design, conduction of the field trials and samplings as well as the evaluation

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

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Keywords

Starter fertilizer · Nitrogen uptake · Nitrification inhibitor · Manure injection

Highlights

Manure injection below maize seeds improves nutrient availability during early growth
Adding a nitrification inhibitor obviates the need for mineral starter fertilizer
The resulting reduction of N- and P-input is beneficial for the environment

Article history

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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 broadcast 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.

Introduction

In northwestern Germany livestock husbandry traditionally has a major share in agricultural production. Long-term use of manures (often at rates higher than the phosphorous (P) demand of the crop) has led to P accumulation in many agricultural soils (Warnecke et al. 2010). Soil test values for P indicate no necessity for P fertilization on a large proportion of farmland in this area (Leinweber 1996). In the last decade biogas plants, producing energy from the digestion of manure and plant biomass came up in the region leading to a further increase in manure formation. As nitrogen (N) and P in manures on many farms outbalance crop nutrient demand by far, large amounts of manure have to be exported out of this region (Warnecke et al. 2010).

Maize (*Zea mays* L.) is the dominating crop in the area, used as fodder and substrate for biogas plants (Keckl, 2015). Low soil temperatures at maize planting typically limit bioavailability of certain nutrients (Barber 1995), as low root zone temperatures reduce the diffusion speed for P as well as nutrient acquisition due to reduced root growth (Imran et al. 2013). Ohlrogge (1962) showed a positive interaction of phosphate and ammonium applied in a band near maize seeds, when compared to the application of each nutrient alone. This interaction typically enhances crop early growth development and thus, farmers commonly apply a mineral starter fertilizer containing ammonium-N and water-soluble P. On farms with livestock, starters are commonly applied on top of the usual application of manure, which in most cases already covers N and P demand of the crop. This practice leads to N and P accumulations in the soil, which are then prone to leaching and runoff (Schröder et al. 2015; Touchton 1988; Withers et al. 2000). According to several studies from Europe (Petersen et al. 2010; Schröder et al. 2015) and North America (Beauchamp 1983; Chen et al. 2010; Sawyer et al. 1991; Schmitt et al. 1995) manure injection appears promising to ensure proper early growth development and high nutrient use efficiencies. Bittman et al. (2012) and Chen et al. (2010) showed the importance of planting maize close to the injection bands, as higher distances impair early development and finally yields when compared to placed injection or banded mineral starter fertilizer. This gain in crop performance is due to increased chemical and spatial nutrient availability. Furthermore, injection of liquid manure is an effective method to mitigate ammonia emissions (Sommer and Hutchings 2001), but N_2O emissions can be higher (Dosch and Gutser 1996).

Using nitrification inhibitors in crop production can contribute to minimize nitrogen losses to the environment, as they are able to reduce nitrate leaching, as well as N_2O emissions and finally increase the nitrogen use efficiency (Ruser and Schulz 2015). However, according to studies from McCormick et al. (1984) and Schmitt et al. (1995), yield response of maize to nitrification inhibitors added to spring injected manure is variable. Several soil and site properties (e.g. temperature, pH and organic matter) as well as management practices (placed injection versus soil incorporation) might impact the effectiveness of nitrification inhibitors (Bundy and Bremner 1973; Keeney 1980). Up to now most research was done with the compounds Nitrapyrin (McCormick et al. 1984; Schmitt et al. 1995), dicyandiamide (Amberger 1986) and Dimethylpyrazolphosphate (Zerulla et al. 2001).

McCarty and Bremner (1989) proved that both active ingredients (1,2,4 Triazol and 3-Methylpyrazol) of the product PIADIN® (SKW Piesteritz, Wittenberg, Germany) are able to inhibit nitrification separately. However, up to now only few publications on the use of this product exist (e.g. Misselbrook et al., 2014; Hu et al., 2013). A series of field trials was established to monitor the growth response of maize to liquid manure broadcast application versus injection. The importance of mineral starter fertilizer was in scope as well as the addition of a nitrification inhibitor. The primary objective of the study was to evaluate placed liquid manure application to obviate the use of mineral starter fertilizers and therefore reduce N and P inputs without impairing maize yield and quality.

Material & Methods

Sites

In 2013 and 2014, maize trials were conducted in cooperation with the extension service (i.e. the Chambers of Agriculture) in Lower Saxony (LS), North Rhine-Westphalia (NRW) and Schleswig-Holstein (SH, see Table 1 for details). Five sites (2; 4; 5; 6; 7) are located in regions with intensive animal farming on sandy soils. Due to long-term manure application, these soils are typically high to very high in soil P test levels (see Table 1). The Luvisols at sites 1 and 3 in cash cropping areas were medium to high in plant available soil P and did not receive high amounts of organic manure over the last decades.

Northwestern Germany is characterized by maritime climate. Mean annual air temperature at the study sites ranges from 8.6°C to 10.4°C from north to south, and mean air temperature from May to September ranges from 13.8°C to 15.4°C. Mean annual precipitation ranges from 742 mm to 880 mm (Ø 826 mm) with rainfall from May to September ranging from 323 mm to 376 mm (Ø 364 mm). In 2013, air temperature was 0.85°C above long-term average and May to September rainfall was 73 mm below average for NRW and LS sites, but 20 mm above for SH sites, with a lack of precipitation in July and August. Temperatures were rather low in April delaying planting of maize at LS and SH sites. In 2014, air temperature was 1.3°C above long-term mean with May to September precipitation rather close to average, but unusual rainfall distribution with high rainfalls in May and July. Despite of above-average air and soil temperatures in spring 2014 manure application and planting was delayed by two weeks at sites 4 and 6 due to unfavorable weather conditions. For further details, see Table 1.

Experimental design

The trials in 2013 were conducted using a complete randomized block design with four replicates and six treatments. The preliminary results demanded a more thorough investigation of the topic and thus in 2014 setup and treatments were adapted to a proper two-factorial setup. It was obvious to change to a two-factorial setup to distinguish the main effects of liquid manure application (LMA) from mineral side dressing (MSD). Therefore, in 2014 it was a split- plot design with four replications. The treatment factors were liquid manure application (LMA, main plot) and mineral side dress (MSD, subplot). Each plot in 2013, as well as each subplot in 2014 were 7 m in length, 3 m in width and consisted of four rows (75 cm row spacing).

Treatments

In 2013 the following treatments were conducted: (1) a control treatment without any fertilization (C-), (2) manure surface banding followed by immediate incorporation into the top 10 cm of soil with MSD (B+), (3) LM injection treatment without and with MSD (I- and I+, respectively) and (4) injection with the nitrification inhibitor 1,2,4 Triazol and 3-Methylpyrazol (PIADIN®, SKW Piesteritz, Wittenberg, Germany) at a rate of 3 l ha^{-1}, also without and with MSD (I(N)- and I(N)+, respectively). For all injection treatments, the top of the manure band was placed 12 cm below the soil surface. The injectors used (Kotte Premaister (Kotte Landtechnik, Rieste, Germany) in NRW and Vogelsang X-Till (Vogelsang Maschinenbau, Essen (Oldb.), Germany) for LS and SH sites) both had four injection shares placed 75 cm apart and were equipped each with a 1000 l slurry tank, a rotary piston pump, and a precision dispenser to provide proper lateral and longitudinal distribution.

Maize was later planted directly above the manure band. Planting density was set to nine plants per m^2 on all sites. The planters had extra shares to place MSD next to the maize row. The MSD treatments were (1) no MSD (-) and (2) with MSD (+) using a blend of calcium ammonium nitrate (CAN, 50 kg ha^{-1}) and diammonium phosphate (50 kg ha^{-1}) to apply 23 kg N ha^{-1} and 10 kg P ha^{-1}. For "no MSD" -plots N-compensation of 23 kg N ha^{-1} of CAN was broadcast post-planting to keep nitrogen levels consistent. The nitrogen fertilization rate was calculated according to local standards (Baumgärtel et al. 2010). The recommended nitrogen fertilization rate is 180 kg N ha^{-1} reduced by preplant soil mineral nitrogen (SMN), N applied via MSD and site-specific conditions like recent organic fertilizer application and catch cropping. Application rates, liquid manure composition and applied nutrients are displayed in Table 2.

In 2014, the treatments for the factor LMA were: (1) control treatment without LM; (2) LM broadcast treatment (B) with surface banding followed by soil incorporation (0-10 cm), (3) LM injection treatment (I) and (4) injection with the nitrification inhibitor (1,2,4 Triazol and 3-Methylpyrazol, PIADIN®, SKW Piesteritz, Wittenberg, Germany) at a rate of 3 l ha^{-1} (I(N)). While for B, I and I(N) treatments the manure application rate was identically (further referred to 100%), another set of I and I(N) treatments was installed with reduced manure rates (66%; Ir and Ir(N), respectively). MSD treatments and manure application rates were as described for 2013 trials.

Crop management practices

At each site crop management was done according to best management practice guidelines for all treatments equally. Different maize varieties had to be chosen to face diverse soil and climate conditions (for more details see Table 3).

Measurements and samplings

In 2014, when the collar of 8th leaf was visible at 50% of the plants (V8-stage) aboveground biomass was collected at six sites (not site 3) to monitor early growth development in the treatments C, B, I and I(N). In the outer rows of each sub-plot, ten plants per plot were cut at stem base, chopped and dried to a constant weight at 80°C. Nitrogen content was determined with the Kjeldahl method (DIN 2005). At silage maturity, in both years, the two center rows of each subplot were harvested with a plot-size field chopper. Fresh weight was measured and a representative sample was taken to determine dry matter content (drying to constant weight) and protein content via near-infrared spectroscopy (DIN 2010).

Calculations

Based on dry matter yield and N concentrations N uptake and nitrogen recovery efficiency (NRE) were calculated according to (Ciampitti and Vyn 2011): nitrogen uptake of the respective treatment minus N uptake of control treatment, divided by the difference in N fertilization for each site:

$$NRE=\frac{N\ uptake_{fert.}\text{-}N\ uptake_{unfert.}}{\Delta\ N\ applied}$$

with N uptakefert. being the N uptake of the respective treatment, N uptakeunfert. being the N uptake of control treatment without MSD of the respective site and Δ N applied MSD being the difference in N fertilization. Furthermore, the N balance was calculated by the subtraction of applied N from N uptake for each site and treatment. The calculations for NRE and N balance are based on total N applied, as the prediction of plant available N from liquid manure is difficult (Gutser et al. 2005).

Table 1: Location, climate and soil properties at the study sites.

State	North-Rhine Westphalia				Lower Saxony						Schleswig-Holstein		
Site	1		2		3		4		5		6	7	
	Haus Düsse		Merfeld		Poppenburg		Sandkrug		Wehnen		Bovenau	Schuby	
Latitude	51°38' N		51°51' N		52°08' N		53°05' N		53°10' N		54°19' N	54°31' N	
Longitude	08°11' E		07°12' E		09°46' E		08°16' E		08°08' E		09°48' E	09°26' E	
Mean annual temp. (°C)*	9.8		10.4		9.6		9.6		9.5		8.9	8.6	
Mean annual precip. (mm)*	836		880		742		841		823		847	844	
Mean temp. May to Sept (°C)*	15.0		15.4		14.8		14.7		14.6		14.0	13.8	
Mean precip. May to Sept (mm)*	376		378		323		376		386		375	372	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2014	2013	2014
Mean Temp. May to Sept (°C)	15.8	16.0	16.0	16.0	15.9	16.0	15.3	16.0	15.3	16.0	15.8	15.2	15.8
Precip. May to Sept (mm)	299	384	295	442	284	362	295	344	295	344	325	379	336
Soil type[a]	LV-ha	LV-ha	LV-gl	PZ-pi	LV-ha	LV-ha	PZ-ha	PZ-ha	PZ-gl	PZ-gl	LV-gl	PZ-ha	PZ-ha
pH ($CaCl_2$)	6.6	6.8	6.3	5.0	6.9	6.7	5.5	5.3	5.4	4.8	6.4	5.5	5.4
SMN 0-60 cm (kg ha^{-1})	47	15	16	15	46	80	15	23	32	13	68	29	23
P CAL 0-30 cm (mg 100 g^{-1})	7	7	12	10	10	9	10	11	11	8	15	12	14

Location, climate and soil properties at the study sites.

[a]Soil type with cm= cambic; gl= gleyic; ha= haplic; LV= Luvisol; PZ= Podzol (IUSS Working Group WRB 2014)

*Long term climatic data (1980-2010)

SMN= soil mineral nitrogen

Table 2: Manure properties and applied nutrients from manure for all trials and treatments.

Site	Manure properties					Applied nutrients (B, I, I(N))*		Applied nutrients (Ir, Ir(N))*	
	Manure type	DM (%)	N (g kg^{-1})	NH_4-N (g kg^{-1})	P (g kg^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})
	2013								
1	Pig	2.9	3.6	2.8	0.6	80	13		
2	Pig	6.5	5.4	4.6	2.0	118	43		
3	Digestate	8.8	5.1	2.1	1.0	157	31		
4	Digestate	11.1	6.7	3.6	2.3	202	68		
5	Pig	3.0	3.7	2.6	0.5	178	23		
7	Digestate	3.9	2.6	1.5	0.3	182	23		
	2014								
1	Pig	4.8	6.0	4.7	1.2	143	29	96	20
2	Pig	7.7	7.1	5.2	2.0	178	50	117	33
3	Digestate	9.7	5.8	2.7	1.0	121	21	80	14
4	Digestate	8.0	7.1	3.5	1.4	164	33	104	21
5	Pig	2.5	4.1	3.6	0.5	180	23	119	15
6	Digestate	6.0	4.7	1.8	0.8	207	33	141	23
7	Digestate	4.5	3.2	1.8	0.3	164	17	107	11

* B= manure broadcast, I= manure injection, I(N)= manure injection with nitrification inhibitor, Ir= manure injection at reduced rate, Ir(N)= manure injection with nitrification inhibitor at reduced rate

Table 3: Tillage and cropping details

Site	Previous crop	Primary tillage[a]	Slurry application	Variety	Planting date	V8 sampling	Harvest date
	2013						
1	Wheat	spring RT	Apr. 16	LG 3216	Apr. 25	-	Oct. 07
2	Maize	spring MP	Apr. 15	LG 3216	Apr. 16	-	Sep. 22
3	Sugar beet	autumn MP	Apr. 24	LG 30222	Apr. 29	-	Oct. 09
4	Maize	spring RT	May 07	LG 30222	May 08	-	Oct. 07
5	Triticale	spring MP	May 07	LG 30222	May 10	-	Oct. 09
7	Maize	spring MP	Apr. 27	Amadeo	May 09	-	Oct. 08
	2014						
1	Sugar beet	autumn MP	Apr. 10	LG 30224	Apr. 23	June 14	Oct. 06
2	Triticale	spring MP	Apr. 09	LG 30224	Apr. 16	June 13	Sep. 20
3	Wheat	autumn MP	Apr. 24	LG 30222	Apr. 29	-	Sep. 18
4	Maize	spring RT	May 05	LG 30222	May 17	June 19	Oct. 02
5	Triticale	spring MP	Apr. 28	LG 30222	Apr. 30	June 19	Oct. 02
6	Wheat	spring MP	Apr. 29	LG 30211	May 15	June 25	Oct. 10
7	Maize	spring MP	Apr. 22	LG 30211	May 06	June 24	Oct. 01

[a] Primary tillage with RT = reduced tillage with a chisel plough, MP = Mouldboard plough

Statistical analysis

All data was analyzed using PROC MIXED in SAS 9.3 (SAS Institute Inc. 2011). For 2013 the model was LMA SITE LMA*SITE with R(SITE) as random factor (LMA = treatment; R = replication). The Model for the split- plot design in 2014 was SITE LMA LMA*SITE MSD MSD*SITE LMA*MSD LMA*MSD*SITE, random factors were LMA*R(SITE) R(SITE). As the site properties were very heterogeneous and therefore could not represent a certain cropping area, sites had to be considered as fixed effect. Means of treatments were compared using the Tukey procedure for fixed effects when significant differences at P<0.05 occurred. Due to a lack of variance homogeneity data of site 6 in 2013 had to be discarded.

Results

Early growth development 2014

In 2014 at V8 stage aboveground plant biomass and N uptake were significantly increased (+28% for biomass and +27% for N uptake, respectively) when MSD was applied (Figure 1). LMA also had significant effects on both biomass and N uptake. Compared to B treatment aboveground biomass was significantly lower for C (-14%), but 11% and 19% higher for I and I(N) treatments, respectively. For N uptake, the differences were even bigger (-16% for C, +16% for I and +27% for I(N) treatment, respectively). Compared to B+ treatment, biomass was slightly decreased for I(N)- (-0.04 Mg ha^{-1}), but significantly for I- treatment (-0.1 Mg ha^{-1}). N uptake did not vary among these treatments (Table 6).

Dry matter yield at harvest

In both seasons LMA and site*LMA influenced maize dry matter production. There was an influence of MSD treatments as well in 2014 (Table 4). While the site*LMA interaction in 2014 is based on the site impact on C treatment and therefore insignificant when C treatment is excluded from the model, in 2013 biomass production was inconsistent for fertilized treatments among sites, especially on the sites with lower yields.

Table 4: Maize aboveground biomass and N uptake as affected by site and LMA treatments for 2013.

Treatments	Aboveground biomass								N uptake							
Sites[a]	mean		1	2	3	4	5	7	mean		1	2	3	4	5	7
LMA[b]	<.0001		0.0113	<.0001	0.0091	<.0001	0.0005	0.0001	<.0001		0.0035	<.0001	0.0004	<.0001	0.0001	<.0001
	Mg ha^{-1}								kg ha^{-1}							
C-	13.8	c[c]	15.1	15.9	18.6	8.9	14.2	10.2	134	d	156	129	192	82	148	93
B+	18.1	b	17.1	20.3	21.0	17.0	17.5	15.6	195	c	196	213	241	156	203	158
I-	18.3	ab	16.4	20.1	21.0	16.7	18.1	17.6	203	bc	196	215	253	166	211	170
I(N)-	18.8	ab	17.0	20.8	21.4	16.9	18.3	17.5	209	ab	209	216	249	183	215	182
I+	18.6	ab	17.5	19.8	21.3	18.0	18.4	17.5	209	ab	201	228	257	165	216	179
I(N)+	19.2	a	17.7	20.3	21.3	18.4	19.5	17.9	216	a	212	221	255	189	231	190

[a] Sites as indicated in Table 1

[b] LMA treatments fertilized with liquid manure (C = no manure, B = manure broadcast, I = manure injection, I(N) = manure injection with nitrification inhibitor) as indicated in Table 2, MSD treatments - = without mineral starter fertilizer, + = with mineral starter fertilizer

[c] different letters indicate significant differences (P<0.05)

Table 5 is on the following page

Table 5: Maize aboveground biomass and N uptake at V8 stage and harvest as affected by site and LMA*MSD treatments for 2014 data

		Aboveground biomass									N uptake								
Sites^a		mean		1	2	3	4	5	6	7	mean		1	2	3	4	5	6	7
		V8 Stage																	
LMA^b		<.0001		0.0002	0.0003		<.0001	0.004	0.0173	0.0171	<.0001		<.0001	0.000		<.0001	0.003	0.003	0.001
MSD		<.0001		<.0001	0.0010		<.0001	<.0001	<.0001	0.002	<.0001		<.0001	0.001		<.0001	<.0001	<.0001	0.002
LMA*MSD		0.424		0.008	0.632		0.670	0.226	0.559	0.232	0.493		0.006	0.616		0.659	0.238	0.673	0.191
LMA	MSD	Mg ha^{-1}									kg ha^{-1}								
C	-	0.49	e^c	0.28	0.66		0.39	0.32	0.71	0.57	19.5	f	10.7	24.4		16.3	12.5	31.3	21.2
C	+	0.70	c	0.44	0.97		0.44	0.54	1.02	0.78	27.6	d	17.4	35.4		18.9	22.0	42.2	29.3
B	-	0.60	d	0.35	0.95		0.49	0.29	0.76	0.77	24.3	e	13.6	35.1		21.9	11.0	33.0	31.1
B	+	0.79	b	0.54	1.13		0.58	0.61	1.00	0.86	31.7	c	21.3	41.9		26.2	24.4	41.8	34.6
I	-	0.69	c	0.60	1.06		0.57	0.38	0.49	0.77	29.5	cd	26.1	41.2		26.7	15.8	34.2	32.7
I	+	0.85	ab	0.74	1.20		0.64	0.70	0.96	0.82	35.5	b	31.8	45.8		29.8	29.0	41.7	34.6
I(N)	-	0.75	bc	0.69	1.09		0.55	0.50	0.94	0.70	32.0	c	29.6	42.6		25.0	20.8	42.9	31.0
I(N)	+	0.91	a	0.70	1.30		0.63	0.68	1.18	0.96	39.0	a	30.2	52.1		29.0	28.2	52.5	41.8
		Harvest																	
LMA		<.0001		<.0001	<.0001	0.013	<.0001	0.006	<.0001	<.0001	<.0001		0.003	0.001	0.000	<.0001	0.011	<.0001	<.0001
MSD		<.0001		<.0001	<.0001	0.005	0.106	0.259	0.023	0.630	0.000		0.001	0.006	0.010	0.084	0.732	0.483	0.287
LMA*MSD		0.282		0.360	0.930	0.547	0.157	0.866	0.413	0.258	0.809		0.143	0.707	0.594	0.242	0.415	0.908	0.352
LMA	MSD	Mg ha^{-1}									kg ha^{-1}								
C	-	18.1	e	19.8	17.8	19.9	13.3	21.5	21.1	13.5	188	e	225	189	204	130	264	204	98
C	+	18.8	e	21.8	19.0	20.4	12.7	22.1	21.8	14.0	195	e	227	205	230	132	265	214	92
B	-	20.6	d	21.1	19.7	20.1	19.0	23.4	23.4	16.8	225	d	234	220	226	190	297	257	147
B	+	21.4	abcd	22.4	20.8	21.9	20.1	23.0	24.7	16.3	232	cd	256	225	244	184	274	261	173
I	-	21.4	abcd	22.8	21.2	20.9	20.5	23.6	24.6	16.5	239	abc	248	233	243	195	292	283	181
I	+	22.0	ab	23.3	21.9	22.2	21.6	24.0	24.8	16.0	245	ab	262	244	245	184	299	287	194
I(N)	-	21.8	abc	22.7	21.5	21.7	21.5	23.5	25.0	16.8	246	a	259	237	246	198	288	280	213
I(N)	+	22.1	a	23.5	22.0	22.2	21.3	23.8	25.1	17.0	249	a	263	237	253	207	292	276	212
Ir	-	20.6	d	22.1	20.0	21.3	19.1	23.5	23.2	15.2	224	d	246	210	235	171	288	264	151
Ir	+	21.5	abc	23.6	20.7	21.9	19.9	23.8	24.4	16.0	233	bcd	255	222	261	169	292	271	162
Ir(N)	-	21.1	cd	22.3	20.3	21.5	20.2	22.0	23.8	17.1	228	cd	250	218	238	192	263	259	174
Ir(N)	+	21.2	bcd	22.3	21.1	21.7	20.3	23.1	23.7	16.0	232	cd	257	229	242	177	282	255	175

Table 6: Comparison, based on the PROC MIXED model, of maize aboveground biomass production (DM) and aboveground N uptake for different methods of liquid manure application (LMA; no LM, LM broadcast, LM injection at two rates, LM injection with nitrification inhibitor at two rates) with and without mineral starter fertilizer (23 kg N ha^{-1} and 10 kg P ha^{-1}), at harvest on six sites in 2013 and at V8 stage and harvest date at seven sites in 2014, respectively.

	2013		2014			
	Harvest		V8		Harvest	
	DM	N uptake	DM	N uptake	DM	N uptake
Site	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
LMA	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Site*LMA	<.0001	0.084	<.0001	<.0001	<.0001	<.0001
MSD			<.0001	<.0001	<.0001	0.000
Site*MSD			0.000	0.000	0.065	0.044
LMA*MSD			0.424	0.493	0.282	0.809
Site*LMA*MSD			0.213	0.141	0.732	0.356
	C Treatment excluded^{a}					
Site	0.003	0.0001	<.0001	<.0001	<.0001	<.0001
LMA	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Site*LMA	0.357	0.933	0.005	0.003	0.310	<.0001
MSD			<.0001	<.0001	<.0001	0.001
Site*MSD			0.005	0.005	0.107	0.054
LMA*MSD			0.641	0.607	0.183	0.666
Site*LMA*MSD			0.212	0.110	0.840	0.306

[a] Separate test with C treatment excluded to check whether highly variable performance of C treatment within sites for yield and N uptake is responsible for significant interactions of site*LMA.

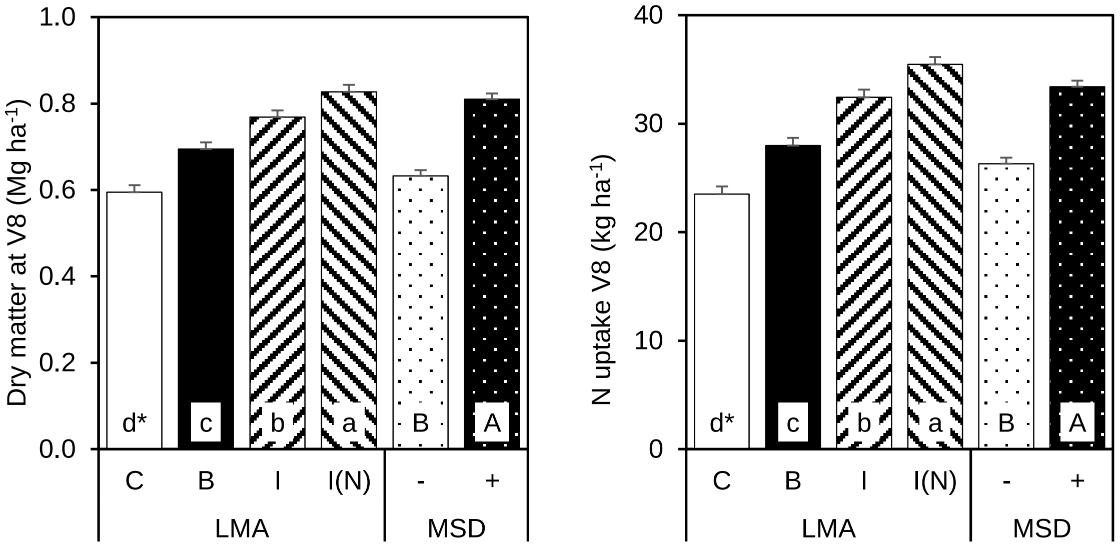

Figure 1: Aboveground dry matter and nitrogen uptake of maize at V8 stage, means and SE for six sites with four replications each in 2014, fertilized with liquid manure (LMA: C = no manure, B = manure broadcast, I = manure injection, I(N) = manure injection with nitrification inhibitor) and mineral side dress (-MSD without and +MSD with mineral side dress of 23 kg N ha^{-1} and 10 kg P ha^{-1})

* Different letters for treatments within LMA and MSD factors indicate significant differences (Tukey $p<0.05$)

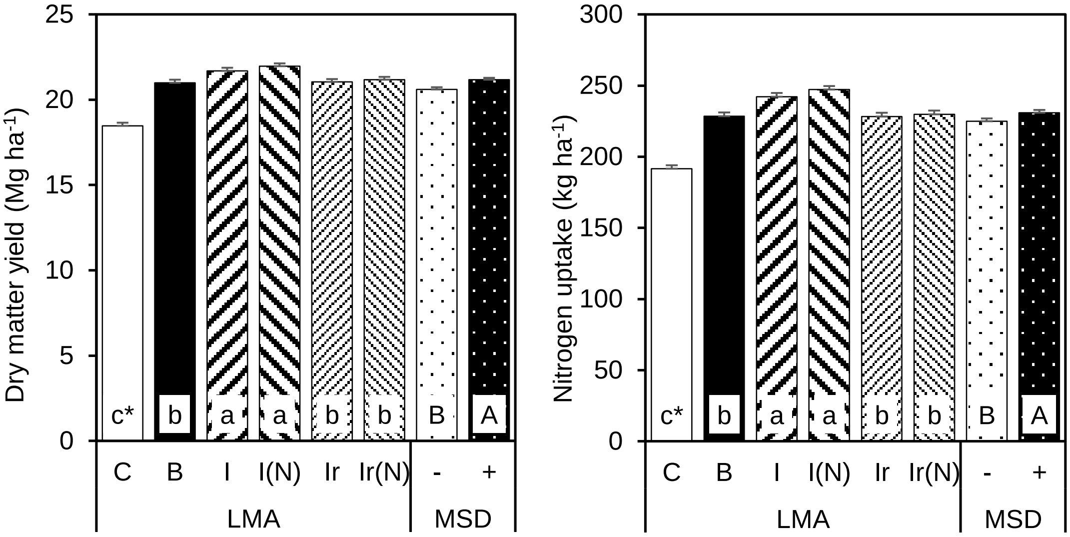

Figure 2: Aboveground dry matter (DM) and nitrogen uptake of maize at harvest, means and SE for seven sites with four replications each in 2014. Fertilization with liquid manure (LMA: C = no manure, B = manure broadcast, I = manure injection, I(N) = manure injection with nitrification inhibitor, Ir = I treatment with reduced manure rate, Ir(N) = I(N) treatment with reduced manure rate) and mineral side dress (-MSD without and +MSD with mineral side dress of 23 kg N ha^{-1} and 10 kg P ha^{-1})

* Different letters for treatments within LMA and MSD factors indicate significant differences (Tukey $p<0.05$)

Maize dry matter yield mean of all sites and treatments was 17.8 Mg ha^{-1} in the 2013 season and 20.9 Mg ha^{-1} in 2014, respectively. Only on site 7 yield was lower in 2014 compared to 2013, while all other sites showed higher yields in 2014. The sites 2 and 3 showed consistent yields over both seasons, which were the highest yields among sites in 2013. In 2014, at sites 1 and 5 maize biomass production was highest, contrasting the 2013 results. The seasonal difference in average yield among sites for biomass production of C- treatment was +4.3 Mg ha^{-1} in 2014 compared to 2013. Mean yield increase of all fertilized treatments, compared to C- treatment, was 4.8 Mg ha^{-1} in 2013 and 3 Mg ha^{-1} in 2014, respectively. Compared to B+ treatment both I- and I(N)- treatments yields averaged over sites were equal in 2013 (+200 kg ha^{-1} for I- and +700 kg ha^{-1} for I(N)-), and in 2014 (+-0 kg ha^{-1} for I- and +400 kg ha^{-1} for I(N)-), as none of these differences was significant (Table 5, 6). Compared to I and Ir treatments the yield difference between −MSD and +MSD for I(N) and Ir(N) treatments is smaller. The performance of treatments with reduced slurry rate injected (Ir and Ir(N)) was equal to B+ treatment for both seasons (Figure 2). Mean biomass accumulation of injection treatments was 20.1 Mg ha^{-1}, while for injection treatments with nitrification inhibitor it was 20.5 Mg ha^{-1}, with a higher difference in the 2013, and a lower in 2014 season.

N uptake

In contrast to biomass production N uptake within fertilized treatments was very consistent in 2013, without significant interaction of LMA*site, while in 2014 there was a variable impact of site*LMA on N uptake, especially on the sites 1 and 5.

Mean N uptake was 194 kg ha^{-1} in 2013 and 228 kg ha^{-1} in 2014, respectively. For nitrogen uptake, the difference between C treatment and fertilized treatments was 72 kg ha^{-1} in 2013 and 45 kg ha^{-1} in 2014, respectively. Nevertheless, N uptake for I(N)- treatment was significantly higher than B+ (+14 kg ha^{-1} in both seasons), with I- treatment in between (+8 kg ha^{-1} in 2013 (Table 5) and +7 kg ha^{-1} in 2014 (Table 6)). As for biomass production, the reduced rate treatments showed no difference to B treatments, and the addition of a nitrification inhibitor increased the nitrogen uptake by 4.5 kg ha^{-1} compared to the injection treatments without nitrification inhibitor. The difference in 2013 also was higher than in the 2014 season.

Nitrogen recovery efficiency (NRE) and N balance

While in 2013 mean NRE for all treatments was 55% (data not shown), in 2014 it was 36%. Some of the sites (4, 7, 3) showed consistent NREs of 40%-50%, while other sites (1, 2) showed higher NREs in 2013 (>70%) and lower in 2014 (<30%). I and I(N) treatments NRE (38% and 42%, respectively) were higher than NRE of B treatment (31%, see Figure 3). Reducing manure application rate led to a further increase of NRE for Ir and Ir(N) treatments (45% and 48%, respectively).

Nitrogen balances were negative for all treatments but not all sites. The sites 4 and 7 showed positive N balances in 2013, at site 7 in 2014 the B treatments also showed positive values. Considering the effect of treatments, the C, Ir and Ir(N) treatments resulted in more negative balances (-157 kg N ha^{-1}, -84 kg N ha^{-1} and -88 kg N ha^{-1}, respectively), than the higher fertilization rate treatments B, I and I(N) (-32 kg N ha^{-1}; -44 kg N ha^{-1} and -50 kg N ha^{-1}, respectively).

Discussion

Early-growth development

Due to visible symptoms of delayed early growth in treatments without MSD, compared to treatments with MSD, in 2013 plant samples were collected at V8 growing stage in 2014. Dry matter (DM) accumulation at V8 stage was significantly enhanced by manure injection, compared to broadcast application of liquid manure, confirming other studies (Schmitt et al. 1995; Schröder et al. 2015).

The addition of a nitrification inhibitor led to even more DM accumulation at this growth stage. This contrasts the findings by Sawyer et al. (1991) as they mention an inconsistent response of the plant to nitrification inhibitors. This impact of the nitrification inhibitor is consistent among sites for this set of trials. While, at least on the loam sites, leaching did not occur and N_2O emissions usually do not reach levels to limit plant available N (Ruser and Schulz 2015), site and soil characteristics could be excluded as reason.

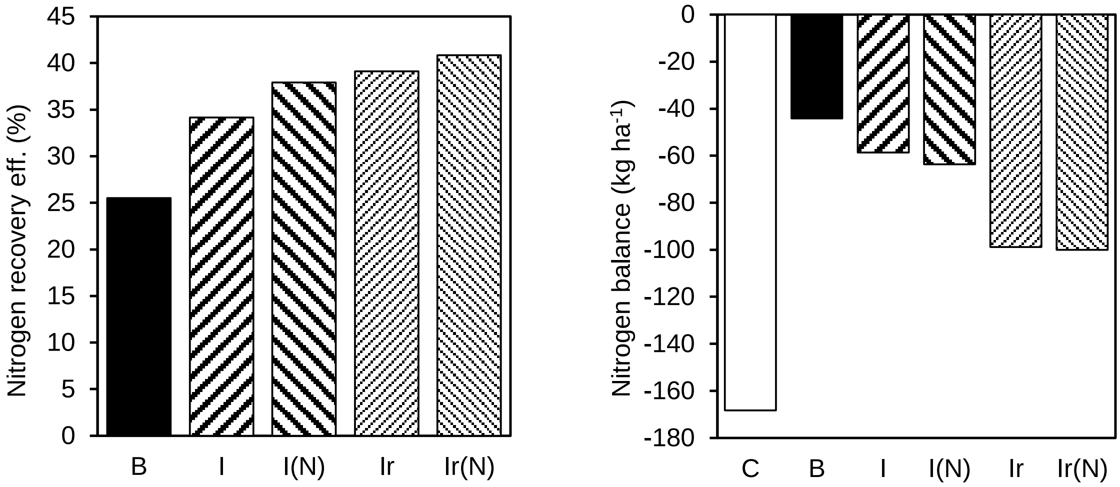

Figure 3: Nitrogen recovery efficiency (left) and nitrogen balance (right) calculated from data of 7 trials in 2014. Fertilization with liquid manure (C= no manure, B= manure broadcast, I= manure injection, I(N)= manure injection with nitrification inhibitor, Ir= manure injection at reduced rate, Ir(N)= manure injection with nitrification inhibitor at reduced rate.

Thus, the nutrient use efficiency from manure must be somehow enhanced by the addition of a nitrification inhibitor, which is probably due to the interaction of ammonium and phosphate in the manure band (Ohlrogge 1962).

Significant Site*LMA interactions are based on variable soil properties. A high P soil test level along with high SMN content at site 6 has led to comparably high biomass accumulation for C treatment without MSD and therefore to a low fertilizer response of the other treatments.

Mean DM of fertilized treatments was only 32% higher than of C treatment at this site. However, at site 1, where SMN and soil P test level, but also N applied via liquid manure was lower, it was 110% higher. As site 4 shows similar response as site 6 and site 2 as site 1, fertilizer response is probably also influenced by latitude and planting date as both affect day length, solar radiation, air and soil temperatures. Mean day length and soil temperatures (10 cm below surface) in the three weeks after planting were 14 h 32 min and 14.1 °C at site 1 and 16 h 08 min and 17.9 °C at site 6, respectively (see Table 1).

Photoperiod until tassel initiation influences total leaf number (Ellis et al. 1992), even though the effect is smaller for maize varieties used in colder climates and higher latitudes (Bonhomme et al. 1991). Tsimba et al. (2013) observed higher crop growth rates from emergence to tasseling, when maize is exposed to higher solar radiation, air and soil temperatures.

Additionally, the use of varieties with different maturity ratings on these respective sites, could account for the low fertilizer response, as varieties with higher chilling tolerance, as used at site 6 tend to produce a higher leaf area at the same thermal time than ones with lower chilling tolerance (Birch et al. 2003).

Furthermore, the higher soil temperatures at site 6 probably improved P availability (Imran et al. 2013), as well as the mineralization of organic nitrogen (Guntiñas et al. 2012).

At V8, the addition of MSD increased DM accumulation for all treatments. Crop development of plants fertilized with injected manure, and nitrification inhibitor did not lag behind broadcast application with MSD and early growth development is not retarded. Thus, the biggest concern of farmers (an unhealthy looking crop that might lead to yield loss (Withers et al. 2000)), is no longer valid, at least for the range of soils tested in this series of experiments. Compared to B treatment, N uptake was significantly enhanced in injection treatments, indicating a higher proportion of plant available nitrogen (Dosch and Gutser 1996). N uptake for I and I(N) treatments without MSD were equal to B treatment with MSD, while biomass production was lower. Thus, either poor P availability restricted plant growth, and/or N uptake was lower in very early growth stages (Sawyer and Hoeft 1990), but increased up to V8 stage. However, placing the manure band close to the seeds and a better understanding of nutrient transformation from manures in the soil could solve the problems with retarded early growth (Sawyer et al. 1991).

Harvest data

While we observed major differences at V8 in 2014, at harvest much of the differences were gone. Yet, still plants in I and I(N) treatments produced higher biomass than plants in B treatments and reduced rate treatments yields were equal to B. Comparing B treatment with MSD, I and I(N) treatments without MSD, yield differences were not significant.

As for early growth development Site*LMA interactions depend on differences in soil properties. Looking at the sites 4 and 5, these differences are most notably, as the fertilizer response is very different. While on site 5 fertilization (mean of fertilized treatments) did only improve yield by 8% in 2014, on site 4 it was improved by 50%, when compared to C treatment without MSD.

The addition of MSD did only increase DM yield by 2% in 2013 and 3% in 2014, respectively, despite the considerable effect on early growth. As the main influence of MSD is only seen in early growth stages and not on yield, farmers having nutrient surpluses can easily go without MSD but probably will not want to risk an unhealthy looking crop, which might lead to yield loss (Schröder et al. 2015; Withers et al. 2000). In the 2014 season I and I(N) treatments increased N uptake, compared to B treatment (+6% and +8%, respectively), while reduced rate treatments were at level. These observations go along with other studies (Schröder et al. 2015) and show the higher nitrogen use efficiency from injected manure.

Reasons for this increase in nitrogen availability may be:

a) reduction of ammonia losses, despite immediate incorporation after surface application of manure in B treatments (Sommer and Hutchings 2001),
b) reduced nitrogen immobilization when manure is injected in a band due to a reduced soil manure interaction (Sørensen and Amato 2002),
c) the addition of a nitrification inhibitor resulting in a mitigation of nitrate leaching and N_2O emissions (Ruser and Schulz 2015).

High variations for N uptake of C treatment between sites did not match the respective V8 data, e.g. at site 6 C treatment showed highest N uptake at V8 and site 1 showed the lowest, at harvest N uptake of C treatment at site 1 was higher.

The low NRE on sites 1 and 2 in 2014 contrast the high fertilizer response at V8, which could be the result of a higher fertilization rate compared to 2013 on the respective sites. Injection treatments however showed higher NRE as N uptake was higher. As expected the highest NRE were calculated for reduced rate treatments.

Nitrogen balances in this set of trials were mainly negative for all fertilized treatments. Sites 4 and 7, showing the lowest yields, however had positive N balances. Yet, still lower yields for reduced rate treatments occurred and NRE's are comparable to other sites, indicate fertilization rates being yield limiting. Lower nitrogen balances reduce the potential of NO_3^- leaching and thus, are beneficial for groundwater resources.

When N uptake by crops is higher than the fertilization rate, nitrogen balances are negative, indicating high mineralization rates from soil organic N. So, soil organic N is being depleted when maize is cropped with a high NUE from manure. This depletion, in long term can decrease N mineralization and therefore lacks sustainability. Thus, soil organic N has to be recovered based on a proper crop rotation containing e.g. catch crops, or legumes.

Conclusion

When liquid manure is injected below maize rows, on a wide range of sites across northwestern Germany the present results indicate no need for additional mineral side dress fertilizer. At harvest, there are no differences in crop yield, but manure injection enhances N uptake, especially when a nitrification inhibitor is added to the injected manure.

However, manure injection without mineral starter fertilizer might lead to retarded early growth, which can be prevented by accurate placement close to the seeds and by the addition of a nitrification inhibitor. Still, based on the present results complex interactions do not allow a final evaluation for the benefit of a nitrification inhibitor. Further research in this area needs combined data on nitrogen transformation in the soil, leaching, plant performance, as well as nitrogen losses due to volatilization and denitrification.

With the potential to reduce nitrogen and phosphor fertilization without impairing crop yield, manure injection can be a strategy to mitigate nutrient surpluses in maize growing and thus being beneficial for the environment.

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2.2 Nitrogen dynamics following slurry injection in maize: crop development

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Matthias Westerschulte contributed to the development of the experimental design, conduction of the field trials and samplings as well as the evaluation

Hans-Werner Olfs supervised Carl-Philipp Federolf

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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.

Introduction

Agriculture in northwestern Germany is traditionally dominated by intensive animal farming. Excessive use of organic manures causes phosphorus (P) accumulation in soils resulting in high soil test values for P in the region indicating only limited need for P fertilization on a large proportion of arable land (Leinweber et al. 1994). Furthermore nitrogen (N) and P from manures outbalance crop nutrient demand on many fields in the region and huge amounts of manure have to be exported (Warnecke et al. 2011). Maize (*Zea mays* L.), used as fodder and substrate for biogas plants is the dominating crop in the region (Keckl 2015).

Despite high soil test levels for P, plant availability of P typically is low in early growth stages of maize as low root zone temperatures reduce P diffusion speed (Imran et al. 2013) and restricted root growth restricts spatial nutrient acquisition (Mollier and Pellerin 1999). Limited P supply usually also impairs crop growth, as it reduces leaf appearance, leaf elongation and final leaf size (Plénet et al. 2000a) and thus, also aboveground biomass (Plénet et al. 2000b). In contrast, Muchow (1988) showed only minor effects of N deficiency on biomass accumulation at very early growth stages of maize. Although N concentrations in leaves can be reduced by 50%, leaf appearance, leaf elongation and final leaf area show only limited reactions to a wide range of N fertilization levels (Vos et al. 2005). Plénet and Lemaire (1999) also did not find major differences in early growth under N limitation, but N concentrations in maize should not fall below a critical value of 3.4% N, if aboveground biomass is below 1 Mg ha^{-1} to obtain maximum yields.

To enhance early growth development and ensure adequate yields by assuring optimum levels of N and P in plants, farmers commonly apply a mineral starter fertilizer (MSF) at planting (Withers et al. 2000). The combination of ammonium N and P proved most effective in several studies (e.g. Ma et al. 2013, Ohlrogge 1962) as both, lateral root proliferation and fine root proliferation are enhanced in zones where high concentrations of ammonium N and P occur (Ma et al. 2013). Additionally, plant uptake of ammonium N induces lower rhizosphere pH, which can increase P availability (Neumann and Römheld 2012). Thus, farmers commonly use starters containing both ammonium N and P (e.g. diammonium phosphate, or blends of calcium ammonium nitrate with diammonium phosphate). On intensive animal farms, these starters are applied in addition to the broadcast application of manure, which usually already covers N and P demand of the crop. This practice results in accumulations of N and P in the soils increasing the risk of nutrient leaching and runoff (Touchton 1988). Especially nitrate leaching is a major problem on sandy soils (Cameron et al. 2013), which are common in northwestern Germany. Reducing reactive N emissions, such as nitrate leaching and N_2O emissions, is a vital task for mankind (Sutton et al. 2011) and a goal of the European Union's water framework directive (European Parliament 2000).

Obviating MSF by slurry injection in a band close to the maize rows was tested in several studies throughout North America (Bittman et al. 2012; Sawyer et al. 1991; Schmitt et al. 1995), Denmark (Petersen et al. 2010), and the Netherlands (Schröder et al. 1997; Schröder et al. 2015). A series of trials in northwestern Germany showed the potential of manure injection to improve nutrient use efficiencies, when compared to broadcast application (Federolf et al. 2016). This might be due to higher proportions of plant available N when manure is injected. Compared to broadcast application of liquid manure, Sommer and Hutchings (2001) refer to reduced ammonia emissions, while Sørensen and Amato (2002) indicate reduced N immobilization as the interaction of soil and manure is lower. Higher soil mineral nitrogen (SMN) concentrations however, might increase denitrification losses (Cameron et al. 2013; Dosch and Gutser 1996). When a nitrification inhibitor (NI) is mixed into the manure prior to application, nitrification of the applied ammonium N from manure is retarded, and thus leads to lower leaching and denitrification losses (Ruser and Schulz 2015). While our previous study (Federolf et al. 2016), showed enhanced early growth when adding a NI, Sawyer et al. (1991) observed inconsistent increases in plant N content at V5 and V6 growth stages, when NIs were added to liquid beef manure. Along with these studies, Schmitt et al. (1995) reported varying effects on yields. Thus, further knowledge on N transformation in the soil after application of liquid manure (LM) with a high spatial resolution and the consequences for crop development is necessary (Westerschulte et al. 2015). Consequently, a field trial was established to monitor SMN dynamics following liquid manure injection, as well as plant growth during maize vegetation in 2014 and 2015. Besides manure injection treatments with and without NI, an unfertilized control, and a local standard treatment where liquid manure was surface banded and incorporated was tested. While plots in the local standard treatment received MSF at planting, no further fertilization was applied to injection treatment plots.

The objective of our study was to compare liquid pig manure injection versus broadcast application in terms of the consequences on plant nutrient acquisition, focusing on obviating the addition of mineral starter fertilizer by slurry injection. Our study is based on the hypotheses that after injection of liquid manure, compared to broadcast application plus MSF, plant availability of nutrients is higher, leading to comparable early growth and equal yields with reduced nutrient input. High manure N concentrations in the injection zones delay turnover of the applied ammonium due to reduced soil-manure interaction (Dosch and Gutser 1996) and thus, reduce the risk of N translocation out of the root zone. We assume that the addition of a nitrification inhibitor to the slurry delays nitrification thereby enhancing ammonium-phosphate interactions, comparable to mineral starter fertilizer. In a corresponding article, Westerschulte et al. (2017) focus on spatial and temporal soil mineral N dynamics.

Material & Methods

Experimental sites, soil conditions and weather conditions

In 2014 and 2015, field trials were conducted in Hollage, Lower Saxony, Germany (52°20’ N, 07°58’ E) at two adjacent fields. On both fields soil type can be categorized as plaggic Podzol (IUSS Working Group WRB (2014)) with sandy soil texture (>87% sand). Organic matter content was 2.0% in 2014 and 2.9% in 2015, respectively (for details see Table 1).

Maritime climate is dominating in northwest Germany. Mean annual air temperature at the study site is 10.0°C and mean annual precipitation 799 mm. On average, monthly precipitation increases from 41 mm in April to 79 mm in August (Table 2). However, in 2014 a mild winter and above average temperatures in March and April led to higher soil temperatures. Higher air temperature throughout July, along with 129 mm of precipitation enabled very high growth rates for the plants. Thus, thermal time from planting to the end of June in 2014 were above long-term average (Figure 1). By contrast, in 2015 May and June (i.e. during the early growth period of maize) were cold and dry. High temperatures in August 2015 however, led to high crop growth rates. Finally, thermal time duration from planting to harvest was 1272 °Cd, being close to the 2014 observation (1450 °Cd).

Table 1: Soil properties

	2014	2015
Sand (%)^a (0.063 < 2.0 mm)	91	87
Silt (%)^a (0.002 < 0.063 mm)	8	9
Clay (%)^a (<0.002 mm)	1	4
pH ($CaCl_2$)^a	5.3	5.5
Corg (%)^a	1.14	1.66
C/N^a	13	16.5
total N (%)^a	0.09	0.1
P_{CAL} (mg 100 g^{-1})^a	8	7.8
SMN (kg ha^{-1})^b	35	45

^{a} soil layer 0-30 cm
^{b} soil layer 0-60 cm
SMN = Soil mineral nitrogen (NH_4-N + NO_3-N)
P_{CAL} = Phosphorus extracted with calcium-acetate-lactate solution.

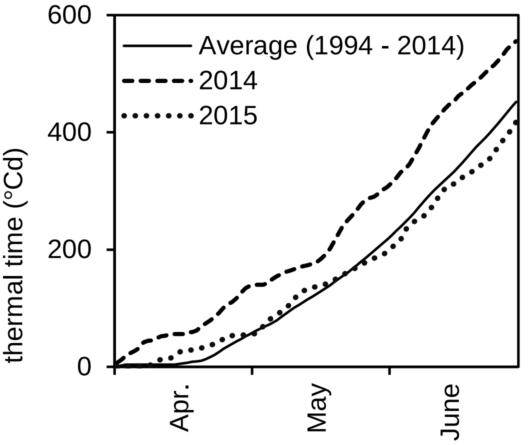

Figure 1: Thermal time according to McMaster and Wilhelm (1997) from April 01 to June 30 at the study site. Comparing long-term average (1994-2014) with 2014, and 2015 growing season.

Experimental design and treatments

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

(1) control (C) without any fertilization to monitor N mineralization from the SMN pool

(2) surface banding (B) of liquid manure with immediate incorporation (disc harrow 0-10 cm in less than 5 minutes after manure application) plus MSF at planting

(3) LM injection treatment (I)

(4) LM injection treatment with a nitrification inhibitor (ENTEC® FL, active ingredient: 3,4-dimethylpyrazol phosphate (DMPP), EuroChem Agro GmbH, Mannheim, Germany) added to the slurry at a rate of 10 l ha^{-1} (1.21 kg DMPP ha^{-1}) prior to application [I(N)].

For both injection treatments, the upper rim of the liquid manure band was 12 cm (2014) and 10 cm (2015) below soil surface. In both years the slurry injector X-Till (Hugo Vogelsang Maschinenbau GmbH, Essen (Oldb.), Germany) adjusted for plot trial operations was used for slurry application. A rotary piston pump and a precision dispenser provided proper longitudinal and lateral distribution of the slurry. The injector had four tines (75 cm apart) followed by injection hoses, allowing the liquid manure to flow into the opened slot. A mineral NP fertilizer was used as MSF in B treatment plots at a rate of 115 kg ha^{-1} to apply 23 kg N ha^{-1} (9.4 kg NO_3-N ha^{-1}, 13.6 kg NH_4-N ha^{-1}) and 10 kg P ha^{-1} (5.6 kg water-soluble P ha^{-1}). MSF was applied 5 cm besides, and 5 cm below the seeds via separate special shares at planting. The N fertilization rate was calculated according to regional advisory standards (Baumgärtel et al. 2010). The recommended fertilization rate is 180 kg N ha^{-1} reduced by preplant SMN, N applied via MSF and site-specific conditions like recent organic fertilization and catch cropping. Liquid manure application rate was equal for the treatments B, I and I(N). As there was no compensation for MSF in the I and I(N) treatments, total N and P rates were lower.

Crop management practices

For the 2014 trial, after harvest of the previous crop maize no tillage operation was done until residue incorporation and seedbed preparation, using a disk harrow twice on March 05 and March 27. Previous crop for the 2015 trial was spring barley

(*Hordeum vulgare* L.), followed by a frost-sensitive catch crop blend consisting of mustard (*Sinapis alba* L.) and oil radish (*Raphanus sativus* L.). A disc harrow was used to incorporate the catch crop on March 04, and for seedbed preparation on April 14, 2015. 23 m^3 ha^{-1} of manure from a nearby pig fattening farm were applied at April 11 in 2014, containing 166 kg ha^{-1} of total N, and 42 kg ha^{-1} of P. In 2015, on April 14, 24 m^3 ha^{-1} of manure from the same farm were applied, containing 130 kg ha^{-1} of total N, and 34 kg ha^{-1} of P (Table 3). Maize (*Zea mays* L. cv. Ricardinio, KWS SAAT AG, Einbeck, Germany) was planted at April 25 in 2014 and April 22 in 2015 4.5 cm below soil surface at a rate of 9.2 seeds m^{-2}. Two herbicide applications according to local recommendations were done each year.

Measurements and samplings

Aboveground biomass was sampled at several vegetative (Vn stage when collar of n^{th} leaf in B treatment was visible) and generative growth stages to monitor plant development (Table 4). Sixteen plants (20 plants at V3 and V4 to ensure that sufficient material for the lab analysis) per plot were cut at stem base in the middle rows and dried at 80 °C to a constant weight. At silage maturity (R5 stage) 7 m in the two center rows of each plot were harvested with a plot sized field chopper and fresh weight was measured gravimetrically. A representative sample was taken to determine dry matter content (drying to constant weight at 80 °C) and for lab analysis. Nitrogen concentrations in all samples were determined using the Kjeldahl method (DIN 2005).

All samples were also analyzed for P concentrations after microwave assisted pressure digestion to dissolve P from organic compounds by ICP-AES (DIN 2012).

Calculations

Based on dry matter accumulation and N concentrations, N uptake, and the N balance (N applied minus N uptake) was calculated for each treatment.

Apparent nitrogen recovery efficiency (NRE) was assessed according to Ciampitti and Vyn (2011). These calculations are based on total N applied.

Thermal time was calculated according to McMaster and Wilhelm (1997):

$$\mathrm{TT} = \left[\frac{(\mathrm{T_{max}}+\mathrm{T_{min}})}{2}\right] - \mathrm{T_{base}} \text{ where if}$$

$$\left[\frac{\mathrm{T_{max}}+\mathrm{T_{min}}}{2}\right] < \mathrm{T_{base}}, \text{ then}$$

$$\left[\frac{\mathrm{T_{max}}+\mathrm{T_{min}}}{2}\right] = \mathrm{T_{base}}.$$

T_{min} and T_{max} were the observed daily minimum and maximum temperatures, respectively. T_{base} was set to 8 °C.

Table 2: Climatic data at the study site

	Mean annual[a]		2014		2015	
	Air Temp.	Precipitation	Air Temp.	Precipitation	Air Temp.	Precipitation
	°C	mm	°C	Mm	°C	Mm
Year	9.4	860	11.3	752	10.2	942
Monthly						
April	9.7	41	11.8	69	9.0	54
May	13.4	59	13.2	113	12.3	40
June	16.2	66	16.2	74	15.9	43
July	18.5	76	20.3	129	18.9	133
August	17.8	79	16.4	83	19.3	187
September	14.1	70	15.8	15	13.5	71
October	10.1	66	13.3	61	9.1	62

[a] Long-term climatic data (1994-2014)

Statistical analysis

Dry matter above ground biomass, N concentration and N uptake were statistically analyzed using the PROC MIXED procedure (SAS Institute Inc. 2011). For both seasons, treatments and replications were tested as fixed effects, the latter to check for heterogeneity of replications (model: treatment replication treatment*replication). Means of treatments were compared using the Tukey procedure for treatments when significant differences at $P<0.05$ occurred. For all sampling occasions that were similar in both years (V6, V10, VT (tasseling) and harvest), a mixed model was performed including years, also as fixed effect to check for year*treatment interactions (model: treatment year treatment*year with replication(year) as random effect). The Pearson's correlation between biomass accumulation (means of treatments) of all samplings and the respective thermal time durations was calculated with EXCEL.

Results

Biomass accumulation

Maize aboveground biomass accumulation was affected by treatment, season and the interaction of both (see Table 5). From emergence to VT biomass in 2015 was lower than in 2014. At V6 in 2015 mean biomass of all treatments was only 20% of the respective 2014 value, while at harvest it was 24% higher (Table 6). In 2014 at V6, significant differences in above ground biomass production for the treatments were observed. Biomass in I(N) treatments was higher than in treatment B (569 kg ha^{-1} versus 466 kg ha^{-1}, respectively), with I treatment in between (523 kg ha^{-1}). For all following growth stages, plants in B treatment showed reduced growth, compared to I and I(N) treatments growth, resulting in 12% and 16% higher yields at harvest for I and I(N), respectively. Treatment C showed significantly lower values than the other treatments for all sampling dates in 2014 (Table 6).

At V3 stage in 2015, aboveground biomass was not significantly influenced by fertilization. Values for B and I(N) treatments were slightly above C and I treatment (Table 6). All following sampling dates in 2015 showed significant differences in plant growth between treatments. Until VT, B treatment showed the highest dry matter accumulation followed by I(N) treatment. Differences between these treatments were significant at V6, V10 and VT samplings, whereas at V3, V4, V8, and harvest samplings they were not. The I and C treatments showed significantly reduced growth from V4 to VT. Throughout the vegetation period biomass for I treatment compared to C treatment was inconsistently higher (no significant differences at V3, V4, V6, and VT samplings and significant differences at V8, V10 and harvest samplings, respectively).

Table 3: Manure properties and fertilization rates

	2014	2015
Manure properties (g kg^{-1})		
Dry matter	93	65
Total N	7.2	5.4
Ammonium N	5.5	3.5
Phosphorus	1.8	1.4
Applied nutrients (kg ha^{-1})		
Total N	166	130
Ammonium N	126	84
Phosphorus	42	34

Table 4: Phenological data

	2014	2015
manure application	Apr. 11	Apr. 14
planting date	Apr. 25	Apr. 22
V3[a] sampling	-	May 22
V4 sampling	-	Jun. 01
V6 sampling	Jun. 10	Jun. 08
V8 sampling	-	Jun. 19
V10 sampling	Jun. 30	Jun. 29
VT sampling	Jul. 22	Jul. 24
harvest date	Oct. 09	Sep. 29

[a]Vn, Vegetative leaf stage n; VT, tasseling

The maximum difference between treatments was found at V10 sampling (B treatments aboveground biomass was 129% larger than C treatment). At harvest, biomass in the three fertilized treatments was 23% higher compared to control.

Nitrogen concentrations

N concentrations were affected by year and treatment for all growth stages (Table 5). The treatment*year interaction was also significant except at harvest. Mean N concentrations for both seasons were significantly enhanced for fertilized plots, when compared to non-fertilized plots at all samplings. The differences between fertilized treatments decreased with proceeding plant development.

As early as V6 stage in 2014, significant differences in nitrogen concentrations within treatments occurred (Table 6). Highest concentrations were found in I(N) treated plots, followed by I treatment (-6.7%, compared to I(N) treatment). Values for B and C treatments were significantly lower than I (-26.5%) and I(N) treatments (-24.6%). In the following weeks, N concentrations and differences between treatments decreased.

At V10 stage I(N) still showed highest N concentrations, followed by I treatment (-10.7%, compared to I(N) treatment). C treatment concentration was significantly lower than I treatment, with B treatment in between.

At tasseling there were no significant differences between treatments. At harvest, the highest concentrations were found in B treatment plots. I and I(N) treatments showed significantly lower values than B treatment (-8.1% and -5.9%, respectively). C treatment showed the lowest N concentrations (-13.6%, compared to B treatment). In 2015, the measured N concentrations were more inconsistent. At V3 sampling, B treatment showed highest values and C treatment lowest, with I(N) and I treatments in between. At V8 and V10 stage however, B treatments N concentration was lower than in C treatment.

Table 5: Statistical analysis for maize aboveground biomass production, aboveground nitrogen concentrations and nitrogen uptake for different methods of manure application treatments in 2014 and 2015 (based on the mixed model)

	V6[b]	V10	VT	Harvest
Aboveground biomass				
Trt[a]	<.0001	<.0001	<.0001	<.0001
Year	<.0001	<.0001	0.0015	0.0008
Year*Trt	<.0001	<.0001	<.0001	<.0001
Nitrogen concentration				
Trt	<.0001	<.0001	<.0001	<.0001
Year	<.0001	<.0001	<.0001	0.004
Year*Trt	<.0001	0.0023	<.0001	0.0955
Phosphorus concentration				
Trt	<.0001	<.0001	0.025	0.71
Year	<.0001	<.0001	0.157	0.251
Year*Trt	<.0001	<.0001	0.003	<.0001
Nitrogen uptake				
Trt	<.0001	<.0001	<.0001	<.0001
Year	<.0001	0.077	<.0001	0
Year*Trt	<.0001	<.0001	<.0001	0.2349

[a] Trt =Treatments (see section ‘Experimental design and treatments’)

[b] Vn = vegetative leaf stage n, VT = tasseling

Table 6: Maize aboveground biomass, nitrogen concentration, and nitrogen uptake as affected by fertilization with liquid manure and mineral starter fertilizer at different growth stages in 2014 and 2015

	2014								2015													
	V6^{c}		V10		VT		Harvest		V3		V4		V6		V8		V10		VT		Harvest	
Treatment^{a}	Aboveground biomass (Mg ha^{-1})																					
C	0.17	a^{b}	1.04	a	5.9	a	10.3	a	0.009	a	0.022	a	0.06	a	0.21	a	0.71	a	7.5	a	16.7	a
B	0.47	b	1.86	b	8.7	b	16.2	b	0.010	a	0.033	b	0.12	c	0.46	c	1.62	d	10.5	c	20.8	b
I	0.52	bc	2.80	c	12.3	c	18.1	c	0.009	a	0.023	a	0.06	a	0.29	b	0.94	b	8.3	a	20.1	b
I(N)	0.57	c	3.06	c	12.8	c	18.9	c	0.010	a	0.029	b	0.11	b	0.46	c	1.47	c	9.2	b	20.9	b
	Nitrogen concentration (g kg^{-1})																					
C	28.7	a	18.1	a	11.1	a	8.8	a	53.5	a	41.2	a	37.6	a	43.0	b	41.9	b	16.8	a	10.0	a
B	28.0	a	18.4	ab	12.1	a	10.2	c	58.5	c	50.6	c	43.4	b	41.7	a	39.4	a	20.0	b	11.4	b
I	35.6	b	20.3	b	11.6	a	9.3	b	56.4	b	47.7	b	43.3	b	49.5	c	45.0	d	21.1	c	11.3	b
I(N)	38.1	c	22.7	c	12.0	a	9.6	b	58.1	bc	50.8	c	46.5	c	50.2	c	43.8	c	20.3	b	11.5	b
	Nitrogen uptake (kg ha^{-1})																					
C	4.8	a	19	a	65	a	91	a	0.47	a	0.9	a	2.3	a	9.2	a	30	a	126	a	167	a
B	13.1	b	34	b	105	b	165	b	0.57	b	1.7	b	5.1	b	19.0	c	64	c	210	c	236	b
I	18.6	c	57	c	143	c	170	bc	0.49	a	1.1	A	2.7	a	14.2	b	42	b	175	b	227	b
I(N)	21.7	d	69	d	155	c	181	c	0.58	b	1.5	B	4.8	b	22.8	d	64	c	187	b	240	b

[a] Treatments fertilized with manure and mineral starter fertilizer (MSF) (C=no manure, no MSF, B=manure broadcast with MSF, I=manure injection without MSF, I(N)=manure injection with nitrification inhibitor without MSF), as indicated in Section 2.2.

[b] different letters within each sampling date indicate significant differences ($P<0.05$)

[c] Vn = vegetative leaf stage n, VT = tasseling

While at V8 the highest values were found in I and I(N) treatments (49.5 g kg^{-1} and 50.2 g kg^{-1}, respectively), at V10 N concentration in I(N) treatment was significantly lower than in I treatment. At harvest, N concentrations of the fertilized treatments were significantly higher than in C treatment.

Nitrogen uptake

Mean N uptake of plants in C treatment, was consistently lower than N uptake of the fertilized treatments for all sampling occasions (Table 6). These differences were significant, except for I treatment at early samplings in 2015 (V3 – V6).

In 2014, compared to I(N) treatment, N uptake in C treatment was 77.7% (V6 stage) to 49.8% (harvest stage) lower. N uptake in B treatment was also consistently lower compared to I(N), but the largest difference was found at V10 stage (-50.9%). At harvest, the difference between B, and I(N) treatments was just -8.7%. Closest to I(N) treatment was I treatment, with significantly lower values at V6 and V10 (-14.2% and -17.9%, respectively) and only marginal differences at later samplings (-7,6% at VT and -6% at harvest, respectively).

In 2015, differences in N uptake occurred as early as V3 stage. Significantly higher values were found in B and I(N) plots (0.57 kg ha^{-1}, and 0.58 kg ha^{-1}, respectively) compared to I and C treated plots (0.49 kg ha^{-1}, and 0.47 kg ha^{-1}, respectively). The same order was observed for the following samplings. At V8 treatment B showed significantly reduced values (-16.6%) compared to I(N) treatment, while I treatment showed 55% higher values than C treatment. These differences between I and C treatments then slowly decreased (+43% at V10, +39% at VT and +35% at harvest, respectively). At V10 B treatment showed the same values as I(N), whereas at VT it was significantly higher (+12.7%). At the final harvest, only C showed lower N uptake (-29%, compared to B) whereas N uptake in I and I(N) was at the same level as B.

Nitrogen balance and apparent nitrogen recovery efficiency

Nitrogen balances were mainly negative, except for B treatment in 2014, where a positive balance (+15 kg N ha^{-1}) occurred (Figure 2). Most noticeable are the major differences for the two seasons. In 2014, the balances for C and B treatments were -91 kg ha^{-1}, and +15 kg ha^{-1}, while in 2015 they were -167 kg ha^{-1} and -83 kg ha^{-1}, respectively. Differences between the treatments were similar in both seasons. Mean apparent nitrogen recovery efficiency (NRE) for fertilized treatments was 49% in both seasons, with only minor differences within the seasons (Figure 2). Lowest values for NRE were calculated for B treatment, and highest for I(N) treatment.

Discussion

Major differences concerning temperature and precipitation between the two seasons led to differences in crop development during early growth. For example, in 2014, thermal time from emergence to V6 was 262 °Cd, while in 2015 it was only 172 °Cd. While Birch et al. (2003) refer to a constant thermal interval between initiation of successive leaves, the present results show a correlation (r^2=0.92) between biomass accumulation and thermal time duration from emergence. We determined sampling occasions based on growth stages, but growth stages proved rather variable as biomass and thermal time duration to V6 in 2014 were quite similar to the respective V8 data of 2015 (Figure 3).

According to Muchow (1988) and Vos et al. (2005), low N concentrations in plants do not interfere leaf area expansion until V6 stage, as maize allows a wide variation of leaf N concentrations.

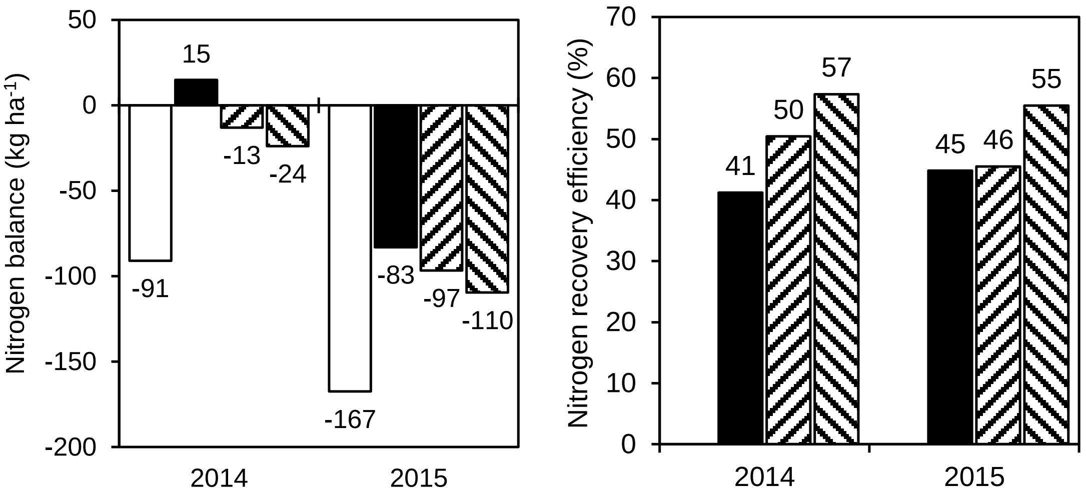

Figure 2: Nitrogen balance (left) and nitrogen recovery efficiency (right) for 2014 and 2015. Treatments fertilized with manure and mineral starter fertilizer (MSF) (C = no manure [□], no MSF, B = manure broadcast with MSF [■], I = manure injection without MSF [▨], I(N) = manure injection with nitrification inhibitor without MSF [▧]).

Although leaf area expansion is not impaired, photosynthetic capacity might be reduced (Vos et al. 2005). Thus, low N concentrations in plants 2014 might have only minor impact on biomass accumulation at the first sampling, at least in fertilized treatments. Applying the critical N approach of Plénet and Lemaire (1999) to our dataset, B and C treatments in 2014 show limiting N concentrations throughout all sampling occasions (Figure 4).

These low to very low plant N concentrations are following major nitrate leaching events in May and June 2014, which lead to very low plant available N in the root zone (for more details on soil mineral N dynamics see Westerschulte et al. 2017), and consequently to low plant N concentrations.

At V10 sampling, SMN in the topsoil layer (0-30 cm) of I and B treatments were nearly at level with C treatment (12.3 kg ha^{-1} for I and 12.8 kg ha^{-1} for B versus 11.4 kg ha^{-1} for C treatment). Only I(N) showed higher values (15.9 kg ha^{-1}), resulting in higher plant N concentration at this growth stage.

At VT, highest SMN values were found in B treatment (47.7 kg ha^{-1} in 0-90 cm), although mainly in the layer 60-90 cm below surface. Wiesler and Horst (1993) found a high proportion of pre-silking N uptake from soil layers up to 45 cm depth, whereas post-silking N was mainly taken up from below 60 cm. Thus, the higher SMN concentrations found in B treatment are most likely not plant available. A reasonable amount of broadcast incorporated slurry N is immobilized shortly after application (Kirchmann and Lundvall 1993), but can be remineralized later (Sørensen and Amato 2002). Thus, B treatment recovered to a certain extent from severe N limitation between tasseling and harvest. As the N concentrations in the I and I(N) treatments also drop below critical values for later sampling dates due to nitrate displacement out of the rooting zone, final yield reductions due to N limitation seem plausible for the 2014 season.

In 2015, no nitrate displacement was found during the vegetation period until harvest sampling (Westerschulte et al. 2017). Thus, N concentrations were always above critical values, even in C treatment (Figure 4). Nevertheless, significant differences of N availability at early samplings might somehow be correlated to differences in biomass accumulation as early as V4 stage. The abundance of ammonium N in the injected slurry band is higher when a NI is added (Westerschulte et al. 2017), and plants under low root-zone temperatures prefer ammonium uptake for energetic reasons (Macduff and Jackson 1991; Subbarao et al. 2006).

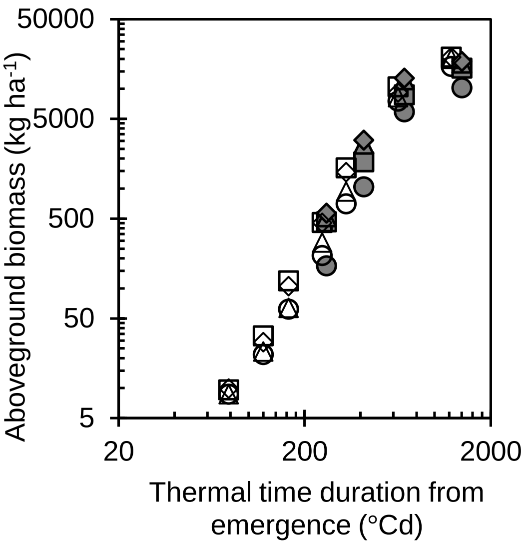

Figure 3: Dry matter related to thermal time of all sampling occasions in the 2014 [filled] and 2015 [no fill] seasons. Means of treatments fertilized with manure and mineral starter fertilizer (MSF) (C = no manure [● / ○], no MSF, B = manure broadcast with MSF [■ / □], I = manure injection without MSF [▲ / △], I(N) = manure injection with nitrification inhibitor without MSF [◆ / ◇]).

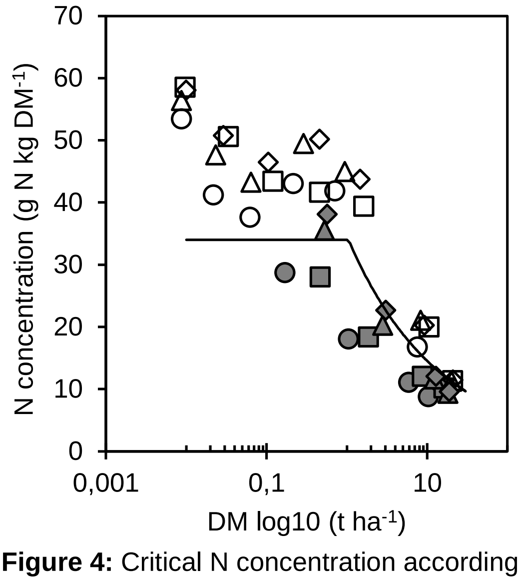

Figure 4: Critical N concentration according to Plénet and Lemaire (1999) [black line] DM versus N concentrations of the samplings in 2014 [filled] and 2015 [no fill]. Means of treatments fertilized with manure and mineral starter fertilizer (MSF) (C = no manure [● / ○], no MSF, B = manure broadcast with MSF [■ / □], I = manure injection without MSF [▲ / △], I(N) = manure injection with nitrification inhibitor without MSF [◆ / ◇]).

Thus, probably more assimilates are available for biomass production, resulting in significantly higher biomass in I(N) treatment, when compared to I treatment. At later samplings B treatments biomass was always the largest, whereas at V8 and V10, N concentration in B treatment was significantly lower than in all other treatments. Only minor precipitation events prior to V8 sampling (8.4 mm within 18 days), might have reduced nitrate availability to a certain extent, as SMN analysis did not show nitrate displacement from the soil zone where the slurry was applied (0-10 cm) to deeper zones (21-30 cm) until tasseling (Westerschulte et al. 2017). However, 30 mm rainfall between V8 and V10 samplings also did not result in higher plant N concentrations in B treatment, compared to the other treatments. At V8, plant N uptake in B treatment (~19 kg ha^{-1}) was more or less at level with the applied N via MSF. Thus, the reduced N concentrations might follow a depletion of MSF N and a sharp decline in SMN below the maize plants (from 58 mg kg^{-1} at V6 to 23 mg kg^{-1} at V10; Westerschulte et al. 2017). Maybe plants needed to adapt to this change in N supply from localized MSF placement to the broadcast slurry N. At tasseling, only C treatment showed N concentrations close to critical values and below critical at harvest. The higher N uptake for C treatment in 2015, compared to 2014, might be due to different weather conditions, or due to differences in soil organic matter content (1.66% in 2015, and 1.14% in 2014, respectively).

Along with the critical N approach according to Plénet and Lemaire (1999) N availability cannot be the sole reason for differences of crop growth between fertilized treatments in this trial. As plants in C, I and to a lesser extent also in I(N) treatments showed visible P deficiency symptoms (purpling of leaves), biomass samples were analyzed for P (Figure 5). Significant differences in P concentrations within treatments were found (B > I(N) > I >= C), ranging from 5.77 g kg^{-1} to 2.78 g kg^{-1} at V4 and from 4.21 g kg^{-1} to

2.17 g kg^{-1} at V6 stage, respectively. Jones (1983) described a function for optimum shoot P concentrations in relation to growth stages, which results in optimum shoot P concentration of 5.76 g kg^{-1} at V4. So, as only B treatment showed optimum P concentrations, differences in biomass can be related to P limitation. We found the greatest differences in P concentrations between treatments at V10 sampling. In a field trial with maize under P deficiency, Plénet et al. (2000b) reported the greatest differences from 8 to 15 visible leaves. Compared to the 2014 season, the period mid-May to late June 2015 was extraordinary cold and dry. Both, soil temperature (Imran et al. 2013), and soil water content (Bhadoria et al. 1991) affect P diffusion speed. Thus, P limitation in C treatment is not surprising.

But the application of 34 kg ha^{-1} P via liquid manure in B, I, and I(N) treatments and another 10 kg ha^{-1} P via MSF in B treatment should lead to sufficient plant available P close to the seedlings.

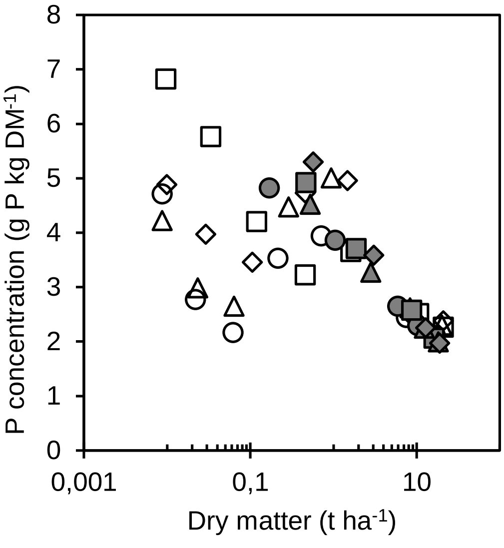

Figure 5: Phosphorus concentrations related to dry matter of the samplings in 2014 [filled] and 2015 [no fill]. Means of treatments fertilized with manure and mineral starter fertilizer (MSF) (C = no manure [● / ○], no MSF, B = manure broadcast with MSF [■ / □], I = manure injection without MSF [▲ / △], I(N) = manure injection with nitrification inhibitor without MSF [◆ / ◇]).

As at V3 only B treatment showed P concentrations superior to C treatment, either spatial or chemical nutrient availability of the injected liquid manure must have been lower compared to MSF. The differences (Figure 5) between I and I(N) treatments for P concentration at V3, V4 and V6 samplings can only be due to the NI, as all other factors (liquid manure, injector, injection depth, application rate) were equal. As shown by Westerschulte et al. (2017), nitrification of NH_4-N in the slurry band was delayed in I(N) treatment, when compared to I treatment. Subbarao et al. (2006) and Withers et al. (2000) refer to better growth, when ammonium and P are applied together, versus each nutrient alone. Application of an ammonium-based fertilizer with NI lowered rhizosphere pH and increased P uptake in a pot experiment with common bean (*Phaseolus vulgaris* L., Thomson et al. 1993). However, the differences in ammonium N found in our experiment are probably not comparable to a pot experiment, where ammonium and nitrate fertilizers were tested. Thomson et al. (1993) furthermore referred to an inhibitory effect on root growth of the used NI (Nitrapyrin). Possibly the compound used in our experiment (DMPP) also influences crop growth.

In two contrasting seasons, despite some restrictions in crop development, the treatments with liquid manure injection showed consistent yields, compared to the manure broadcast treatment. Major SMN displacement in 2014 reduced yields in all treatments, but to a lesser extent when slurry was injected. In 2015, a cold and dry period during early growth lowered P availability resulting in major growth differences. At harvest, however no significant differences between fertilized treatments were found. In both years, the addition of a NI to manure did not lead to significantly increased yields and N uptake, but it increased early growth by assuring higher P concentrations in the plant. Compared to B treatment, I(N) treatment showed equal N uptake and significantly higher yields, despite a noteworthy reduction in N (-23 kg ha^{-1}) and P (-10 kg ha^{-1})

fertilization. Thus, the lower N balances and higher nutrient recovery efficiencies for slurry injection treatments found in this experiment are comprehensible, and go along with the findings of other studies (Federolf et al. 2016; Schröder et al. 1997). Negative N balances indicate a reduced potential for nitrogen losses to the environment, thus, major sustainability targets (European Parliament 2000; Sutton et al. 2011) are met to a greater extent. Although Westerschulte et al. (2017) showed a significant delay in nitrification of the applied ammonium via liquid manure, the inconsistent and insignificant effects on yields and N uptake were also found by others (Federolf et al. 2016; Sawyer et al. 1991; Schmitt et al. 1995).

Thus, for a final evaluation of the agronomic effects of nitrification inhibitors further studies, and a more detailed knowledge on the mode of action of DMPP, as well as the interactions on soil microbiology and plant nutrition are necessary. Even if the agronomic value of nitrification inhibitors is variable, the environmental impact needs to be regarded as well. As nitrification inhibitors are able to reduce leaching and denitrification (Barneze et al. 2015; Ruser and Schulz 2015; Subbarao et al. 2006), they might be able to meet the target of reducing reactive nitrogen emissions into the environment (Sutton et al. 2011). On the other hand possible discharge of nitrification inhibitor compounds and their metabolites into aquatic environments needs to be taken into account (Scheurer et al. 2016).

Five sinks for fertilizer nitrogen are known, (i) plant uptake, (ii) ammonia emissions, (iii) trace gas emissions, (iv) nitrate leaching, and (v) the soil nitrogen pool (organic and inorganic). When cumulating the data for SMN of Westerschulte et al. (2017) with our observations, among all tested treatments the highest proportions of applied N were found in I(N) treatment throughout all sampling occasions. Yet, still major pathways for N losses and N contents of plant roots were not quantified. Thus, further research on this topic measuring all possible N sinks ideally using labelled N is necessary.

Conclusion

Injection of liquid manure close to maize seedling ensured optimal nutrients supply for the crop. Under cold conditions, the addition of a nitrification inhibitor seems to promote phosphorus availability in early growth stages. The impact of nitrification inhibitors on soils under field conditions however, needs further studies.

Manure injection showed a huge potential to reduce nitrogen and phosphor fertilization rates, without impairing maize yields on sandy soils in northwestern Germany. Thus, farmers can use this technology to decrease nutrient surpluses and benefit the environment.

Acknowledgements

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2.3 Assessing crop performance in maize field trials using a vegetation index

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Matthias Westerschulte contributed to the development of the experimental design, conduction of the field trials and samplings as well as the evaluation

Hans-Werner Olfs supervised Carl-Philipp Federolf

Gabriele Broll supervised Carl-Philipp Federolf

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Assessing crop performance in maize field trials using a vegetation index

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Abstract

New agronomic systems need scientific proof before being adapted by farmers. To increase the informative value of field trials, expensive samplings throughout the cropping season are required.

In a series of trials where different application techniques and rates of liquid manure in maize were tested, a handheld sensor metering the red edge inflection point (REIP) was compared to conventional biomass sampling at different growth stages and in different environments. In a repeatedly measured trial during the 2014, 2015, and 2016 growing seasons, the coefficients of determination between REIP and biomass / nitrogen uptake (N_{upt}) ascended from 4 leaves stage to 8 leaves stage, followed by a decent towards tasseling. In a series of trials in 2014, and 2015, the mean coefficients of determination at 8 leaves stage were 0.65, and 0.67 for biomass and N_{upt}, respectively. The predictability of biomass or N_{upt} by REIP however, is limited to similar conditions (e.g. variety). In this study, REIP values of e.g. ~721, represent Nupt values from ~8 kg ha^{-1} to ~38 kg ha^{-1}. Consequently, the handheld sensor derived REIP used in this series of experiments can show growth differences between treatments, but referential samples are necessary to assess growth parameters.

Introduction

Nitrogen (N) is a crucial nutrient for crop growth and below optimal plant N concentration can lead to significant yield losses (Mollier and Pellerin 1999, Plénet and Lemaire 2000). Excess N fertilization however, can have a negative impact on ecosystems (Sutton et al. 2011). To acquire reliable information on balanced N fertilization, researchers usually use multi-location and/or multi-annual field trials to acquire fundamental information (Gomez and Gomez 1984). Regarding maize (Zea mays L.) fertilization trials in areas with higher contents of soil organic matter, major differences in early-growth might become insignificant at harvest due to a high N mineralization potential (Federolf et al. 2017). If the harvest is the only sampling, it might be difficult to explain differences in yield and quality due to early-growth nutritional status (Clewer and Scarisbrick 2001). Retarded early-growth in maize however, might influence farmers decisions when it comes to adopting new strategies or they may stick to a known “safety first system” (Withers et al. 2000). Thus, detailed knowledge about nutrient interactions in the soil-plant system are crucial, therefore sampling throughout the whole vegetation period is necessary (Clewer and Scarisbrick 2001).

Visual scoring is cheap and easy, but it is non-quantitative, difficult to standardize, and biased by human error (Montes et al. 2011, Olfs et al. 2005). Soil and plant sampling is more accurate, but these practices are time consuming and costly. They lead to great quantities of samples which require a workforce to obtain, process and analyze (Olfs et al. 2005, Rambo et al. 2010). Furthermore, destructive sampling needs additional space in plots (Clewer and Scarisbrick 2001), increasing the area needed for each plot and thus, decreasing the chances of finding adequate and homogeneous sites for a field trial. In a series of trials, additional difficulties appear when crops (especially rapidly developing spring crops like maize; Birch et al. 2003) reach a planned sampling stage simultaneously at different sites. To decrease the number of samples, researchers increasingly try to use chlorophyll-meters (Rashid et al. 2005), or measure vegetation indices via spectral nondestructive plant analysis to get information on biomass and crop nutritional status (Winterhalter et al. 2011, Osborne et al. 2002).

In the last decades several spectral indices have been evaluated for their ability to describe aboveground biomass. For example, leaf area index (LAI) and N uptake of broadacre crops such as maize, wheat (Triticum aestivum L.), or oilseed rape (Brassica napus L., Erdle et al. 2011, Thoren and Schmidhalter 2009) have been considered. Although a huge number of different indices exist, the normalized differenced vegetation index (NDVI, Rouse et al. 1974), and the red edge inflection point (REIP, Guyot and Baret 1988) have been used most frequently. The NDVI is very sensitive at low LAI values, but tends to saturate at moderate to high LAI (Baret and Guyot 1991). Thus, for crops with LAI values higher than 2, the REIP is a better predictor due to inclusion of red-edge information (Sticksel et al. 2004). Mistele and Schmidthalter (2008a) showed a consistently useful correlation of the REIP and a NIR/NIR ratio (R780/R740) and the aboveground N uptake of maize crops, whereas limitations for the use of the NDVI and other single ratios, which combine the reflection in the red or green ranges with NIR, were found. However, they also experienced the need for a minimum of biomass for the REIP to return useful values. Compared to other vegetation indices, Sticksel et al. (2004), found a high consistency of REIP values throughout different light conditions during a day.

The vegetation indices are mainly obtained by using multispectral cameras, which require significant post-processing, or ready to use sensors that measure, calculate and directly display obtained values. They are available as handheld devices (Tavakoli et al. 2014), mounted on purpose adjusted sensor platforms (Montes et al. 2011, Winterhalter et al. 2011), tractors (Mistele and Schmidhalter 2008b), unmanned aerial vehicles (UAV, Rasmussen et al. 2016), or satellites (Thenkabail et al. 2000, Malenovský et al. 2012). Satellite sensing, such as the ESAs Sentinel mission, provides several wavebands in the red edge and near infrared spectra with a spatial resolution of 10 m per pixel (Malenovský et al. 2012), which is not sufficient for plot trials. UAVs, although offering certain opportunities and being available, require deep knowledge of image processing and data interpretation (Rasmussen et al. 2016). Thus, tractor or platform mounted sensing devices seem the most appropriate version for experimental stations, whereas handheld sensors can easily be transported from field to field, which makes them convenient for diverse types of experimental series.

The purpose of our study was to evaluate the use of the REIP obtained with a handheld sensor to describe growth differences of maize stands in field trials, where different combinations of liquid manure (broadcast application, and subsurface injection without and with a nitrification inhibitor) and mineral starter fertilizers were tested in different environments (Federolf et al. 2016, Federolf et al. 2017). The research questions were how soil-plant-fertilizer interactions influences on crop growth change sensor values obtained using REIP, and whether it is possible to link sensor measurements to crop parameters via regression analysis independent of growth stage and crop management practices?

Material & Methods

Experimental sites, soil and weather conditions

To obtain a reasonable amount of data for the study, two different approaches were used. One experiment was conducted at Hollage, close to Osnabrück (Germany) in the 2014 - 2016 seasons, to gain in-depth insight into the possibilities of sensor use especially during the early-growth development of maize. To check the usability of the sensor at different sites, a series of experiments was established in cooperation with the Chambers of Agriculture in North Rhine-Westphalia, Lower Saxony and Schleswig-Holstein at six sites in the years 2014, and 2015.

The Hollage experiment

In 2014, 2015 and 2016, field trials were conducted at Hollage, Lower Saxony, Germany (52°20’ N, 07°58’ E) on three adjacent fields. The soil types can be categorized as plaggic, or gleyic Podzols (IUSS Working Group WRB 2014) with sandy soil texture (>87% sand; for details see Table 1).

Maritime climate is dominating in northwestern Germany. Mean annual air temperature at the study site is 10.0°C and mean annual precipitation is 800 mm. On average, monthly precipitation increases from 41 mm in April to 79 mm in August. In 2014, a mild winter and above average temperatures in March and April led to higher soil temperatures compared to the long-term average. Higher air temperature throughout July, along with 129 mm of precipitation enabled very high growth rates of the maize plants. Thus, thermal time from planting to harvest in 2014 were above the long-term average (Figure 1). In 2015, May and June (i.e. the early growth period of maize) were cold and dry, but elevated temperatures in July and August 2015 led to reasonable crop growth rates. Finally, thermal time duration from planting to harvest was 1272°Cd, being close to the 2014 observation (1450°Cd). In 2016, April and May were extraordinarily dry. Most of the total precipitation during the season was due to a rainy June contributing 231 mm to a total of 424 mm of precipitation (Figure 2). A cold period after planting until the second week of May led to delayed emergence, but afterwards temperature was above average, especially in late August and September, leading to thermal time duration of 1401°Cd from planting to harvest.

Table 1: Locations, climate and soil conditions, fertilization details and crop management practices.

location	Hollage			Haus Düsse		Merfeld		Sandkrug		Wehnen		Bovenau		Schuby	
latitude	52°20' N			51°38' N		51°51' N		53°05' N		53°10' N		54°19' N		54°31' N	
longitude	07°58' E			08°11' E		07°12' E		08°16' E		08°08' E		09°48' E		09°26' E	
Climate															
mean an. precip. (mm)	860			836		880		841		823		847		844	
mean an. air temp. (°C)	9.4			9.8		10.4		9.6		9.5		8.9		8.6	
soil conditions															
soil type^1	PZ-pi			LV-ha		PZ-pi		PZ-ha		PZ-gl		LV-gl		PZ-ha	
texture^2	sand			silt		sand		sand		sand		loam		sand	
	2014	**2015**	**2016**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
pH^2 (CaCl$_2$)	5.3	5.5	5.4	6.8	6.8	5.0	4.6	5.3	5.4	4.8	5.3	6.4	6.2	5.4	5.3
SMN^3 (kg ha^{-1})	35	45	20	15	40	15	63	23	23	13	41	68	31	23	29
P$_{CAL}$^{4} (mg 100 g^{-1})	8.0	7.8	7.2	7.0	5.7	10.0	9.2	11.0	8.0	8.0	11.0	15.0	8.6	14.0	11.2
nutrients applied via manure															
total N (kg ha^{-1})	166	130	182	143	100	178	87	164	136	180	118	207	198	164	180
P (kg ha^{-1})	42	34	50	29	28	50	24	33	32	23	19	33	21	17	15
crop management practices															
variety	Ricardinio			LG30224	LG30254	LG30224	LG30254	LG30222		LG30222		LG30211	P7524	LG30211	P7524
planting	Apr 25	Apr 22	Apr 19	Apr 23	Apr 23	Apr 16	Apr 15	May 17	Apr 27	Apr 30	Apr 29	May 15	Apr 27	May 06	Apr 30
V8 sampling	-	Jun 19	Jun 13	Jun 14	-	Jun 13	Jun 15	Jun 19	Jun 25	Jun 19	Jul 06	Jun 25	Jul 02	Jun 24	Jul 03
harvest	Oct 09	Sep 29	Sep 19	Oct 06	Oct 05	Sep 20	Sep 29	Oct 02	Oct 06	Oct 02	Oct 26	Oct 10	Oct 27	Oct 01	Nov 04

[1] Soil type with cm= cambic; gl= gleyic; ha= haplic; pi= plaggic; LV= Luvisol; PZ= Podzol (IUSS Working Group WRB 2014)

[2] Soil layer 0-30 cm

[3] SMN = Soil mineral nitrogen (NH4-N + NO3-N) prior to manure application (soil layer 0-60 cm)

[4] PCAL = Phosphorus extracted with calcium-acetate-lactate solution (soil layer 0-30 cm)

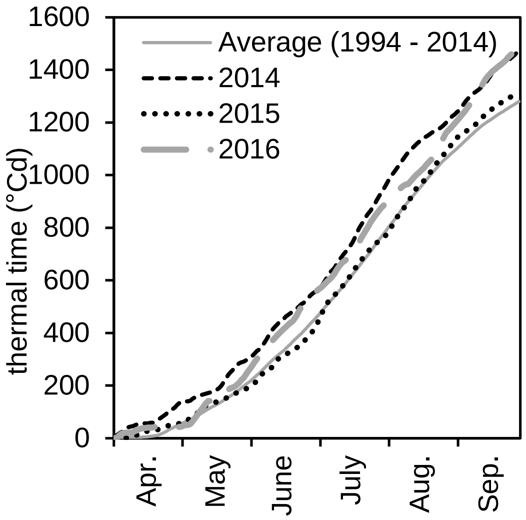

Figure 1: Thermal time according to McMaster and Wilhelm (1997) from April to September at the Hollage experiment. Comparing long average (1994-2014) with 2014, 2015 and 2016 growing seasons.

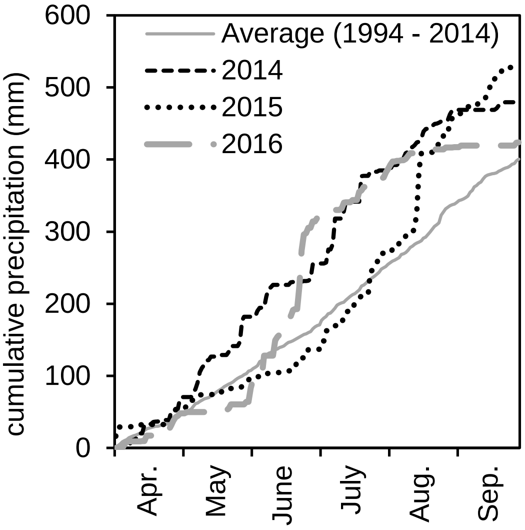

Figure 2: Cumulative precipitation (mm) from April to September at the Hollage experiment. Comparing long average (1994-2014) with 2014, 2015 and 2016 growing seasons.

The experimental series

In 2014 and 2015, maize fertilization trials were conducted in cooperation with the extension service (i.e. the Chambers of Agriculture) in Lower Saxony, North Rhine-Westphalia and Schleswig-Holstein (see Table 1 for details). The five sites were in regions with intensive animal farming on sandy soils. Due to long-term manure application, these soils are typically high to very high in soil P test levels (see Table 1). One site in a cash crop dominated area, with little livestock, showed a medium to high level of plant available soil P but this site did not receive considerable amounts of organic manure over the last decades. Mean annual air temperature at the study sites varies from 8.6°C to 10.4°C from north to south, and mean air temperature from May to September ranges from 13.8°C to 15.4°C, respectively. Mean annual precipitation ranges from 742 mm to 880 mm (Ø 826 mm) with rainfall from May to September being from 323 mm to 376 mm (Ø 364 mm). In 2014, the air temperature was 1.3°C above the long-term average with May to September precipitation being rather close to the average, but with unusual rainfall distribution, having high rainfall in May and July. In 2015, air temperature and precipitation in the months of April, May and June were lower than in the 2014 season. There were major rainfall events after the eight-leaf sampling (V8) in July however, these led to similar total precipitation across both seasons.

Experimental designs and treatments

In the experimental series, a split-plot design with the two factors "liquid manure application" and "mineral starter fertilizer" was used with four replications. Main plots were randomized in each replication and subplots were randomized within main plots. Treatments in the factor "liquid manure application" were an unfertilized control (C), liquid manure broadcast (B), liquid manure injection (I) and liquid manure injection with nitrification inhibitor (I(N); see also Federolf et al. 2016). All plots were split into two 7 m long subplots (4 rows with 75 cm row spacing). Half of the plot received mineral starter fertilizer ("+"; 23 kg N ha^{-1} and 10 kg P ha^{-1}) at planting, whereas the other half did not ("-"). The Hollage experiment was reduced to the four treatments of major interest, out of the experimental series. These were set up as a randomized complete block design with four replications. Besides an unfertilized control treatment (C-), a liquid manure broadcast treatment (B+) with mineral starter fertilizer (23 kg N ha^{-1} and 10 kg P ha^{-1}) was compared to two liquid manure injection treatments with (I(N)-) and without nitrification inhibitor (I-), respectively. The plots consisted of four maize rows with 75 cm row spacing and were 25 m long to allow destructive analysis (see also Westerschulte et al. 2017). Nitrogen fertilization levels of all trials were adapted to local recommendations (Table 1).

Crop management practices

Crop management and crop protection in all experiments was done according to best management guidelines adapted to local needs for all treatments equally at each site. Different varieties were used in the experiments due to different soil and climate conditions. Planting dates, ranging from April 15 to May 17 were chosen individually for each site (see Table 1). Plant density was 9.2 plants m^{-2} at the Hollage experiment and 9 plants m^{-2} in the experimental series.

Measurements and samplings

Biomass samplings

Aboveground biomass was sampled at several vegetative stages (Vn stage when collar of nth leaf in B treatment was visible, details in Table 2) in the Hollage experiment, while only one sampling was done at V8 in the experimental series. At each sampling occasion, sensor measurements were conducted within two days before, or after biomass sampling, depending on weather conditions. To obtain biomass samples at Hollage, sixteen plants (20 plants at V3 and V4 to ensure sufficient material for lab analysis) per plot were cut at the stem base in the center rows. In the experimental series, ten plants were cut in the outer rows of the plots, as plot size did not allow destructive measurements in the growing season. All samples were dried at 80°C to constant weight. Nitrogen concentrations from representative subsamples of the dried material were then determined using the Kjeldahl method after fine grinding of the plant material (DIN 2005).

Sensor measurements

To determine the red edge inflection point (REIP) vegetation index a modified handheld device of the Fritzmeier ISARIA sensor (Fritzmeier Umwelttechnik, Großhelfendorf, Germany) was used along with a smartphone to run the software (Haas 2013). The measuring device comprises four of LED elements, which emit monochrome light at predetermined wavelengths (670 nm, 700 nm, 740 nm, and 780 nm, respectively) in a measurement cycle, a light receiving element, and a control device to determine the intensity of light reflectance (Haas 2010). The software calculates the REIP index with the following equation:

$$REIP = 700 + 40 * \frac{\frac{R_{670} + R_{780}}{2} - R_{700}}{R_{740} - R_{700}}$$

(Haas 2010). The frequency of measurement cycles per second depends on light conditions and was always above 100 cycles per second in our trials. For each second, means of all measurement cycles are stored into a single file per plot, which then contains >10 obtained values. The sensor head was walked along the two center rows of each plot in a round-trip at a height of 60 cm above the whorl of the plants. While walking the sensor along the plots, the sensor head was always kept at nadir view to reduce the influence of the general anisotropy of spectral reflectance measurements (Casa et al. 2010). The field of view in this respective distance is a circle with approximately 25 cm diameter. The sensor measurements at the Hollage experiment were done on a weekly basis, depending on weather conditions, from roughly two-leaf stage to tasseling (only to 10 leaf stage in 2016, see Table 3). As the REIP shows little sensitivity to diurnal variations (Sticksel et al. 2004), the measurements were done at different respective ambient light conditions.

Table 2: Biomass samplings at the Hollage experiment.

	2014	2015	2016
V4[1] sampling	-	June 01	May 24
V6 sampling	June 10	June 08	June 03
V8 sampling	-	June 19	June 13
V10 sampling	June 30	June 29	June 24
VT sampling	July 22	July 24	-
harvest date	Oct. 09	Sep. 29	Sep. 19

[1] Vn = vegetative leaf stage n, VT = tasseling

Calculations and data analysis

Based on dry matter accumulation and N concentrations, the plant N uptake was calculated for each plot. For further processing the obtained REIP values were averaged for each plot. For the Hollage experiment analysis of variance was performed with PROC MIXED in SAS, followed by a LSD post hoc test, if $P<0.05$ (SAS Institute Inc. 2011).

The following exponential regression was used (Thenkabail et al. 2000).

$$a = m * e^{b*REIP}$$

(with "a" being either DM, or N_{upt}; Eq. 1). The respective correlations between aboveground biomass (DM), plant N uptake (N_{upt}) and the red edge inflection point (REIP) derived from the sensor measurements were computed using the "lm" function in the R software environment (R Core Team 2016) for each sampling occasion separately. Eq. 1 was linearized

$$\ln(N_{upt)}) = \ln(m) + b * REIP$$

and the Cook's distance (Di) was used to identify outliers when D_i>4/n (with n being the number of datapoints; Bollen und Jackman 1990). The regression was computed again after removal of outliers. For the nitrogen concentrations (N_{conc}), a linear regression was fitted

$$N_{conc} = \mathrm{m} + b * REIP,$$

the Cook's distance and removal of outliers were performed as with the linearized data.

Table 3: Dates of sensor measurements the Hollage experiment.

2014		2015		2016	
date[1]	DAP[2]	date	DAP	date	DAP
May 22	27	May 20	28	May 24	35
May 27	32	May 26	34	May 31	42
June 03	39	June 01	40	June 03	45
-	-	June 08	47	June 08	50
June 10	46	June 11	50	-	-
June 18	54	June 16	55	June 16	58
June 26	62	June 19	58	June 23	65
June 28	64	June 24	63	-	-
July 04	70	June 29	68	-	-
July 10	76	July 09	78	-	-
July 15	81	July 16	85	-	-
July 24	90	July 24	93	-	-

[1] date = date of sensor measurement in the respective year

[2] DAP = days after planting

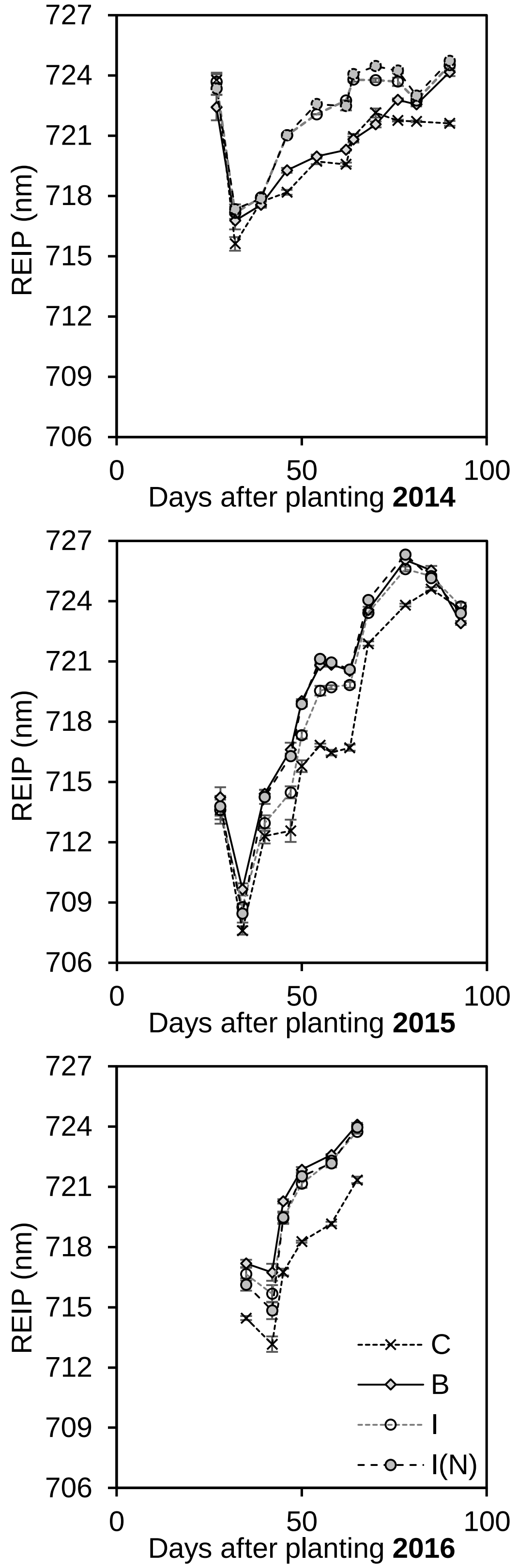

Figure 3: Development of REIP values for treatments in the Hollage experiment in 2014, 2015, and 2016. Means of treatments fertilized with manure and mineral starter fertilizer (MSF) [C = no manure, no MSF, B = manure broadcast with MSF, I = manure injection without MSF, I(N) = manure injection with nitrification inhibitor without MSF].

Results

Devolution of sensor values

From the first (27, and 28 days after planting in 2014, and 2015, respectively) to the second (32, and 34 days after planting in 2014, and 2015, respectively) measurements, a sharp decline in REIP values was observed (-6.6 points from 723.3 to 716.7 in 2014, and -5.2 points from 713.8 to 708.6 in 2015, respectively; Figure 3; Supplementary Table 6). In the following measurements, the values increased until peaking at 76, and 78 days after planting in 2014, and 2015, respectively. In 2016, there was also a slight decline from the first to the second measurement (-1 point, Figure 3; Supplementary Table 6). However, the first and second measurements were later (35 DAP, and 42 DAP, respectively) than in the previous years and there were no measurements after V10 (65 DAP). The differences between treatments obtained by plant analysis go along with the REIP measurements.

Correlations between REIP and biomass, N concentrations and nitrogen uptake

The Hollage experiment

The coefficients of determination for the Hollage experiment are displayed in Table 4, and the raw data in Supplementary Table 6. In the 2014 season, for all measurements (V6, V10, and VT), the coefficients of determination for biomass and N_{upt} were above 0.7 and highly significant. In 2015, the coefficients of determination for biomass ($r^2 = 0.27^*$), as well as for N_{upt} ($r^2 = 0.23^{n.s.}$) were low at the V4 measurement. With ongoing crop development, the relationship between the values obtained by plant analysis and the sensor measurements increased, peaking at V8, when r^2 for the relation REIP / biomass was 0.82***, and for REIP / N_{upt} at 0.92***, respectively. At V10, and VT, the coefficients of determination decreased again.

Table 4: Coefficients of determination (r^2) and the results of the correlation analysis (p values) between biomass, N concentration, N uptake and the red edge inflection point (REIP) for different growth stages at the Hollage experiment in the growing seasons 2014 - 2016.

	2014						2015						2016					
	biomass		N_{conc}		N_{upt}		biomass		N_{conc}		N_{upt}		biomass		N_{conc}		N_{upt}	
growth stage	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p
V4[1]	no data						0.27	*	0.30	*	0.23	n.s.	0.80	***	0.69	***	0.81	***
V6	0.75	***	0.81	***	0.87	***	0.60	***	0.23	n.s.	0.60	***	0.81	***	0.37	*	0.80	***
V8	no data						0.82	***	0.23	n.s.	0.92	***	0.87	***	0.70	***	0.85	***
V10	0.77	***	0.81	***	0.83	***	0.66	***	0.02	n.s.	0.68	***	0.83	***	0.57	**	0.88	***
VT	0.80	***	0.21	n.s.	0.76	***	0.43	**	0.17	n.s.	0.40	*	no data					

[1] Vn = vegetative leaf stage n, VT = tasseling

Table 5: Coefficients of determination (r^2) and the results of the correlation analysis (p values) between biomass, N concentration, N uptake and the red edge inflection point (REIP) at V8 stage of maize in the experimental series for the seasons 2014 and 2015.

	2014						2015					
	biomass		N_{conc}		N_{upt}		biomass		N_{conc}		N_{upt}	
site	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p
Bovenau	0.69	***	0.02	n.s.	0.61	***	0.59	***	0.38	***	0.66	***
Haus Düsse	0.69	***	0.48	***	0.68	***	no data					
Merfeld	0.47	***	0.13	*	0.50	***	0.52	***	0.01	n.s.	0.54	***
Sandkrug	0.81	***	0.44	***	0.81	***	0.73	***	0.12	n.s.	0.82	***
Schuby	0.52	***	0.04	n.s.	0.49	***	0.57	***	0.10	n.s.	0.62	***
Wehnen	0.80	***	0.36	***	0.81	***	0.76	***	0.01	n.s.	0.81	***

In 2016, the coefficients of determination were above 0.8*** for biomass and N_{upt} at all sampling occasions. The relationship between REIP and N_{conc} was variable throughout the study period. While in 2014, at V6, and V10, the coefficients of determination were high (r^2 = 0.81***), for the VT measurement it was only $0.21^{n.s.}$. In 2015, only at the V4 measurement, a significant relationship was observed, while all other samplings did not show significant relations. In 2016, again the relationships were significant, but the coefficients of determination were lower (ranging from 0.37 to 0.7) than in 2014.

The experiment series

Although major differences in temperature and precipitation occurred between the two seasons, there was no obvious trend for a certain nutrient deficiency to be more expressed in one of the years. The Wehnen site, for example showed severe P deficiency symptoms in 2015 with a mean P concentration of only 2.32 g kg^{-1} in shoot biomass, whereas the neighboring site Sandkrug showed high values (mean 4.60 g kg^{-1} in 2015). This P deficiency at the Wehnen site led to major differences in crop growth as the unfertilized control treatment (treatment C-) only produced 201 kg DM^{-1} biomass until V8, whereas treatment I(N)+ produced 1475 kg DM ha^{-1} at the same stage (Supplementary Table 7). For N concentrations, there was also no clear trend within the seasons. The coefficients of determination between REIP and N_{conc} were also low in this series (means for all sites are 0.24 and 0.12 in 2014 and 2015, respectively; Table 5). However, at some sites there were significant relations (Haus Düsse, Sandkrug and Wehnen in 2014, and Bovenau in 2015). The relations between REIP and biomass ranged from 0.47 and 0.53 (Merfeld 2014 and 2015, respectively) to 0.81 and 0.76 (Sandkrug 2014 and Wehnen 2015, respectively) with a mean of 0.65. Comparable results were observed for the coefficients of determination between REIP and N_{upt}. Lowest values were calculated for Schuby and Merfeld (0.49 and 0.50 in 2014, 0.62 and 0.52 in 2015, respectively) and highest for Sandkrug and Wehnen (both 0.81 in 2014, 0.82 and 0.81 in 2015, respectively).

Discussion

The Hollage experiment

The REIP values at the very early measurement dates in 2014 and 2015 were quite variable and the sharp decline to the second measurement five to six days later indicates a shift from mainly soil borne reflectance in the first, to a rather plant borne reflectance in the second measurement. Behrens et al. (2005) showed an influence of soil reflectance on the NDVI. As both NDVI wavebands are also used for the REIP, the influence of soil reflectance at low biomass levels most likely also affected the first measurements in our trials. For maize aboveground biomass significant differences between treatments at the Hollage experiment in 2015 occurred as early as V4 (Federolf et al. 2017). At this stage, the obtained values were lower than in the earlier measurement in both 2014 and 2015, but the differences between treatments became detectable. Thus, for sensing differences in agronomic maize trials with the device used, the plants should at least have four leaves to guarantee sufficient leaf area and biomass, as other studies also showed a need for minimum biomass for the REIP to give accurate readings (Mistele and Schmidthalter 2008a). At V6 the coefficients of determination were higher and the estimations of biomass, and N uptake were quite good throughout the three seasons. In 2015 however, the coefficient of determination was lower compared to 2014 and 2016 (0.60 versus 0.87, and 0.80 for REIP and N uptake in 2015, 2014 and 2016, respectively). Due to complex nutrient interactions when fertilizing with liquid manure, in the 2015 season the plants in some treatments showed P deficiency symptoms, low P concentrations and reduced biomass (Federolf et al. 2017). In contrast to N deficiency, P deficiency has no major effect on leaf chlorophyll content (Al-Abbas et al. 1974) and photosynthesis per unit leaf area but leads to reduced leaf growth and LAI (Plénet et al. 2000). According to Osborne et al. (2002) early season P deficiency influences near infrared (NIR) reflectance, especially between V6 and V8 growth stages. In our trials, P deficiency symptoms decreased with ongoing development until V8 stage (Federolf et al. 2017). The coefficients of determination peaked between V6 and V10 in the Hollage experiment. Within these growth stages, also the growth differences between treatments were high, no matter whether N or P was limiting. Although under P deficiency, biomass and N uptake might be overestimated to a certain degree by reflectance measurements due to changing spectral properties.

At tasseling, the coefficients of determination were lower than at the previous stages. This might be due to difficulties concerning canopy architecture and to saturation effects in VI's (Rambo et al. 2010), as the field of view (FOV) of the tested sensor is only 25 cm in diameter and thus does not properly represent the field situation with 75 cm row distance. Drouet und Bonhomme (1999) investigated the leaf area density in row canopies of maize. In a stand like ours, leaf area density and leaf N per area was distributed very heterogeneously between intra- and inter-row spaces. They also found a positive correlation of irradiance interception of a certain leaf area and the respective laminal N content, indicating that N translocation processes tend towards photosynthetically active leaf area (Drouet und Bonhomme 1999). Winterhalter et al. (2012) found a decrease in total N contents from the top to the bottom of the plants. Thus, at growth stages after stem elongation, as the field of view of any sensor above the canopy is not able to obtain data from lower leaves, the informative value of hyperspectral data is probably reduced. Furthermore, at tasseling, plant height (>2.4 m) and tassels hamper the definition of the crop canopy and the sensors usually measure reflectance on leaves (Winterhalter et al. 2012).

Regarding the changes in REIP values and their respective coefficients of determination to biomass and N uptake from the Hollage experiment, the most appropriate timing of measurement seems from V6 until stem elongation. However, the sensor values might be influenced by other stress factors than N deficiency.

The experiment series

Due to small plots, the plant sampling had to be done in the outer rows of each plot whereas the sensor measurement was done in the center rows. Although root proliferation of the inter row space, and thus also in the “inter-plot” space, in maize stands of 75 cm row width is unlikely prior to V8 growth stage (Schröder et al. 1997), edge effects between treatments cannot be totally excluded. This might have influenced the coefficients of determination, which were mainly lower than in the Hollage experiment. Furthermore, there was only one sampling per site per year, which hinders the evaluation of crop development differences in this series of trials.

Especially in 2015, due to low temperatures at all sites, limited growth was observed. At the Bovenau site, frost damage was still visible on the plants during V8 sampling, leading to heterogeneous conditions throughout the trial. Additionally, as was also seen in the 2015 data from the Hollage experiment after a cold period, nutrient deficiency symptoms other than N may occur, hampering the precision of the sensing data. The most severe P deficiency symptoms occurred at Wehnen 2015, the least at Sandkrug 2015, though at these trials the highest coefficients of determination were found. Thus, as also stated by Osborne et al. (2002), for later growth stages, the influence of P on spectral measurements seems to decrease.

Repeatability of measurements

Regarding all combinations of measured REIP and biomass or N uptake of our experiments (n = 448), a simple regression cannot easily be drawn (see Figure 4), although there is a dependency between the factors. The interference of soil reflectance is a weakness of vegetation indices in early growth stages (Hatfield et al. 2008; Rambo et al. 2010). As our trials were performed on a range of soils (from sandy Podzols to silty Luvisols, see Table 1), in different regions, which themselves influence crop growth and canopy architecture due to different photoperiods (Liu et al. 2013; Bonhomme et al. 1991). Growth differences were observed between the sites (Federolf et al. 2016) and also found in the sensor values. Furthermore, we also used different varieties with different traits like earliness, canopy architecture, or nutrient uptake dynamics, which might also lead to differences in the sensor values (Montes et al. 2011). Sellers (1985) reported a strong influence of leaf inclination on reflection of solar radiation when maize LAI was low, especially at higher solar angles. This influence however, might be lower when active sensors are used. Moreover, there is a general consent that other stress factors, such as herbicides, diseases or drought also reduce chlorophyll contents in plant leaves and hence reflection properties, leading to a "blue shift" of the red edge (Carter und Knapp 2001). In the present study, when looking at REIP values of e.g. ~721 (±0.2), the respective N uptake varied from ~8 kg ha^{-1} to ~38 kg ha^{-1} and the biomass from 180 kg ha^{-1} to 1280 kg ha^{-1}. Thus, for any conversion of REIP values into biomass or N uptake (or for producing fertilizer application recommendation maps), reference samples for field specific calibration need to be taken (Hatfield et al. 2008, Olfs et al. 2005).

Compared to visual scoring however, the VI from a sensor is less prone to human bias, it can be standardized and is able to quantify and add value to visual impressions. As spectral sensors are relatively cheap and easy to use, such measurements might increase the explanatory power of field trials. Still, it must be kept in mind, that N deficiency is not the only influencing factor on sensor derived VI, thus these values always need to be regarded with respect to current and previous conditions. The field of view of the sensor used in this study however is only 25 cm in diameter, which is insufficient for typical maize stands with 75 cm row spacing. When used as a handheld sensor, this is not an issue as one can easily center walk the sensor head on the row. One upside of this is the reduction of background noise due to limited inter-row reflectance interference at early growth stages. Increasing the field of view of the sensor by increasing the sensing diameter to at least covering one row width would reduce the necessity of accurate row detection. More robust oblique view systems (Schmidthalter et al. 2008) however, need larger plot sizes.

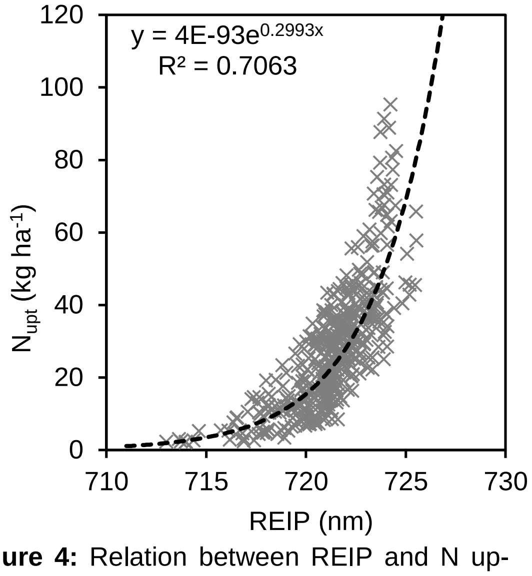

Figure 4: Relation between REIP and N uptake (N_{upt}) and the respective exponential regression (dotted line) from the Hollage experiment and the experimental series.

Conclusion

In a range of experiments, the used sensor could gather useful data describing biomass and nutritional status of maize. Although the coefficients of determination and a shift of the obtained red edge inflection point (REIP) due to different background noise or canopy architecture do not allow a direct calculation of crop parameters from the REIP values, the used handheld sensor is a viable tool to quantify growth differences within one field trial. Regarding the objectivity and easiness of spectral handheld sensors, they can be a powerful tool in field trials.

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Supplementary Table 6: Raw data means of four replications for aboveground dry matter biomass (DM), whole plant nitrogen concentrations (N_{conc}), plant nitrogen uptake (N_{upt}) and red edge inflection point (REIP) for the treatments in the Hollage experiment (control (C-), broadcast (B+), injection (I-), and injection with nitrification inhibitor (I(N)-)).

		2014				2015				2016			
growth stage[1]	treatment[2]	DM kg ha^{-1}	N_{conc} g kg^{-1}	N_{upt} kg ha^{-1}	REIP nm	DM kg ha^{-1}	N_{conc} g kg^{-1}	N_{upt} kg ha^{-1}	REIP nm	DM kg ha^{-1}	N_{conc} g kg^{-1}	N_{upt} kg ha^{-1}	REIP nm
V4	C-	no data				21.8	41.21	0.92	712.3	13.4	42.7	0.57	714.5
	B+					33.5	50.60	1.74	714.4	29.1	52.9	1.55	717.2
	I-					23.3	47.68	1.14	713.0	16.8	48.3	0.81	716.0
	I(N)-					29.0	50.77	1.50	714.3	21.2	52.5	1.11	716.1
V6	C-	169	28.7	4.8	718.2	57.6	37.53	2.21	713.6	71.2	39.9	2.84	716.7
	B+	466	28.0	13.1	719.3	116.7	43.42	5.18	716.6	172.6	44.6	7.69	720.3
	I-	523	35.6	18.6	721.0	62.3	43.30	2.75	714.5	97.5	43.2	4.22	718.3
	I(N)-	569	38.1	21.7	721.0	102.8	46.51	4.89	716.3	138.6	46.2	6.42	719.5
V8	C-	no data				213.9	43.05	9.41	716.5	328	33.0	10.84	719.2
	B+					457.3	41.67	19.47	720.8	1011	37.7	38.24	722.6
	I-					288.1	49.48	14.55	719.7	675	40.5	27.19	722.0
	I(N)-					455.3	50.17	23.33	721.0	839	40.5	34.03	722.2
V10	C-	1044	18.1	18.9	721.0	707	41.90	30.17	721.9	1217	21.4	32.43	721.3
	B+	1860	18.4	34.1	720.8	1617	39.43	65.14	723.6	2812	25.5	89.21	724.1
	I-	2803	20.3	57.0	723.8	937	44.98	43.10	723.4	2269	24.5	69.29	723.6
	I(N)-	3055	22.7	69.5	724.1	1465	43.76	65.54	724.1	2435	25.9	78.64	724.0
VT	C-	5880	11.1	65.1	721.6	7509	16.76	128.68	723.6	no data			
	B+	8706	12.1	104.8	724.2	10513	19.99	214.78	722.9				
	I-	12294	11.6	143.2	724.5	8293	21.09	178.76	723.7				
	I(N)-	12835	12.0	155.0	724.7	9212	20.26	190.67	723.4				

[1] Vn = vegetative leaf stage n, VT = tasseling

[2] treatments fertilized with liquid manure (C = Control without manure; B = Manure broadcast; I = Manure injection; I(N) = Manure injection with nitrification inhibitor) and without (-), or with (+) mineral starter fertilizer, as described in section ‘Experimental design and treatments’

Supplementary Table 7: Raw data means of four replications for aboveground dry matter biomass (DM), whole plant nitrogen concentrations (N_{conc}), plant nitrogen uptake (N_{upt}) and red edge inflection point (REIP) for the treatments an V8 growth stage in the experimental series.

	2014				2015			
treatment[1]	DM	N_{conc}	N_{upt}	REIP	DM	N_{conc}	N_{upt}	REIP
	kg ha^{-1}	g kg^{-1}	kg ha^{-1}	nm	kg ha^{-1}	g kg^{-1}	kg ha^{-1}	nm
Bovenau								
C-	752	42.88	32.32	720.9	275	36.44	10.10	719.3
C+	1015	41.83	42.22	721.6	331	37.20	12.40	720.6
B-	761	43.48	33.02	720.5	289	38.64	11.18	719.9
B+	999	41.87	41.84	721.9	391	38.87	15.21	721.3
I-	758	45.05	34.17	720.9	270	39.86	10.84	720.3
I+	964	43.17	41.67	721.7	320	38.81	12.66	720.2
I(N)-	943	45.47	42.87	721.6	255	38.65	9.95	720.2
I(N)+	1177	44.58	52.49	722.6	302	39.97	12.10	721.4
Haus Düsse								
C-	278	38.36	10.67	717.7	no data			
C+	442	39.43	17.44	720.1				
B-	352	38.46	13.63	718.3				
B+	542	39.19	21.29	720.2				
I-	602	43.29	26.06	720.2				
I+	745	42.74	31.84	721.0				
I(N)-	694	42.58	29.56	720.7				
I(N)+	695	42.98	29.85	722.0				
Merfeld								
C-	658	37.15	24.42	721.6	351	46.30	16.25	722.3
C+	973	36.39	35.40	722.6	669	44.04	29.38	723.2
B-	946	37.05	35.06	722.2	371	44.64	16.57	721.3
B+	1134	36.99	41.89	723.0	705	42.69	30.01	723.4
I-	1063	38.77	41.24	723.3	542	45.01	24.39	722.1
I+	1197	38.25	45.76	723.4	652	44.77	29.16	723.8
I(N)-	1087	39.34	42.63	723.3	582	47.66	27.75	722.8
I(N)+	1300	40.02	52.07	723.2	640	45.52	29.37	723.3
Sandkrug								
C-	387	42.17	16.32	720.5	388	41.06	15.91	718.0
C+	443	42.74	18.91	721.0	745	40.97	30.50	721.1
B-	486	45.18	21.93	721.3	461	43.47	20.09	718.4
B+	581	45.04	26.18	721.9	841	41.89	35.22	721.0
I-	574	46.53	26.73	721.5	511	49.65	25.34	719.7
I+	641	46.55	29.84	722.2	680	50.36	34.29	721.4
I(N)-	551	45.47	25.04	721.5	556	48.05	26.67	720.3
I(N)+	632	45.88	29.01	722.2	736	49.38	36.40	721.4
Schuby								
C-	567	37.15	21.15	721.2	186	41.36	7.72	720.0
C+	776	37.70	29.31	722.0	413	41.66	17.22	721.1
B-	772	40.31	31.14	721.7	259	44.10	11.47	721.2
B+	864	40.07	34.59	721.8	382	43.20	16.51	721.5
I-	773	42.33	32.70	722.0	240	44.72	10.72	720.6
I+	825	41.99	34.64	722.1	389	43.85	17.09	721.6
I(N)-	697	44.48	31.01	721.9	294	44.24	13.16	720.8
I(N)+	959	43.69	41.82	722.6	436	43.67	19.01	721.3
Wehnen								
C-	317	39.06	12.50	718.0	201	37.17	7.54	721.0
C+	542	40.58	22.04	721.5	1121	32.89	36.88	723.3
B-	289	37.98	10.98	718.1	264	36.86	9.74	720.9
B+	607	40.00	24.38	721.2	1240	32.35	40.26	723.1
I-	382	41.36	15.83	719.4	612	39.80	24.33	722.7
I+	699	41.37	28.97	722.0	1114	38.28	42.64	724.4
I(N)-	502	41.05	20.78	720.9	1145	37.58	43.06	725.1
I(N)+	682	41.50	28.22	722.5	1475	37.95	55.81	725.4

[1] treatments fertilized with liquid manure (C = Control without manure; B = Manure broadcast; I = Manure injection; I(N) = Manure injection with nitrification inhibitor) and without (-), or with (+) mineral starter fertilizer, as described in section ‘Experimental design and treatments’

Chapter 3

3. General discussion

3.1 Reflection of research questions

Understanding the fate of nutrients from manure applied to farmland, the complex interactions between climate, soils, nutrients and plants is crucial for an economically successful and environmentally sound production of food, feed and fiber for a growing global population. Several experiments were conducted to gather better insight to support advisors, decision makers, producers of technologies and supplies, agronomists and farmers to sustainably intensify maize production in northwestern Germany. The following questions served as guideline to improve nutrient use efficiencies from liquid manures in maize:

a) is it possible to obviate mineral starter fertilizer when injecting liquid manure below the maize row?

b) how long does the nitrification of slurry-ammonium-nitrogen to nitrate take under field conditions and what are the consequences on nitrate displacement?

c) are nitrification inhibitors able to delay nitrification and what are the consequences on crop nutrition?

d) can hyperspectral sensors be used to improve understanding of crop nutritional status throughout the cropping period?

The aim of the project was to gather scientific knowledge about nutrient interactions following slurry injection to improve decision support by exchanging information with farmers, contractors, advisors, companies and scientists.

3.2 Manure injection to obviate mineral starter fertilizers –potentials and limitations

Regarding the results of Section 2.1 on crop performance comparing liquid manure broadcast and injection treatments on a range of sites throughout northwestern Germany, the results indicate no need for mineral starter fertilizers. In all trials only minor differences between fertilized treatments in maize yields and silage quality were observed. The experiments in Section 2.2 allow a very detailed discussion comparing common farm practice system (manure broadcast plus mineral starter fertilizer) with manure injection systems without starter fertilizer. In the 2014 season of the experiment, sustained precipitation in May and June promoted nitrate leaching and thus, favored the performance of injection treatments. Alternatively, in 2015 no leaching of nitrate occurred due to minor precipitation, but cold temperatures led to P deficiency in treatments without mineral starter fertilizers. The results indicate comparable yields for injection treatments for both seasons, although N and P rates were reduced. This leads to the conclusion that manure injection is a slurry application strategy capable to enhance nutrient use efficiency from manure. Additional investigations showed a strong increase of N_2O emissions when manure is injected prior to planting (Zurheide et al. 2016). Besides N_2O emissions during nitrification of NH_4^+, high NO_3^- concentrations in the manure band following manure application until V10 stage provide ample substrate for denitrification. A peak of N_2O emissions was observed from beginning of May until the end of June. This finding should be considered when evaluating the environmental impacts of manure injection.

The trials have also shown the importance of nitrification inhibitors when manure is applied prior to planting of maize, but major N uptake starts 8 to 10 weeks after fertilization. Mixing nitrification inhibitors into the slurry prior to application significantly delayed NO_3^- formation, which was a key to the reduction of leaching. This delay in NO_3^- formation furthermore reduced the N_2O emissions by 50%, compared to injection without NI (Zurheide et al. 2016). Additionally, under low soil temperatures, the addition of a nitrification inhibitor to the applied manure improved early growth nutrition and thus, crop growth. This might be due to:

a) higher preference for ammonium uptake under low root zone temperatures, as it requires less energy (Macduff and Jackson 1991).
b) interaction of ammonium and P, which is widely recognized and the reason for using N and P containing starter fertilizers (Ma et al. 2013; Ohlrogge 1962).
c) physiological effects of the nitrification inhibitor on plant growth (Thomson et al. 1993).

Plant growth inhibitory effects have been shown by Thomson et al. (1993) for the compound nitrapyrin. However, an inhibitory effect is unlikely for the azol-based compounds of the products used in our study (1,2,4-Triazol; 3-Methylpyrazol; 3,4-Dimethylpyrazol-Phosphate), as azol-compounds are widely used as fungicides. In contrast to the crop growth promoting effects of Strobilurin-fungicides (Ruffo et al. 2015), for azol-compounds no physiological effects on plants have been shown so far. Summing up, the use of nitrification inhibitors in combination with manure injection brings certain advantages, but for a better understanding of the processes further knowledge of the implications of nitrification inhibitors on nitrification, their interference in the soil-plant-system and the environment are necessary.

Although manure injection significantly reduced nitrate displacement at Hollage in 2014 (Westerschulte et al. 2017), the considerable time lag between manure application (April) and major N uptake of maize (end of June) still led to leaching of NO_3^-. Thus, on sites with increased leaching risk, applying the total amount of N prior to maize planting is questionable.

A major benefit of splitting manure applications is the possibility to adjust the second dose to current crop needs. Besides common methods as pre-side dress SMN sampling, plant-sap nitrate concentrations, and tissue analysis, the experiments in Section 2.3 have shown the possibility to use spectral measurements for the assessment of crop performance. These measurements provide the possibility to adapt fertilization rates on a site-specific level in high spatial resolution. Therefore, reflectance sensors mounted to either tractor, drone, or satellite can be used.

As also shown in Section 2.3, vegetation indices are precise in detecting growth differences but so far, they are not able to provide stand-alone fertilizer application maps without any referencing. Thus, ideas evolved to fuse vegetation indices with soil parameters, current cropping details and historic vegetation data allow a better prediction of crop nutrient demand. The so called “digital farming” approach also allows for the integration of satellite imaging and other crop sensing technologies with models for crop growth and soil nutrient availability to increase the precision of fertilizer requirement prediction.

Another major invention of recent years is the on-the-go determination of manure nutrient composition via near infrared spectrometry (Zimmermann et al. 2008). With the possibility to measure the composition of manure, it is possible to switch manure fertilization from the rather “disposal” based rating in “m^3 ha^{-1}” to the rather “fertilizer value” based rating in “kg N ha^{-1}”, or “kg P ha^{-1}”.

Together, these technological advances ease appropriate fertilization and allow higher nutrient use efficiency from manure applied at later growth stages. Further positive side effects of split applications might result from a reduction of high nitrate concentrations that are prone to denitrification. Split applications are common in other monocots like grass and cereals, allowing a certain adjustment to current crop needs. In these cropping systems N losses can be reduced. Thus, on sites with a very high leaching risk, switching cropping and fertilizing systems, or converting these sites to permanent grassland to reduce leaching is reasonable. It is quite common to top dress liquid manure in maize at 6 to 10 leaf stages with trailing hoses mainly to relieve manure storage capacities, or to adapt N application to pre-side dress SMN test. This practice, however, can lead to substantial N losses via ammonia volatilization and, consequently, to low N use efficiency (Ball Coelho et al. 2005), especially when dry weather follows manure application.

Injection of manure in growing crops might result in root damage but increases nutrient availability. Compared to preplant applications, lower soil moisture reduces the risk of soil compaction and N losses (i.e. nitrate and nitrous oxide). Still, substantial soil movement is required, which disturbs herbicide layers and promotes germination of thermophilic weeds. On the other hand, late injection might be combined with mechanical weed control, establishment of an undersown crop or an herbicide application.

Nevertheless, certain limitations for slurry injection exist. First, manure injection requires significant tillage to provide space for the slurry, which is applied at significant volumes. As there are no possibilities for further seedbed preparation after slurry injection, the injectors need to finalize the seedbed on the go. With the injectors currently available for farmers, this restricts the use of slurry injection to coarse textured soils, where formation of clods is unlikely during tillage operations. When using auto-guidance systems to trace the manure bands however, shallow seedbed preparation with active or passive harrows could be an option to overcome this restriction. Additionally, for finer textured soils, when nitrification inhibitors are used, injecting earlier in the season (e.g. 4 to 6 weeks prior to maize planting) would favor the weathering of clods into smaller aggregates and increase soil temperatures, compared to later tillage dates.

A major issue with manure injection compared to broadcast application is the need for heavy machinery with reduced working width. While trailing hose applicators can use existing tramlines (15 to 36 m), the drag requirements of injectors currently limit the working width to 6 m. Thus, one in four rows is rolled over by the manure tanker-injector combination typically leading to soil compaction and reduced yields (Nevens and Reheul 2003).

When soil is deep-tilled prior to manure application, these tracks are to be tilled again and adequate machine setting needs special attention. To reduce costs for tillage as well as roll-over compaction, farmers combine manure injection with strip tillage where the only tillage operation is done when injecting manure, after rolling over untilled soil. Although strip tillage requires highly trained machinery operators, increased attention to crop protection and is only possible if there is no soil compaction, farmers prefer strip till injection instead of injection in tilled soils.

This is mainly based on the fear of increased costs for manure management and application. Unfortunately, during the project there was no opportunity to get insight into the economics of different manure injection strategies.

3.3 Implications on regional nutrient flows

Comparatively high nutrient use efficiency from manure can be achieved in maize production. Consequently, in regions where ample amounts of manure are available, maize plays a key role in crop rotations. However, this is to a certain extent due to high mineralization potential from organicaly bound soil and manure N and leads to a decomposition of organic matter. Thus, negative N balances in maize cropping are common and N cycles on silage maize fields for biogas or fodder can only be closed when organic matter is somehow replenished through a proper crop rotation.

The average N and P uptake of silage maize in the trials was ~220 kg N ha^{-1}, and ~40 kg P ha^{-1}, respectively, reflecting the mean nutrient uptake for silage maize in the region. Fertilizer recommendations based on a recommended value of 180 kg N ha^{-1} typically allows organic N application rates of ~170 kg N ha^{-1}, which is the current ceiling level for N fertilization with animal manure according to the German Fertilizer Ordinance (DüV 2007). In the revised German Fertilizer Ordinance, this ceiling level of 170 kg N ha^{-1} will most likely be expanded to all types of organic N fertilizers (Bundesministerium für Ernährung und Landwirtschaft 2017).

Crop P withdrawal should be the ceiling for any P fertilization as soil P status is typically high to very high in the region, and reduced P fertilization is recommended (Kerschberger et al. 1997; Taube et al. 2015). Consequently, the obviation of mineral starter fertilizers containing 10 kg P ha^{-1}, would significantly relieve P balances in maize cropping. As maize is cropped on 50% of the cropland in many regions in north-western Germany, obviation of mineral starter fertilizers at a standard rate of 23 kg N ha^{-1} and 10 kg P ha^{-1} could decrease nutrient imports. Consequently, for many farmers manure export requirements could be reduced.

Only forage ley shows higher requirements than silage maize. If cropped leys are used to reduce feed imports, nutrient balances might be unburdened. Thus, a shift of crop rotations to other crops than maize will not significantly relieve N and P balances. Similar research approaches in the Netherlands (Schröder et al. 2015), Denmark (Petersen et al. 2010), Canada (Bittman et al. 2012), and the US (Schmitt et al. 1995) led to likewise conclusions. Consequently, the present results might also be of interest for researchers and farmers in the aforementioned, as well as other regions where intensive livestock and/or biogas production is combined with intensive maize cropping. Furthermore, these results might also be used to enhance nutrient use efficiency from manure in other spring sown row crops like sugar beet, potato, or sunflower.

Thus, in areas of intensive livestock farming, obviating mineral starter fertilizers with manure injection is a step towards consequent nutrient cycling and increases the sustainability of agriculture. However, additional measures to decrease imports of N and P (i.e. increasing nutrient use efficiency in animal husbandry or shifting cropland from biogas substrate production to cropping of feed) need to be applied, as the potential for higher crop N and P uptake is limited.

Chapter 4

Conclusions and outlook

Conclusions and outlook

To overcome early growth nutrient deficiency, maize needs starter fertilizers to achieve optimum growth rates. The present results show the potentials of using injected slurry as starter fertilizer for maize to reduce imports of mineral fertilizer in areas of intensive livestock farming.

In a series of field experiments (22 site-years), slurry injection led to equal yields, compared to slurry broadcast plus mineral starter fertilizer. Mean nitrogen uptake of maize was enhanced when manure was injected. Together with reduced nitrogen rates, it led to increased nitrogen use efficiency.

When major precipitation events during early growth stage favor the displacement of nitrate out of the root zone, the nitrification is retarded. A further slowdown of nitrification and nitrate movement can be achieved by adding a nitrification inhibitor to the injected slurry. Under severe leaching conditions this leads to higher yields and nitrogen uptake. When air and soil temperatures as well as precipitation during early growth are low, phosphorus availability is limited. The „starter fertilizer value“ of manure can be enhanced by adding a nitrification inhibitor as the duration of high ammonium-nitrogen and phosphorus concentrations in close proximity of maize seedlings increases nutrient availability in cold soils.

Nevertheless, our trials showed that manure injection only delays nitrification and nitrate displacement out of the root zone but does not totally inhibit nitrate leaching. Additionally, high concentrations of nitrate in the soil for a prolonged period promotes denitrification and nitrous oxide emissions. Although nitrification inhibitors significally decreased N losses, on sites with high leaching or denitrification potential, splitting of nitrogen fertilization to improve the synchrony with crop nitrogen requirements is advised.

Compared to the current common farm practice of broadcasting manure and using mineral starter fertilizers at maize planting, slurry injection showed a higher resilience to disadvantageous weather extremes. Furthermore, nutrient use efficiency from liquid manure was always enhanced. Thus, imports for mineral starter fertilizers might be reduced.

The adoption of manure injection to obviate mineral starter fertilizers by farmers would align with upcoming legislation concerning manure management, increase on-farm nutrient cycling and, thus, meeting the goals of sustainable intensification of agricultural production in Europe. Concerns like potentially higher nitrous oxide emissions and the fate of nitrification inhibitors as well as their metabolites need further attention in upcoming research activities.

Field trials with high spatial and and adequate temporal resolution during critical growth stages of a crop are necessary to improve agronomic systems. To reduce costs and increase objectivity, vegetation indices based on spectral measurements of plants can be used. Furthermore, these indices might be used for in-season, site-specific fertilizer recommendations and variable rate application maps.

For a final comparison of the tested fertilization strategies, a life cycle assessment would help with adjusting legal frameworks. Furthermore, an economic cost-benefit analysis could affect farmer’s decisions and show potentials for agri-environmental compensation payments.

Summary

The expansion of livestock husbandry and biogas production in large parts of northwestern Germany during the last two decades increased the amount of accruing manure, as well as the demand for maize as fodder crop and substrate for biogas plants. To overcome phosphorus deficiency symptoms during early growth of maize, farmers commonly apply mineral starter fertilizers containing ammonium-nitrogen and phosphorus on top of the usual manure applications required to meet crop nutrient demand. This practice typically leads to overfertilization of N and P and the excess nutrients are then prone to be lost into the environment.

Recent developments of agricultural machinery allow for the injection of slurry bands into the soil prior to maize planting. Due to high concentrations of ammonium and phosphorus in the manure band, chemical transformation and translocation of these nutrients is reduced. When the bands are placed near the seeds, even the radicles can access the applied nutrients. Hence, application of mineral starter fertilizers might be obviated. Earlier investigations showed insufficient knowledge of nutrient transformations in manure bands and their consequences on crop growth. To resolve these problems a research project at the University of Applied Sciences Osnabrück was conducted in close cooperation with the local agricultural extension services, machinery producers and farmers.

In a series of field trials, broadcasting of liquid manure was compared to injection with and without a nitrification inhibitor in three consecutive growing seasons (2013 to 2015). The trials were conducted in a split-plot design, where all liquid manure treatments were divided in subplots with and without a mineral starter fertilizer. Biomass samplings at eight leaves stage and harvest gave insight into the performance of the treatments. Compared to broadcast application with starter fertilizer, manure injection showed slightly retarded early growth in some trials. However, yields and nitrogen uptake at harvest were similar. When a nitrification inhibitor was added to the injected manure, early growth was not retarded, yields were alike broadcast and injection treatments, but nitrogen uptake was higher in all seasons (on average ~7%).

To further investigate nitrogen dynamics and crop growth, another field trial was conducted on a sandy soil close to Osnabrück in 2014 and 2015. Manure injection with and without a nitrification inhibitor was compared to broadcast application with mineral starter fertilizer and an unfertilized control treatment. Plant samplings were taken at regular intervals. Major precipitation events in May and June 2014 led to significant nitrate leaching, especially in the broadcast treatment. Manure injection delayed the nitrification of slurry ammonium and consequently the translocation out of the root zone. Thus, plants in injection treatments could accumulate more nitrogen in their biomass and showed less nitrogen deficiency symptoms. This led to increased yield (+16.5%) and nitrogen uptake (+9.6%) for injection treatment with nitrification inhibitor compared to broadcast treatment. In 2015, low temperatures impaired seminal root growth and phosphorus availability. The mineral starter fertilizer in the broadcast treatment led to better early growth than injected slurry. When a nitrification inhibitor was added to the injected manure, less P deficiency symptoms were observed, and the crop growth was only slightly retarded. Due to the high compensation potential of silage maize, these differences were equalized until harvest. Nevertheless, the mean apparent nitrogen recovery efficiency of both seasons was higher in injection treatments with and without nitrification inhibitor, compared to broadcast with mineral starter fertilizer (48%, 56% and 43%, respectively).

To ease the handling of field trial series by decreasing the number of tissue samplings, the use of a handheld sensor was tested during vegetative growth of maize. In the series of field trials with the local extension service, the derived vegetation index showed significant correlations to biomass and nitrogen uptake at eight leaves stage. Measurements of the vegetative growth observed during the nitrogen dynamics trial showed that the sensor needs sufficient leaf area to deliver precise data, but also tends to saturate when maize tassels evolve. The best estimates were found between six and ten leaves. Thus, the sensor can be a valuable tool to reduce numbers of tissue samples and, thus, time and effort needed in fertilization trials.

Altogether, these results should encourage farmers to obviate mineral starter fertilizers by using manure injection when cropping maize on sandy soils. The advantages that come along with manure injection based on the present research indicate higher shares of manure nutrients find their way into the plants due to delayed biochemical transformations. These nutrients are consequently not lost into the environment. Nitrification inhibitors have shown a positive effect on crop performance and led to a further reduction of nitrogen losses. However, further knowledge of their decomposition with special regard to the ecological impact of their compounds and metabolites need to be thoroughly evaluated.

Zusammenfassung

Die Intensivierung der Viehhaltung und die Ausweitung der Biogasproduktion in Nordwestdeutschland führte in den vergangenen Dekaden zu einem steigenden Anfall an organischen Düngern und zur Ausweitung des Maisanbaus. Um eingeschränkten Nährstoffverfügbarkeiten in der frühen Jugendentwicklung des Maises entgegenzuwirken setzen Landwirte mineralische NP-Unterfußdünger ein, obwohl ausreichende Wirtschaftsdüngergaben den Nährstoffbedarf üblicherweise decken. Die aus dieser Praxis resultierenden Nährstoffüberschüsse führen zu Nährstoffakkumulationen in den Böden und können in nicht agrarische Ökosysteme verlagert werden.

Neuere Entwicklungen der Agrartechnik ermöglichen es, Gülle in Bändern unter der später gelegten Maisreihe zu platzieren. Durch die hohen Konzentrationen von Ammonium-Stickstoff und Phosphat im Gülleband werden sowohl die Umwandlung als auch die Verlagerung dieser Nährstoffe verzögert. Somit kann die Nährstoffversorgung über den Entwicklungszyklus hinweg sichergestellt werden. In vorausgegangenen Untersuchungen fiel häufig eine verzögerte Jugendentwicklung bei Gülleunterfußdüngung auf, die Erträge waren jedoch vergleichbar. Zur Klärung offener Fragen wurde an der Hochschule Osnabrück in Zusammenarbeit mit den Landwirtschaftskammern Niedersachsen, Nordrhein-Westfalen und Schleswig-Holstein, Firmen der Agrarindustrie, Lohnunternehmern und Landwirten ein von der Deutschen Bundesstiftung Umwelt finanziertes Forschungsprojekt durchgeführt.

In den Jahren 2013–2015 wurde in einer Versuchsserie auf acht Standorten die flächige Gülleeinarbeitung mit der Gülleunterfußdüngung mit und ohne Nitrifikationshemmstoff verglichen. Die Versuche wurden im Split-Plot Design angelegt, wobei alle Gülledüngungsvarianten jeweils mit und ohne mineralischer Unterfußdüngung angelegt wurden. Pflanzenproben zum 8-Blattstadium und zur Ernte gaben Aufschluss über Unterschiede im Wachstum der Pflanzen. Verglichen mit dem ortsüblichen Standardverfahren (flächige Gülleeinarbeitung mit mineralischer Unterfußdüngung), zeigten die Varianten mit Gülleunterfußdüngung auf manchen Standorten Nährstoffmangelsymptome und eine verzögerte Entwicklung. Zur Ernte waren Erträge und Stickstoffaufnahmen jedoch vergleichbar. Wurde der injizierten Gülle ein Nitrifikationshemmstoff zugesetzt, zeigten sich die Pflanzen in der Jugendentwicklung häufig vitaler und wüchsiger, was zwar nicht zu Ertragsunterschieden, jedoch zu erhöhten Stickstoffaufnahmen führte.

Um die Stickstoffdynamik in Boden und Pflanze detaillierter untersuchen zu können, wurde auf Schlägen mit sandigem Boden bei Osnabrück in den Jahren 2014 und 2015 ein weiterer Versuch angelegt. Dort wurden lediglich Gülleunterfußdüngung mit und ohne Nitrifikationshemmstoff mit der flächigen Gülleeinarbeitung mit mineralischer Unterfußdüngung sowie einer ungedüngten Kontrolle verglichen. Regelmäßige Boden- und Pflanzenuntersuchungen gaben Einblicke in die Stickstoffdynamik in den geprüften Verfahren. Starkniederschläge im Mai 2014 führten zu erheblicher Nitratverlagerung aus dem Wurzelraum, vor allem wenn die Gülle flächig eingearbeitet wurde. In den Güllebändern wurde das ausgebrachte Ammonium langsamer in Nitrat umgewandelt, die Stickstoffverlagerung war reduziert. So konnten die Pflanzen in den Gülleunterfußdüngungsvarianten mehr Stickstoff aufnehmen und zeigten geringere Mangelsymptome.

Folglich wurde in den durch Gülleunterfußdüngung mit Nitrifikationshemmstoff gedüngten Beständen bis zur Ernte 16,5% mehr Biomasse bilden und 9,6% mehr Stickstoff aufnehmen als im Standardverfahren.

Im Jahr 2015 hingegen waren Spross- und Wurzelwachstum sowie die Phosphorverfügbarkeit durch niedrige Temperaturen bis Ende Juni eingeschränkt. Das Phosphat aus dem mineralischen Unterfußdünger in der Standardvariante war in der Jugendentwicklung besser verfügbar als das aus der injizierten Gülle. Die Beimischung eines Nitrifikationshemmstoffs führte zu geringeren Ausprägungen von P-Mangelsymptomen, folglich war das Pflanzenwachstum besser. Bis zur Ernte verwuchsen sich diese Unterschiede jedoch weitestgehend. Zusammen mit einer geringeren Düngung konnte im Mittel der beiden Jahre höhere Anteile des gedüngten Stickstoffs im Aufwuchs wiedergefunden werden (48%, beziehungsweise 56% des gedüngten Stickstoffes in den Gülleunterfußdüngungsvarianten ohne und mit Nitrifikationshemmstoff, 43% des Stickstoffs in der Standardvariante).

Um innovative Produktionssysteme in die landwirtschaftliche Praxis zu bringen, sind umfangreiche Untersuchungen über mehrere Jahre und Standorte nötig. Die Anzahl anfallender Pflanzen- und Bodenproben sind entsprechend hoch und kostenintensiv. Als günstigere Alternative zur destruktiven Pflanzenprobennahme wurde der Einsatz eines handgeführten Reflexionssensors getestet. In der Versuchsserie zeigten die Sensormesswerte signifikante Zusammenhänge mit Biomasseaufwuchs und Stickstoffaufnahme im Acht-Blattstadium.

Im Stickstoffdynamikversuch in Osnabrück wurde zu verschiedenen Entwicklungsstadien die Messwerte mit den Aufwüchsen verglichen. Die beste Abschätzung für Pflanzenparameter lieferte der REIP zwischen Sechs- und Zehn-Blattstadium des Maises. Grundsätzlich ist der Sensor zum Vergleich unterschiedlicher Varianten hinsichtlich Biomasse und Stickstoffaufnahme gleichermaßen geeignet. dadurch können destruktive Parzellenbereiche entfallen. Es kann allerdings nicht unabhängig von Umwelteinflüssen vom erhobenen Messwert auf die Wachstumsparameter rückgeschlossen werden.

Zusammengefasst sollen die Ergebnisse Landwirte dazu ermutigen, die mineralische Unterfußdüngung durch eine platzierte Gülledüngung zu ersetzen. Die vorliegenden Untersuchungen haben gezeigt, dass die Nährstoffausnutzung aus organischen Wirtschaftsdüngern durch Gülleinjektion im Maisanbau auf sandigen Böden erheblich gesteigert werden kann. Insbesondere unter widrigen Bedingungen helfen Nitrifikationshemmstoffe, Stickstoffverluste zu vermindern und die Nährstoffverfügbarkeit zu steigern.

Detaillierte Untersuchungen zum Verbleib und zu den ökologischen Auswirkungen ihrer Wirkstoffe und Metaboliten sind jedoch für eine ganzheitliche Betrachtung mit einzubeziehen.

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Appendix

Abstracts of co-authored Papers

List of publications

a) peer reviewed publications
b) other publications

Conference contributions

a) talks
b) posters

Curriculum vitae

Abstracts of co-authored papers

Journal of Plant Nutrition (2018) 41 1381-1396

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

Matthias Westerschulte · Carl-Philipp Federolf · Dieter Trautz · Gabriele Broll · Hans-Werner Olfs

Keywords

Fertilizer placement · Starter fertilizer · Nutrient balances · Rhizosphere acidification · Micronutrients · Recovery efficiency

Article history

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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 2015: +33% P, +25% Zn, +39% Mn). Under P deficiency due to cold weather conditions broadcast application showed higher P uptake until 6-leaf (36–58%), while it was lower at 8- (32%) and 10-leaf (19%) stage compared to slurry injection (+NI). Zn availability was enhanced for slurry injection (+NI) during early growth and Zn and Mn uptakes were higher at harvest. Slurry injection decreased P balances by 10–14 kg P ha^{-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.

Journal of Plant Nutrition and Soil Science (2018) 181 69-77

A new chamber design for measuring nitrous oxide emissions in maize crops

Hans-Werner Olfs · Matthias Westerschulte · Nicolas Ruoss · Carl-Philipp Federolf · Tim Zurheide · Maria Elena Vergara Hernandez · Nikolas Neddermann · Dieter Trautz · Herbert Pralle · Roland Fuß · Reinhard Well

Keywords

Air tightness · Gas sampling · N_2O flux measurements · Split chamber · Row crops · *Zea mays*

Article history

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DOI: 10.1002/jpln.201700008

Abstract

Nitrous oxide (N_2O) emissions from agricultural land are often estimated by measuring changes in N_2O concentrations over a given period in the headspace of a gas-sampling chamber covering a specific soil area. This technique is particularly challenging in tall growing row crops such as maize (*Zea mays* L.), to which farmers regularly apply fertilizer banded below the seeds to ensure good crop development. Placing chambers in the inter-row space leads to bias in flux measurements, due to exclusion of fertilized and rhizosphere soil. Chambers for N_2O flux measurements should therefore be placed centered over the row. A new split chamber for gas sampling was developed in this study from a closed, rectangular chamber (original chamber: 78 cm × 78 cm, 51 cm height). The new chamber is applicable for use for the complete maize growing cycle until harvest. For each flux measurement, the two parts of the chambers are placed in a gas-tight seal on a collar previously inserted into soil covering a representative area of land. In a later growth stage, when plant height exceeds chamber height, stalks of developed maize plants can be fixed between the two chamber parts through a rubber-tightening opening on the top of the chamber. Air tightness of the split chamber was tested in the laboratory and the split chamber was compared with the original chamber in a field experiment with slurry injection under maize seeds. The laboratory test demonstrated similar air tightness of both chamber types. The field test yielded almost identical N_2O fluxes for the original chamber (244 µg N_2O-N m^{-1} h^{-1}) and the split-chamber (254 µg N_2O-N m^{-1} h^{-1}). It can be concluded that the split chamber is an adequate gas-sampling unit, with particular advantages when flux measurements are conducted in tall growing row crops.

Nutrient Cycling in Agroecosystems (2017) 107 1-17

Nitrogen dynamics following slurry injection in maize: soil mineral nitrogen

Matthias Westerschulte · Carl-Philipp Federolf · Dieter Trautz · Gabriele Broll · Hans-Werner Olfs

Keywords

Spatial nitrogen distribution · Nitrate leaching · Nitrification inhibitor · Nitrogen displacement · Liquid manure

Article history

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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.

Journal of Plant Nutrition and Soil Science 178 (2015) 923-934

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

Matthias Westerschulte · Carl-Philipp Federolf · Herbert Pralle · Dieter Trautz · Gabriele Broll · Hans-Werner Olfs

Keywords

Band application · Slurry injection · Mineral nitrogen · Soil monolith · Soil sampling method

Article history

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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.

List of publications

Peer reviewed publications

Federolf C-P, Westerschulte M, Olfs H-W, Broll G, Trautz D (2018) Assessing crop performance in maize field trials using a vegetation index. Open Agriculture (2018) 3:250-263. doi: 10.1515/opag-2018-0027

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Olfs H-W, Westerschulte M, Ruoss N, **Federolf** C-P, Zurheide T, Vergara Hernandes M-E, Neddermann N, Trautz D, Pralle H, Fuß R, Well R (2018) A new chamber design for measuring nitrous oxide emissions in maize crops. Journal of Plant Nutrition and Soil Science 181: 69-77. doi: 10.1002/jpln.201700008

Westerschulte M, **Federolf** C-P, Trautz D, Broll G, Olfs H-W (2018) Slurry injection with nitrification inhibitor in maize: Plant phosphorus, zinc and manganese status. Journal of Plant Nutrition 41:1381-1396. doi: 10.1080/01904167.2018.1452940

Westerschulte M, **Federolf** C-P, Trautz D, Broll G, Olfs H-W (2017) Nitrogen dynamics following slurry injection in maize: soil mineral nitrogen. Nutrient Cycling in Agroecosystems 107:1–17. doi: 10.1007/s10705-016-9799-5

Westerschulte M, **Federolf** C-P, Pralle H, Trautz D, Broll G, Olfs H-W (2015) Soil nitrogen dynamics after slurry injection in field trials: Evaluation of a soil sampling strategy. Journal of Plant Nutrition and Soil Science 178:923–934. doi: 10.1002/jpln.201500249

Other publications

Federolf C-P, Westerschulte M, Olfs H-W, Trautz D (2016a) 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 (2016b) Gülleunterfußdüngung zu Silomais im Nordwesten – Die Nährstoffbilanz entlasten. Landwirtschaft ohne Pflug 5:23–27

Federolf C-P, Westerschulte M, Olfs H-W, Trautz D (2016c) Gülleunterfußdüngung zu Mais – Nährstoffaufnahme in der Jugendentwicklung. Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften 28:48–49

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Olfs H-W, **Federolf** C-P, Westerschulte M, Trautz D (2016) Gülle-Injektion im Maisanbau - Mineralische Unterfußdüngung kann ersetzt werden. Mais 2:64–67

Olfs H-W, **Federolf** C-P, Westerschulte M, Trautz D (2015) Nitratauswaschung stoppen. DLZ Agrarmagazin Spezial Gülledüngung:16–18

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Trautz D, **Federolf** C-P, Westerschulte M, Neddermann N, Zurheide T, Olfs H-W, Vergara-Hernandez M-E (2017b) Effizienter geht es kaum. DLG-Mitteilungen 3:28-31

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2016a) Wirkung unterschiedlicher Nitrifikationshemmstoffe zur Stabilisierung des Ammoniumstickstoff bei Gülledepot-Applikation. VDLUFA-Schriftenreihe Bd. 72/2016:192–203

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Westerschulte M, Federolf C-P, Pralle H, Trautz D, Olfs H-W (2016c) Entwicklung einer Beprobungsmethode zur Beschreibung der Bodenstickstoffdynamik nach Gülleinjektion in Maisfeldversuchen. 47. DLG-Technikertagung: Aspekte des Versuchswesens in den Bereichen: Technik (insbesondere mobile Datenerfassung), Pflanzenbau, Pflanzenschutz und Qualität von Feldversuchen. Vorträge der Fachtagung vom 26. und 27. Januar 2016 in Hannover, 127–136

Westerschulte M, Federolf C-P, Pralle H, Trautz D, Olfs H-W (2015) Entwicklung einer Beprobungsmethode zur Beschreibung der Bodenstickstoffdynamik nach Gülleinjektion in Maisfeldversuchen. VDLUFA-Schriftenreihe Bd. 71/2015:194–205

Zurheide T, Pralle H, Westerschulte M, Federolf C-P, Vergara M-E, Trautz D, Olfs H-W (2016) Untersuchung von Lachgasemissionen bei Gülledepot-Applikation mit Zugabe von Nitrifikationshemmstoffen am Standort Osnabrück. VDLUFA-Schriftenreihe Bd. 72/2016: 52–62

Conference contributions

Talks

Federolf C-P, Westerschulte M, Olfs H-W, Trautz D (2017a) Gülleunterfußdüngung zur Optimierung der Stickstoffnutzungseffizienz im Maisanbau. Fachtagung Pflanzenbau, Staader Saatzucht e.G., 14.02.2017, Selsingen, Germany

Federolf C-P, Westerschulte M, Olfs H-W, Trautz D (2017b) Gülleunterfußdüngung zu Mais – Effizienterer Einsatz von Wirtschaftsdüngern. Informationsveranstaltung rund um Gewässerschutz und Nährstoffmanagement, Landwirtschaftskammer Nordrhein-Westfalen, 31.01.2017, Espelkamp/Frotheim, Germany

Federolf C-P, Westerschulte M, Olfs H-W, Trautz D (2016a) Gülleunterfußdüngung zur Optimierung der Stickstoffnutzungseffizienz im Maisanbau. SKW-Fachtagung ‚Düngung 2016' – Stickstoff in der Pflanze. 22.11.2016, Leipzig; 23.11.2016 Butzbach; 24.11.2016, 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 (2016c) 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 (2015a) Applikation von Wirtschaftsdüngern im Depot als Beitrag zur nachhaltigen Landbewirtschaftung. Projektpräsentation für Rainer Spiering (MdB), 20.10.2015, Osnabrück, Germany

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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 (2015d) 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 (2015e) Applikation von Wirtschaftsdüngern im Depot als Beitrag zur nachhaltigen Landbewirtschaftung 1. Pflanzenbauliche Aspekte. Osnabrücker Geographisches Kolloquium, University of Osnabrück, 27.05.2015, Osnabrück, Germany

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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

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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 (2016a) 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

Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2016b) Gülle-Depotapplikation im Maisanbau – Entwicklung einer neuen Bodenbeprobungsmethode. 5. Doktoranden-Symposium 2016, Fachhochschule Münster und Hochschule Osnabrück, 18.11.2016, Münster, Germany

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Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2015b) 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

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Westerschulte M, Federolf C-P, Trautz D, Olfs H-W (2014b) N_{min}-Bodenbeprobung bei platzierter 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) Gülledepot zu Mais II. Ertragsleistung, Landwirtschaft und Wasserschutz – Feldtag für Kooperationslandwirte und Wasserschutzberatung, Landwirtschaftskammer Niedersachsen, 15.06.2016, Oldenburg, Germany

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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 phosphor 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, Landwirtschaftskammer Niedersachsen, 23.–24.09.2014, Oldenburg, Germany

Curriculum vitae

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Professional career

2013 - 2017	Research associate University of Applied Sciences Osnabrück, Faculty of Agricultural Sciences and Landscape Architecture, Working group 'Sustainable Agro-Ecosystems' Research Project 'Optimizing nitrogen and phosphor use efficiencies from liquid manure by slurry injection to reduce environmental pollution'

Education

2014 - current	PhD candidate University of Osnabrück Institute of Geography
2011 - 2013	Master of Science University of Hohenheim, Master programme in Agricultural Sciences - Major: Crop production systems Thesis: 'Einfluss von Bodenbearbeitung und Vornutzung auf Zugkraftbedarf, Feldaufgang und Ertrag von Silomais (*Zea mays*)'
2007 - 2011	Bachelor of Science University of Hohenheim, Bachelor programme in Agricultural Sciences - Major: Agricultural Engineering Thesis: 'Einfluss des Reihenabstandes auf das Bestandesklima und den Ertrag von Winterweizen'
2004 - 2007	Secondary school Gewerbliche Schule Öhringen - Technisches Gymnasium Degree: Abitur
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1993 - 1998	Primary school Grund- und Hauptschule Neuenstein

Erklärung über die Eigenständigkeit der erbrachten wissenschaftlichen Leistung

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