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Cattle slurry acidification and application method can improve initial phosphorus availability for maize

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Abstract

Background and aims The utilization of phosphorus (P) in cattle slurry as a starter fertilizer in maize cropping is poor. To improve this and to obviate the use of additional mineral starter-P fertilization, we examined if slurry acidification, placement strategy and application time could increase maize yield and phosphorus uptake (PU) in the early growth stages.

Methods In a climate-controlled pot experiment, untreated (pH 6.5) and acidified (pH 5.5 or pH 3.8) cattle slurry was injected in narrow or broad bands two or 30 days before sowing of maize on a coarse sandy and a sandy loam soil and compared with mineral P fertilizer.

Results After broad band slurry injection, the P concentration in maize tissues at the five-leaf stage and the dry matter yield at the seven-leaf stage were equal to or higher than the mineral P fertilizer treatment. Treatments with strongly acidified slurry (pH 3.8) had 49% higher PU at the seven-leaf stage compared to untreated slurry, but only on the sandy soil, suggesting an indirect pH effect on PU. Application time had no effect.

Conclusion Broad band slurry injection or strong acidification can improve early-stage growth of maize and potentially obviate the use of mineral P.

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Abbreviations

P	Phosphorus
N	Nitrogen
PU	Phosphorus uptake
DM	Dry matter
DAS	Days after sowing
pH 3.8	Strongly acidified slurry
pH 5.5	Moderately acidified slurry
pH 6.5	Untreated slurry
NB	Narrow band
BB	Broad band
L	Late application
E	Early application
WEP_i	Dissolved reactive water-extractable P
WEP_t	Total dissolved water-extractable P
WEP_u	Dissolved Unreactive water-extractable P

Introduction

Phosphorus (P) is an essential plant nutrient and crucial for future food security (Klinglmair et al. 2015). Mineable rock phosphate, the main source of P for production of mineral P fertilizers, is a non-renewable resource (Shepherd et al. 2016), which is very unevenly distributed globally (van Dijk et al. 2016). In developed countries mineral P is often used in excess, whereas in developing countries P fertilization is often limited because of lacking mineral resources and/or financial

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constraints (Shepherd et al. 2016). The local, the regional and the global P imbalances call for better management strategies of the finite P resource and development of circular economies with minimum use of fertilizers based on rock phosphates and reduced environmental damage (EC 2014; Withers et al. 2015).

Sufficient P supply from planting to the six-leaf stage can have a large impact on the final crop yield in maize (Barry and Miller 1989). Remobilization of seed-P reserve is completed after one week of growth, and in the critical period hereafter the maize plants are highly dependent on exogenous P uptake (Nadeem et al. 2011). The low mobility of P in soil combined with the high P demands from the fast-growing young maize plants with a not yet fully developed root system will often result in sub-optimal P nutrition of maize at this growth stage (Mollier and Pellerin 1999). The release of P from soil and fertilizer and the diffusion towards the roots will often be too slow to meet the demands of the maize plant (Syers et al. 2008). At the same time, low root zone temperatures in early spring in Northern Europe may further limit growth, since low root zone temperatures reduces both the root growth (Engels and Marschner 1990) and the diffusion rate of P (Grant et al. 2001). These conditions explain why maize often responds positively to the increase in soluble P concentrations in the vicinity of the young roots resulting from the application of mineral P as a starter fertilizer.

In 2015 silage maize was cropped on 183,000 ha in Denmark, corresponding to 7.0% of the agricultural area. In Denmark maize for silage is typically grown on livestock farms with excess of P in the supplied manure relative to crop demand. In spite of this, 10–15 kg P ha^{-1} mineral P fertilizer is routinely placed at the time of sowing to ensure sufficient P supply in the early growing stages (Knudsen 2010), corresponding to 16% of the annual mineral P fertilizer consumption (14.000 t P in 2014) in Denmark. The resulting P surplus in maize cropping contributes to the accumulation of P in soil (Rubæk et al. 2013), leading to a higher risk of P losses to the aquatic environment (Kronvang et al. 2009). It is generally accepted that the fertilizer effect of manure P is 80–100% of the effect of mineral P fertilizer (e.g. Rubæk and Sibbesen 1995), but some experiments indicate that manure P is not available to plants shortly after application to soil (Petersen et al. 2010).

The placement of the slurry affects the spatial distribution of P in the soil, and hence could partially overcome the low mobility of P and enhance the degree of exposure of slurry P to the root surface area. According to some studies (e.g. Bittman et al. 2012; Schröder et al. 2015), planting maize close to an injection band can promote early P uptake and growth of maize. However, Sawyer and Hoeft (1990) found an unfavorable root growth environment within the cattle manure injection zone, which could be due to the formation of phytotoxic organic acids under local anaerobic conditions in this zone (Lynch 1980), which may inhibit maize growth. To ensure a large contact zone between the roots and the slurry P and at the same time avoid unfavorable root conditions in a concentrated manure band, we speculated that injection of the slurry in a broad band with maize planted above the injection band could be advantageous, but we have found no studies reporting this approach.

Slurry acidification can minimize ammonia volatilization (e.g. Fangueiro et al. 2015; Kai et al. 2008) and increase the amount of dissolved P in manure (Christensen et al. 2009; Fordham and Schwertmann 1977). Since the solubility of manure inorganic P is pH dependent (Pagliari 2014), acidification may improve P availability in manure and the suitability of manure as a replacement for mineral P as a starter fertilizer. However, the pH and the buffer capacity of the soil might be important moderators of the initial effect of slurry acidification. Little is known about how strong the acidification has to be in order to improve P availability and enhance early maize growth, and even less is known about how these effects are moderated by different soil types and soil properties.

In practice, mineral P fertilizer is applied at the time of maize sowing. Applying slurry in advance of sowing could be beneficial since it would allow organic P compounds as well as compounds unfavorable for root growth to mineralize before plant emergence (Lynch 1980; Oberson and Joner 2005).

The question is whether it is possible to obviate the use of mineral P fertilizer by treating and applying manure P in a manner and time that would supply sufficient readily-available manure P to young maize plants and hence avoid over-fertilization and crop deficiencies of P. The aim of the present pot experiment was to study effects of cattle slurry acidification, application technique and application time on maize yield and P uptake (PU) at early growth stages compared with using mineral P starter fertilizer.

We hypothesized that: 1) Placement of slurry in a broad fertilizer band would improve early maize growth compared to placement in a narrow band; 2) acidification

of cattle slurry would increase the P solubility, providing a higher availability of exogenous P to maize and hence a better early maize growth; and 3) application of cattle slurry in advance of sowing would increase the P availability because the organic P compounds can be mineralized and made plant-available and/or growth-inhibiting organic substances can be degraded.

Materials and methods

Design

The study was conducted in pots in climate-controlled chambers as a fractional factorial experiment with three factors: 1) Acidification of cattle slurry (untreated slurry [pH 6.5], moderately acidified slurry [pH 5.5] and strongly acidified slurry [pH 3.8]), 2) application technique (narrow band [NB] or broad band [BB] injection) and 3) application time (two or 30 days before sowing, only tested for the narrow band injection). In total, nine different combinations with cattle slurry and two reference treatments receiving mineral N with and without mineral P fertilizer were tested (Table 1). The pot treatments were set up in a randomized complete-block design with three replicates. To minimize the positional effects in the growth chamber, the pots were re-randomized weekly within each chamber during growth.

Soils and cattle slurry acidification

The treatments were tested on a sandy loam and a coarse sand topsoil (0–15 cm) typical for maize cropping in Denmark (Table 2). The soils were sieved through a 2-cm sieve and mixed before application to pots.

Cattle slurry was obtained directly from a cattle house with dairy cows in October 2014 at Research Centre Foulum, Aarhus University. The slurry was mixed for three hours in a small tank before sampling and then stored at 2 °C until use. Acidification of slurry was carried out 32 days before sowing by adding 18 M sulfuric acid (H_2SO_4) while stirring until pH was 5.5 and 3.8, respectively. Slurry pH tended to increase slightly by storage, and the slurry pH was adjusted again just before application. In total, 1.70 ml H_2SO_4 per kg cattle slurry was added to reach pH 5.5, and 6.75 ml H_2SO_4 kg^{-1} cattle slurry was added to reach pH 3.8. Slurry properties after acidification are given in Table 3.

Pot preparation and fertilization

The soil was carefully packed and compacted in trapezoidal pots (upper length 27 cm, lower length 24 cm, upper width 14 cm, lower width 12 cm, height 20 cm). In each pot 4.8 kg dry weight soil was added, equivalent to approx. 13 cm height. Slurry injection was simulated by applying slurry to this lower soil layer in either a 4-cm narrow band (NB) or in a 13-cm broad band (BB, Fig. 1). A furrow was made in the lower soil layer in order to apply slurry in a NB, and an equivalent amount of soil was removed from the BB treatments and added to the upper soil layer. A total of 6.86 kg dry weight soil was added per pot, and compacted equivalent to an initial bulk density of 1.31 g cm^{-3}. The pots were placed in a greenhouse (day temperature 10 °C, night temperature 5 °C) until sowing.

Slurry was dosed according to the total P in slurry at a rate of 57 mg P kg^{-1} soil (490 g fresh slurry to each pot). This corresponds to 20 kg P ha^{-1}, assuming a distance of 75 cm between plant rows. Two days before sowing, 389 mg P was applied as Na_2HPO_4 dissolved in 50 ml water to the 20P treatment. All slurry treatments received 143 mg ammonium-nitrogen (NH_4^+-N) kg^{-1} soil in the slurry, corresponding to 50 kg NH_4^+-N ha^{-1}. Taking the expected immobilization of NH_4^+ in the slurry into account, about 40 kg NH_4^+-N ha^{-1} in slurry was expected to be available initially and the 0P and 20P treatments received 114 mg NH_4^+-N kg^{-1} soil as $(NH_4)_2SO_4$ dissolved in 20 ml water corresponding to 40 kg NH_4^+-N ha^{-1}. The liquid mineral fertilizers were applied to the lower soil layer similar to the placement of slurry in the NB treatments. In total, 20 and 70 ml water was applied in the 0P and 20P treatment, respectively.

Before sowing, soil moisture was adjusted to 60% of the pot capacity (Table 2). Three maize seeds (Zea mays L. var. Adept) were sown per pot at 3 cm depth and 3 cm above the fertilizer band (Fig. 1). The pots were placed in a greenhouse until germination (day temperature 15 °C, night temperature 12 °C). Non-germinated seeds were replanted with spare maize seedlings. To eliminate other nutrient deficiencies but P and potential salt damages due to high salt concentrations during germination, additional nutrient solutions were surface-applied twenty-six days after sowing (DAS) at rates (in mg kg^{-1} soil) of: 52 N (as $Mg(NO_3)_2$ and KNO_3), 42 K (as KNO_3), 8 S (as $MgSO_4$), 1.5 Mn (as $MnSO_4$), 1.3 Zn (as $ZnSO_4$), 0.6 B (as H_3BO_3), 1.5 Cu (as $CuSO_4$), 0.02 Co (as $CoCl_2$) and 0.3 Mo (as $NaMoO_4$).

Table 1 Treatment codes and the combinations of the three factors

Treatment code	Acidification level, slurry pH	Application time, days before sowing	Application technique, band width (cm)
0P		2 (late, L)	
20P		2 (late, L)	
pH 3.8 ENB	3.8	30 (early, E)	4 (NB[a])
pH 5.5 ENB	5.5	30 (early, E)	4 (NB)
pH 6.5 ENB	6.5	30 (early, E)	4 (NB)
pH 3.8 LNB	3.8	2 (late, L)	4 (NB)
pH 5.5 LNB	5.5	2 (late, L)	4 (NB)
pH 6.5 LNB	6.5	2 (late, L)	4 (NB)
pH 3.8 LBB	3.8	2 (late, L)	13 (BB[b])
pH 5.5 LBB	5.5	2 (late, L)	13 (BB)
pH 6.5 LBB	6.5	2 (late, L)	13 (BB)

[a] NB, Narrow band

[b] BB, Broad band

Climate conditions and irrigation

After germination (13 days after sowing) the pots were placed in climate-controlled chambers. The average temperature was 15 °C with a controlled fluctuation of ±4 °C day^{-1}, a mean temperature increase of 0.1 °C day^{-1} after 10 days, and a constant relative air humidity of 75 ± 10%. Plants were grown in 16-h photoperiods with light intensities ranging from 170 to 1060 µE m^{-2} s^{-1}. The pots were irrigated with demineralized water to a water content of 60% of the pot capacity during the first 35 DAS with increasing frequency from twice weekly to daily. After 35 days the pots were irrigated daily to 70% of the pot capacity to avoid draught until

Table 2 Characterization of the two soils used for the pot experiment. Standard deviation is given in parentheses ($n = 2$, except single point PSC $n = 3$)

	Coarse sandy soil	Sandy loam
Clay (<2 µm), g 100 g^{-1} soil	3.0 (0.4)	6.8 (0.3)
Silt (2–20 µm), g 100 g^{-1} soil	4.0 (0.3)	11.5 (0.6)
Coarse silt (20–63 µm), g 100 g^{-1} soil	1.9 (0.2)	11.3 (0.3)
Fine sand (63–200 µm), g 100 g^{-1} soil	22.1 (0.8)	30.1 (0.9)
Coarse sand (200–2000 µm), g 100 g^{-1} soil	66.8 (1.5)	38.0 (1.5)
Water content at pot capacity[a], %	33	34
pH (water)	6.3 (0.2)	6.5 (0.1)
Bicarbonate extractable P[b], mg 100 g^{-1} soil	4.8 (0.2)	4.0 (0.1)
Total C, g 100 g^{-1} soil	1.3 (0.1)	1.7 (0.0)
Exchangeable K, mg 100 g^{-1} soil	3.4 (0.2)	7.0 (0.3)
Total N, g 100 g^{-1} soil	0.09 (0.01)	0.14 (0.0)
Single point PSC[c], mg P 100 g^{-1} soil	23.7 (7.0)	35.3 (0.9)
CEC[d][e], mmol$_c$ kg^{-1}	60.7	93.2
Base saturation[e], kg 100 kg^{-1}	61.6	77.0

[a] Defined as the amount of water remaining in the pot after full irrigation and visible drainage has ceased (24–48 h of drainage) as described by Kirkham (2004)

[b] After Banderis et al. (1976) described in Sørensen and Bülow-Olsen (1994)

[c] PSC, Phosphorus sorption capacity after Bache and Williams (1971)

[d] CEC, cation exchange capacity

[e] After Kalra and Maynard (1991)

Table 3 The effect of slurry acidification on water-extractable reactive P (WEP_i), water-extractable unreactive P (WEP_u), total water-extractable P (WEP_t), ammonium-N (NH_4^+-N), N-total, dissolved Ca and Mg in slurry. Different letters within columns denote statistical significance (Tukey, $P < 0.05$)

Acidification level	DM %	Total P[*] g kg^{-1} dry weight slurry	WEP_i	WEP_u	WEP_t	NH_4^+-N	Total N	Ca	Mg
Strong (pH 3.8)	11.8	7.13	4.51[b]	0.37[a]	4.89[b]	14.7[b]	35.9[b]	6.94[a]	4.58[a]
Moderate (pH 5.5)	11.3	7.13	4.92[a]	0.40[a]	5.32[a]	16.2[a]	38.8[a]	4.70[b]	4.37[b]
Untreated (pH 6.5)	11.1	7.13	2.25[c]	0.40[a]	2.65[c]	16.6[a]	39.7[a]	2.61[c]	2.17[c]

[*] Measured before acidification

harvest. Occasional weeds were handpicked. Pest control was not required.

Plant and soil sampling

Entire pots with three plants were sampled destructively 45, 60 and 75 DAS corresponding to the five-leaf stage, six-leaf stage and seven-leaf stage, respectively. The plants were cut 1 cm above the soil surface and oven-dried (60 °C) to constant weight for determination of DM weights. The dried plant samples were ground to pass a 1-mm screen.

At each harvest, soil was sampled in the 0P, 20P, pH 5.5ENB, pH 6.5ENB, pH 5.5LNB and pH 6.5LNB treatments with a special sampling design, where samples originated from well-defined distances from the narrow fertilizer band. Soil was sampled from two different positions from the fertilizer band, but at the same depth: One soil sample below the narrow fertilizer band, and one soil sample at 5 cm horizontal distance from the narrow fertilizer band and 8 cm below the maize seed (Fig. 1). From each position a 25-mm diameter soil core was taken horizontally along the whole pot length (25 cm).

Analytical methods

Dissolved P, Mg and Ca in slurry were defined in this work as P, Mg and Ca present in the supernatant after centrifugation of the slurry for 30 min at 20 °C with a relative centrifugal force (RCF) of 15,029 x g. Dissolved, reactive water-extractable P (WEP_i) and total water-extractable P (WEP_t) in the cattle slurry were measured in the supernatant after extractions of 3 g slurry (fresh weight) shaken with 30 ml of deionized water for 1 h at 20 °C followed by centrifugation as described above. WEP_i concentrations were determined by spectrophotometry on the centrifuged sample using the molybdic blue method (ISO 6878 2004) modified after Murphy and Riley (1962). WEP_t in the slurry was determined in a subsample of the supernatant using acid persulfate digestion in an autoclave (120 °C, 200 kPa) (Koroleff 1983) followed by measurement of WEP_t concentrations with the colorimetric method described above. The concentration of dissolved Mg^{2+} and Ca^{2+} in the supernatant was measured using atom absorption spectroscopy. Slurry DM was determined by drying for 24 h at 80 °C. Total ammonium-N was measured by flow colorimetry (Sommer et al. 1992) and total N in slurry was analyzed using a Kjeldahl method. Total P in slurry was determined after digestion in perchloric and sulfuric acid (ISO 6878 2004).

Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) in soil were determined by flow colorimetry (Autoanalyzer III, Bran + Luebbe GmbH, D 22803 Nordersted, Germany) after extracting fresh soil immediately after sampling with 2 M KCl by shaking for 1 h (1:4, w/w). After extraction the soil was oven-dried for 24 h at 105 °C

Fig. 1 Cross-sectional view of the pot (*left* and *centre*). The pot seen from above (*right*) in the narrow band (NB) treatments
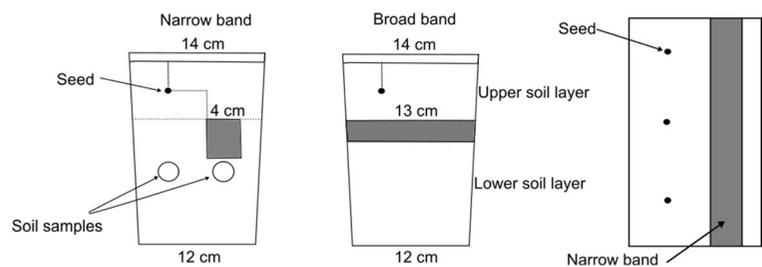
for dry weight determination. Soil samples for pH determination and P analyses were air-dried and sieved (2-mm sieve). Soil pH was measured in water suspensions (1:2.5, w/w). WEP_i and WEP_t in soil were measured in the supernatant after extraction of 1 g of air-dried soil shaken in 50 ml of deionized water for 1 h at 20 °C followed by centrifugation for 10 min at 20 °C with RCF at 1831 x g. Concentrations of WEP_i and WEP_t in soil extracts were determined as described for manure above.

The P concentration in plant tissue was determined by digesting 300 mg dried plant material in 3 ml H_2O_2 (9.7 M) and 6 ml HNO_3 (14.3 M) by pressurized microwave digestion. The P concentration in the diluted digest was determined by ICP-OES according to Liu et al. (2013).

Data calculation and statistical analysis

The fraction of unreactive water-extractable P (WEP_u) was determined as the difference between WEP_t and WEP_i. Total inorganic N (Nmin) in soil was calculated as the sum of NO_3^--N and NH_4^+-N. Total P uptake (PU) was calculated from dry matter weights and the P concentration of the whole plant.

The statistical analysis was conducted using the R-Project software package Version 3.2.3 (R Development Core Team 2015). Linear models were applied for the dependent variables P concentration, DM yield and PU. The ordinary least square approach was used for estimating the parameters in the linear models. In cases where the treatment effect was found to be significant in a one-way analysis of variance (ANOVA), further analyses were made to isolate differences between treatments (pairwise comparisons) using the general linear hypotheses (*glht*) function implemented in the *multcomp* package in R. The treatment effect was split up into the three factors (acidification, application technique and application time) and their interactions to identify significant predictors by performing a three-way ANOVA (type II) implemented in the *car* package in R. Direct comparisons of balanced grouped treatments were done by orthogonal main effects contrast statements by calculating the differences in LS means between levels of a significant factor implemented in the *contrast* package in R. DM yield at 75 DAS on the sandy loam, P concentration at 45 DAS on the sandy loam and PU at 60 DAS on the sandy soil were log-transformed to obtain normal distribution of residuals. Relationships between continuous variables were examined by calculating Pearson's correlation coefficient (r). Significance was declared at the $P \leq 0.05$ level of probability if nothing else is stated.

Results

### Acidification effects on cattle slurry composition

The concentration of WEP_i and WEP_t was significantly higher in the moderately acidified slurry (pH 5.5), whereas the untreated slurry had the lowest concentration of WEP_i and WEP_t (Table 3). Contrastingly, the concentration of WEP_u was not affected by the acidification. WEP_i represented 32, 69 and 62% of the total P in slurry in untreated, moderately acidified (pH 5.5) and strongly acidified slurry (pH 3.8), respectively. The concentrations of water-extractable Ca and Mg were highest in the strongly acidified slurry and lowest in the untreated slurry (Table 3).

P concentrations in maize tissue

On both soils the P concentration in maize tissue was significantly higher 45 DAS in 20P compared to 0P treatments (Fig. 2). Injection of slurry in a broad band led to P concentrations at 45 DAS similar to the 20P treatment on both soils. The P concentration in maize tissue decreased drastically from 45 DAS to 60 DAS. The P concentration did not differ among the treatments at 60 and 75 DAS, except on the sandy soil 75 DAS, where 20P had a significantly higher P concentration compared to 0P. A significant correlation was found between the maize P concentration 45 DAS and DM yield 75 DAS ($r = 0.69$, $P < 0.01$) on the sandy loam, whereas no significant correlations were found between maize P concentration 60 or 75 DAS and DM yield 75 DAS.

DM yield

On both soils, the response of mineral P fertilizer (20P) on DM yield was positive compared to the 0P treatment, but only significant at 75 DAS on the coarse sandy soil (Fig. 3). On the coarse sandy soil, all treatments receiving slurry as starter fertilizer had a DM yield equal to or higher than the 20P treatment at 75 DAS. Treatments with strongly acidified slurry (pH 3.8) resulted in DM yields higher than the 20P treatment (on average 16%)
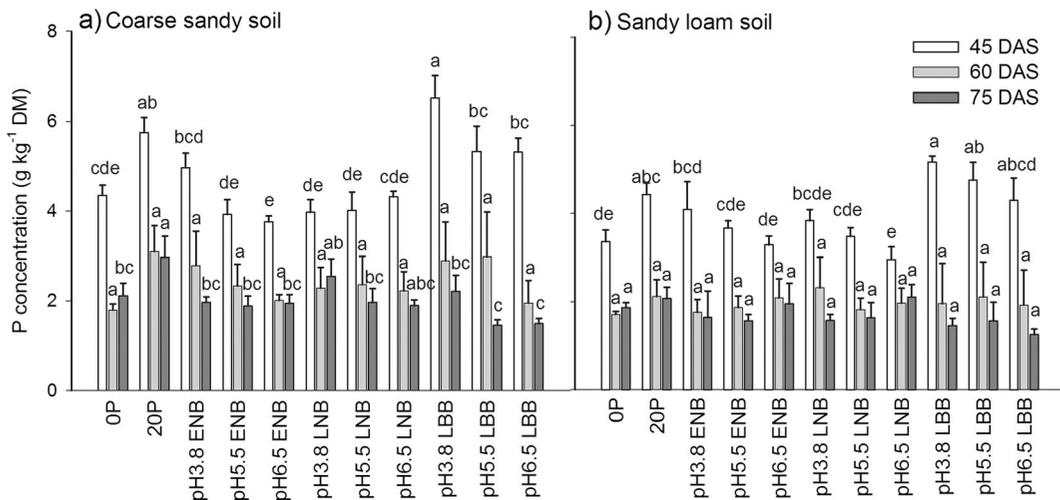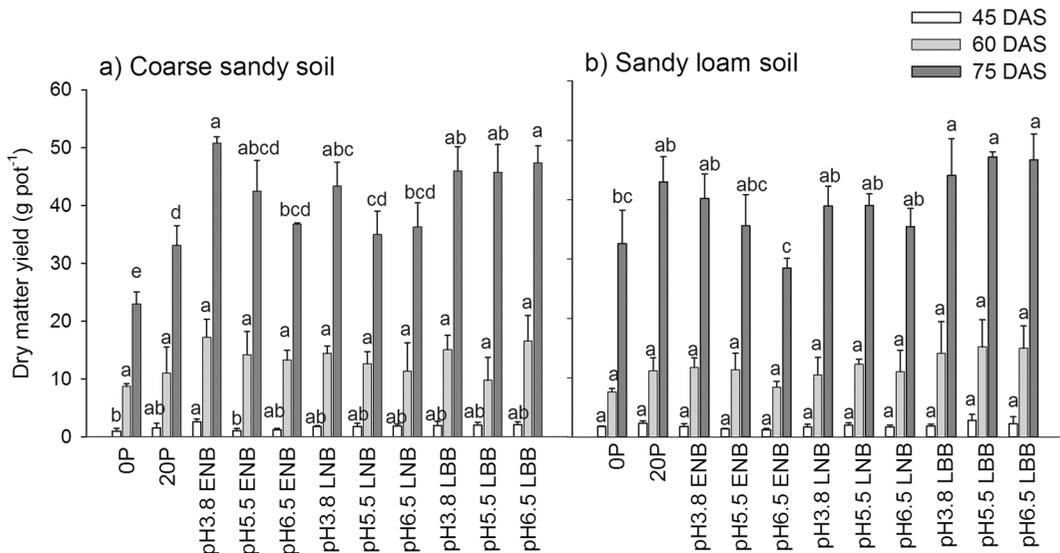
Fig. 2 P concentration (g kg^{-1} DM) in whole maize plants for the different fertilizer and manure treatments on a) coarse sandy soil and b) sandy loam. DAS: Days after sowing (45: five-leaf stage; 60: six-leaf stage; 75: seven-leaf stage). Different letters denote significant differences between treatments within each harvest time (Tukey, $P < 0.05$). Error bars represent the standard deviation ($n = 3$). For treatment abbreviations see Table 1

in this soil, irrespective of the application time and technique. Remarkably high (on average 40% higher than 20P) DM yield was observed 75 DAS on the coarse sandy soil, when the slurry was injected in a broad band, irrespective of the acidification level. Similarly, a significant increase in DM yield compared to the 0P treatment was observed at 75 DAS when the slurry was injected in a broad band on the sandy loam. Injection

of untreated slurry in a narrow band 30 days before sowing (pH 6.5 ENB) in the sandy loam, did not result in an increased DM yield compared to 0P.

On both soils, the DM yield 75 DAS was significantly higher in treatments receiving strongly acidified slurry compared to treatments with untreated slurry when the slurry was applied 30 days before sowing in a narrow band, whereas these differences were not

Fig. 3 Dry matter yields (g pot^{-1}) for the different fertilizer and manure treatments on a) the coarse sandy soil and b) the sandy loam. DAS: Days after sowing (45: five-leaf stage; 60: six-leaf stage; 75: seven-leaf stage). Different letters denote significant differences between treatments within each harvest time (Tukey, $P < 0.05$). Error bars represent the standard deviations ($n = 3$). For treatment abbreviations see Table 1

Table 4 Total P uptake in maize plants measured 45, 60 and 75 days after sowing (DAS) (45: five-leaf stage; 60: six-leaf stage; 75: seven-leaf stage) and selected pot treatment contrasts.

Different letters within columns denote statistical significance among treatments (Tukey, $P < 0.05$). For treatment abbreviations see Table 1

Treatment	Treatment abbrevation	P uptake (mg P pot^{-1})					
		Coarse sandy soil			Sandy loam		
		45	60	75	45	60	75
		----DAS----					
1	0P	4.4[b]	15.6[c]	48.2[c]	5.9[ab]	13.1[c]	60.5[a]
2	20P	9.0[ab]	32.5[ab]	98.4[ab]	10.1[ab]	23.2[abc]	88.2[a]
3	pH 3.8 ENB	12.9[a]	47.0[a]	100.1[ab]	7.2[ab]	20.4[abc]	66.8[a]
4	pH 5.5 ENB	4.0[b]	32.1[ab]	79.4[abc]	5.0[b]	21.1[abc]	55.5[a]
5	pH 6.5 ENB	4.3[b]	26.7[abc]	71.4[bc]	4.1[b]	17.3[bc]	55.8[a]
6	pH 3.8 LNB	6.9[ab]	33.3[ab]	111.3[a]	6.3[ab]	23.0[abc]	61.1[a]
7	pH 5.5 LNB	7.2[ab]	28.9[abc]	68.0[bc]	6.8[ab]	22.4[abc]	63.6[a]
8	pH 6.5 LNB	7.9[ab]	23.9[bc]	68.4[bc]	4.9[b]	20.8[abc]	75.2[a]
9	pH 3.8 LBB	13.0[a]	42.6[ab]	102.6[ab]	8.8[aa]	24.9[ab]	65.0[a]
10	pH 5.5 LBB	10.5[ab]	27.0[abc]	67.2[bc]	13.0[a]	29.8[a]	73.6[a]
11	pH 6.5 LBB	11.0[ab]	31.1[ab]	70.5[bc]	9.5[ab]	28.1[ab]	58.9[a]
Pot treatment contrasts							
Acidification differences							
A) 3,6,9 vs. 4,7,10	pH 3.8 vs. pH 5.5	3.7[ns]	11.6[*]	33.1[*]	-0.8[ns]	-1.6[ns]	0.1[ns]
B) 3,6,9 vs. 5,8,11	pH 3.8 vs. pH 6.5	3.2[ns]	13.7[*]	34.6[*]	1.3[ns]	0.7[ns]	1.0[ns]
C) 4,7,10 vs. 5,8,11	pH 5.5 vs. pH 6.5	-0.5[ns]	2.1[ns]	1.4[ns]	2.1[ns]	2.4[ns]	1.0[ns]
Application time differences							
D) 3,4,5 vs. 6,7,8	Early (E) vs. late (L)	-0.3[ns]	6.6[ns]	1.1[ns]	-0.6[ns]	-2.5[ns]	-7.3[ns]
Application technique differences							
E) 6,7,8 vs. 9,10,11	Narrow band (NB) vs. broad band (BB)	-4.2[*]	-4.9[ns]	2.5[ns]	-4.6[**]	-5.5[*]	0.8[ns]

* and ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively. ns, not significant

observed when the slurry was applied in a narrow band two days before sowing.

Total P uptake

On the coarse sandy soil, linear regression analyses revealed that acidification level was the only significant treatment variable influencing PU at 60 and 75 DAS. At 60 DAS strong slurry acidification (pH 3.8) significantly increased PU by 40% and 60% compared to the pH 5.5 treatments and the untreated slurry treatments, respectively. At 75 DAS PU was 44 and 49% higher in pH 3.8 treatments, compared to pH 5.5 treatments and untreated slurry treatments, respectively (Table 4, contrast A and B). The PU in the pH 3.8 treatments was significantly higher than PU in the 0P treatment at 60 DAS and 75 DAS, and not significantly different from the 20P treatment.

On the sandy loam, The linear regression analysis revealed that of the slurry treatments, application technique was the only significant treatment variable influencing PU at 45 and 60 DAS. Injection of slurry in a broad band significantly increased PU by 80 and 26%, compared to injection in a narrow band at 45 and 60 DAS, respectively (Table 4, Contrast E).

Soil pH, water-extractable P and inorganic N in soil

Soil was sampled at different distances from the slurry placed in a narrow band and from the mineral fertilizer treatments to document the spatial variability in soil P and N status arising from fertilizer placement. Sampling was restricted to the slurry treatments receiving untreated slurry and moderately acidified slurry (pH 5.5), which are directly comparable to common acidification

practices in Denmark. Soil from both the application times was sampled in order to demonstrate changes over time.

On the coarse sandy soil, the soil pH ranged from 4.5 to 7.0 below the band and from 4.6 to 6.1 distant from the band (Fig. 4a). On the sandy loam pH had a range of 5.4 to 6.9 compared to a soil pH range of 5.9 to 6.4 distant from the band (Fig. 4b). On both soils, the soil pH in the untreated slurry treatments (pH 6.5 ENB and pH 6.5 LNB) below the band was similar to or higher than the initial soil pH, while the moderately acidified slurry decreased soil pH below the band. The 0P and 20P treatments on the coarse sandy soil had the lowest soil pH both below and distant from the band (Fig. 4).

On the coarse sandy soil, early application of moderately acidified slurry (pH 5.5 ENB) resulted in a significantly higher concentration of WEP_i and WEP_u in the soil below the band than with all the other treatments at 45 DAS (Fig. 5a). At 75 DAS the highest concentration of WEP_i below the band was found with the late application of moderately acidified slurry (pH 5.5 LNB). The numerical differences of WEP for the treatments within each harvest time distant from the band were small for both soils (Fig. 5). To study the mobility of P and N in soil from 45 DAS to 75 DAS, correlation coefficients were calculated. The correlation between the WEP_i measurements below and distant from the slurry band was insignificant on both the sandy soil ($r = 0.14$, $P = 0.38$) and the sandy loam ($r = 0.00$, $P = 0.99$), and likewise for WEP_u with the slurry treatments on the sandy soil ($r = 0.23$, $P = 0.17$), whereas a significant

correlation was found between WEP_u below the band and WEP_u distant from the band for the slurry treatments on the sandy loam ($r = 0.59$, $P < 0.001$).

At 60 DAS and 75 DAS Nmin decreased rapidly on both soils (Fig. 6). There was a strong linear relationship between NO_3^--N in the soil below and distant from the band for the slurry treatments on both the coarse sandy soil ($r = 0.96$, $P < 0.001$) and the sandy loam ($r = 0.94$, $P < 0.001$), whereas there were no significant correlations between NH_4^+-N below and distant from the band on either the coarse sandy soil ($r = 0.09$, $P = 0.61$) or the sandy loam ($r = -0.27$, $P = 0.11$).

Discussion

Effects of application technique

At the first plant sampling P concentrations were significantly higher in the 20P treatment compared to 0P on both soils. The positive effect of the 20P treatment was later on reflected in significantly higher DM yields and PU at 75 DAS on the sandy soil and this tendency was also seen on the sandy loam. This underlines the importance for maize growth of exposing the roots to sufficient exogenous P while plants are young and the root surface area limited.

Treatments receiving slurry in a broad band had significantly higher DM yields compared to the 0P treatment on both soils at 75 DAS irrespective of the acidification level. On the sandy soil the final DM yield
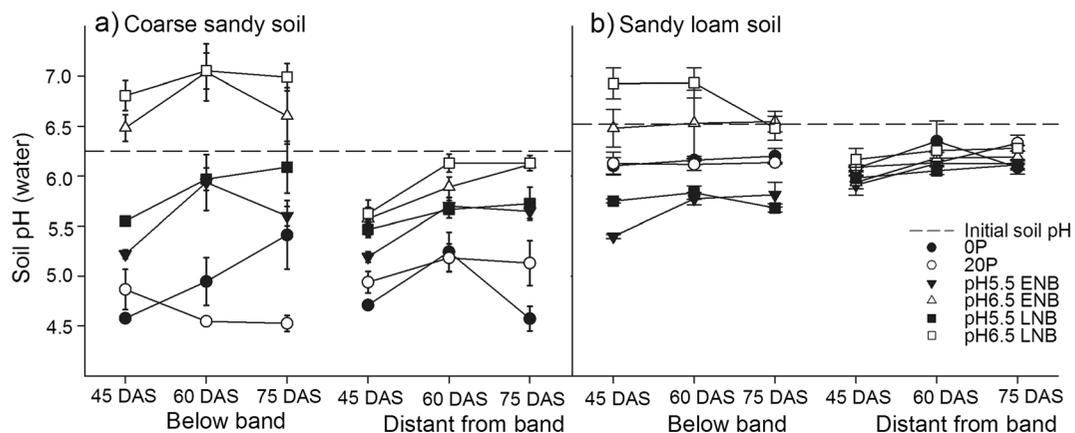
Fig. 4 Soil pH (water) measured 45, 60 and 75 DAS after amendment of mineral fertilizer and cattle slurry to a) the coarse sandy soil and b) the sandy loam. Distant from band: Soil sampled 5 cm distant (horizontally) from the narrow fertilizer band. Below band: Soil sampled below the narrow fertilizer band. DAS: Days after sowing (45: five-leaf stage; 60: six-leaf stage; 75: seven-leaf stage). Error bars represent the standard error of the mean ($n = 3$). For treatment abbreviations see Table 1
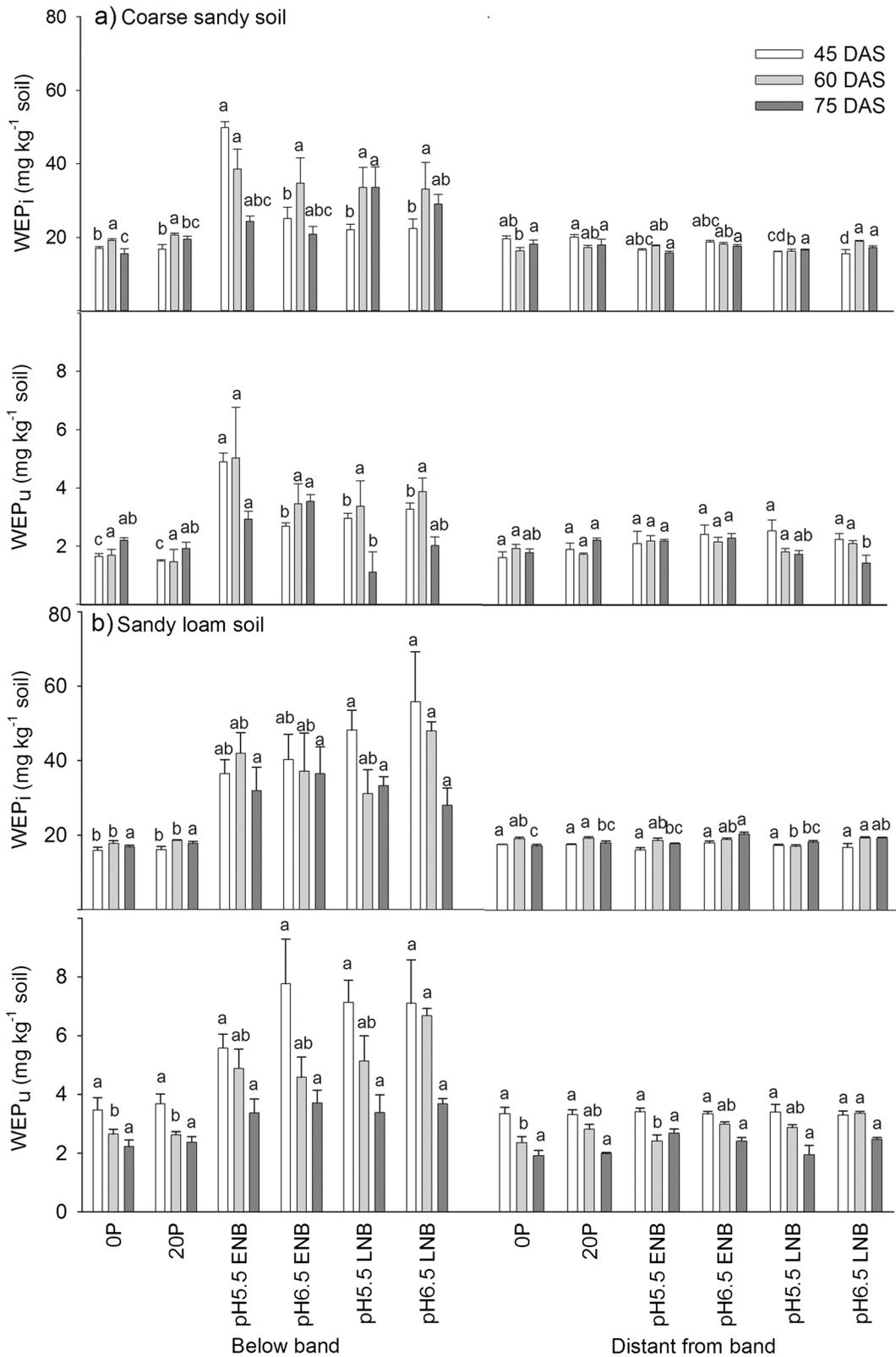
◀ **Fig. 5** Water-extractable reactive P (WEP_i) and unreactive P (WEP_u) in soil after amendment of mineral fertilizer and cattle slurry to a) the coarse sandy soil and b) the sandy loam. Below band: Soil sampled below the narrow fertilizer band. Distant from band: Soil sampled 5 cm distant (horizontally) from the narrow fertilizer band. DAS: Days after sowing (45: five-leaf stage; 60: six-leaf stage; 75: seven-leaf stage). Different letters denote significant differences between treatments within each harvest time (Tukey, $P < 0.05$). Error bars represent the standard error of the mean ($n = 3$). For treatment abbreviations see Table 1

was even higher with broad-banded slurry than in the 20P treatment, indicating that the higher DM yield was not only a result of higher P availability, but also related to other growth promoting effects of the slurry such as higher availability of other nutrients in the broad-banded slurry. On both soils broad-banded slurry treatments had P concentrations in maize tissue at 45 DAS similar to the 20P treatment (Fig. 2). Moreover, the strong correlation between P concentration at 45 DAS and DM yield at 75DAS on the sandy loam implies that the maize plants were able to benefit from slurry P applied in a broad band. The lack of a response in the P concentrations 60 and 75 DAS could be due to a proportional increment in DM yield, as also pointed out by Schröder et al. (2015). The beneficial effect of the broad band injection could be due to: a) The broad band being placed closer to the seed both horizontally and vertically than the narrow band giving plants quicker access to N and P, b) in the narrow band unfavorable conditions for root growth persisted for a longer period (Sawyer and Hoeft 1990), limiting root access to P, and c) maize plants were forced to grow through the layer of broad-banded slurry resulting in fast access to nutrients.

The lack of a relationship between WEP_i below and distant from the narrow band clearly demonstrated the limited mobility of WEP_i in the soil, whereas the opposite was observed for NO_3^--N, which moved readily with soil water by mass flow and by diffusion. This confirms the rationale for placing the exogenous P pool close to the seed and preferably in a broad band, which may ensure quicker access to the manure nutrients and enlarge the root surface area exposed to slurry P compared to the narrow band. These findings support explanation a) and c) above.

We interpret the significant correlation between WEP_u below and distant from the band on the sandy loam as an indication that WEP_u is more mobile than WEP_i, and to some degree resembles the mobility of NO_3^--N. Glaesner et al. (2011) also demonstrated

increased mobility of some forms of organic P compared to inorganic P in a leaching study. Magid et al. (1996) suggested that organic P compounds might be held less strongly by the soil matrix, because P bound to or in organic matter may be masked from potential sorption sites. WEP_u is prone to mineralization and could potentially be measured as WEP_i after mineralization, but, unfortunately, it was not possible to determine which role WEP_u played for PU in the present study.

Mineral P fertilizer application mimicked the placement of slurry in the narrow band, but the 20P treatment had higher plant P concentrations similar to the broad-banded slurry treatments (Fig. 2). The lower P concentrations in treatments with narrow-band slurry injection could be due to unfavorable conditions in the concentrated narrow band with possible salt stress or anaerobic conditions and formation of organic acids, as described by Sawyer and Hoeft (1990) and Lynch (1980) and suggested in explanation b). Whether the higher DM yields in treatments with slurry applied in a broad band only is the result of higher availability of P is questionable. The better growth response at 75 DAS could also be due to a general improvement of nutrient availability in the broad band, as suggested in explanations a) and c). Nmin decreased rapidly from 45 to 75 DAS in slurry treatments, and we cannot exclude that the maize plants were N-limited at 75 DAS. However, DM yield increased significantly from 60 DAS to 75 DAS and the soils still contained inorganic N at 60 DAS, indicating that inorganic N could only have been the limiting factor in a short part of the growth period between 60 and 75 DAS. Moreover, we did not observe visible N deficiency symptoms on the plants. The mean Nmin concentration in soil was highest in the treatment with N fertilizer alone (0P) at 75 DAS in the sandy soil (Fig. 6). This could be explained by the poor plant growth, and hence a lower N uptake by maize.

It is concluded that the maize planted above a broad slurry band benefited from the quick and improved access to the manure nutrients, including P. The improved growth response with broad-banded slurry applications could also be due to less toxic conditions to root growth compared to the narrow slurry band. However, the improved effect of broad banding could also be a result of reduced distance to the seed in the broad band treatments compared to the narrow band. A narrow slurry band placed just below the seed at similar distance might have given similar results as the broad
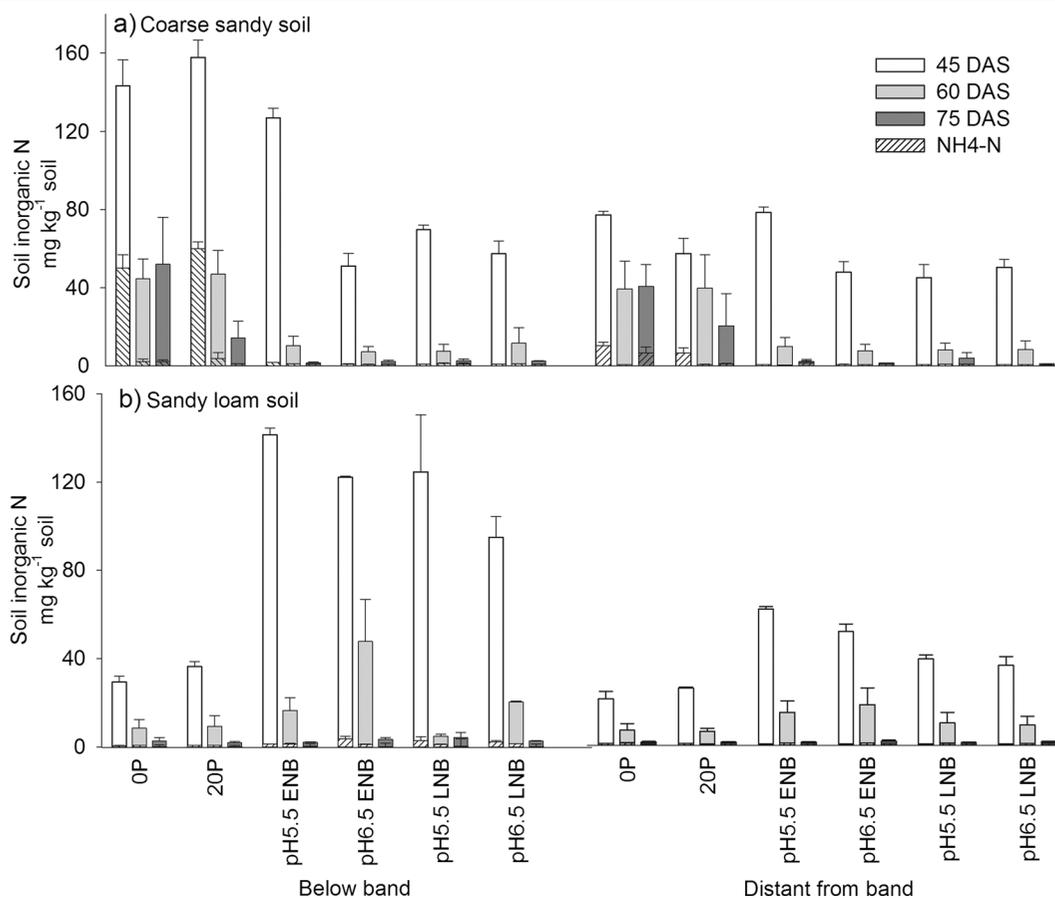
Fig. 6 Soil inorganic N after amendment of mineral fertilizer and cattle slurry to the a) coarse sandy soil and b) sandy loam. Below band: Soil sampled below the narrow fertilizer band. Distant from band: Soil sampled 5 cm distant (horizontally) to the narrow band. DAS: Days after sowing (45: five-leaf stage; 60: six-leaf stage; 75: seven-leaf stage). Error bars represent the standard error of the mean ($n = 3$). For treatment abbreviations see Table 1

band. To clarify this further studies are needed on slurry band placement, where also growth and spatial distribution of roots are determined.

Effects of slurry acidification

Moderate (pH 5.5) and strong slurry acidification (pH 3.8) increased the concentration of WEP_i in slurry compared to untreated slurry, whereas the acidification did not have an effect on WEP_u (Table 3), indicating that the acidification mainly affected the inorganic P in slurry. By lowering the pH to 5.5 and 3.8 it was possible to dissolve 76 and 72% of total P in the slurry, respectively, confirming that acidification can be used to adjust the amount of dissolved P in slurry. Dissolved Mg and Ca increased drastically from pH 6.5 to 5.5, indicating that struvite ($NH_4 MgPO_4$) and P bound to Ca phosphate minerals are dissolved. Christensen et al. (2009) also

found an increase in the concentration of dissolved P in pig slurry by lowering the pH from 6.3 to 5.5, and this was mainly attributed to the dissolution of struvite. Fordham and Schwertmann (1977) found that most of the P dissolved was attributed to Ca phosphate minerals in the pH range from 6.7 to 5.5, whereas no additional P was dissolved when pH dropped below 5.5, which is in line with our results.

On the sandy soil, PU was significantly higher 60 and 75 DAS, when the slurry was strongly acidified (pH 3.8) compared to moderately acidified slurry (pH 5.5) and untreated slurry (pH 6.5), whereas no effect of acidification was observed on the sandy loam. The contrasting effect of slurry acidification on PU on the two soils might be explained by their different buffer capacities. Soil pH below the band was highly affected by the treatments on both soils, but the range of the soil pH was higher in the sandy soil compared to the sandy loam

(Fig. 4), which is also reflected in the higher CEC and base saturation of the sandy loam (Table 2), providing a better buffer against soil acidification. The lacking effect of acidification on the sandy loam with respect to PU could also be due to the significantly higher P sorption capacity (PSC) of the sandy loam (Table 2), and hence increased capacity to rapidly remove dissolved reactive P from the soil solution.

Similar PU values in treatments receiving acidified slurry on the sandy soil would be expected, since the numerical difference in slurry WEP_i between the two acidification levels was small. However, a significantly higher PU was observed in treatments receiving strongly acidified slurry compared to moderately acidified slurry on the sandy soil. We did not measure the pH in soil amended with strongly acidified slurry, but expect that soil pH would be lower than the pH in soil amended with moderately acidified slurry and that a lower soil pH level will be maintained for a longer period compared to the soil pH in treatments with moderately acidified slurry that slightly increased from 45 to 75 DAS on the sandy soil (Fig. 4). Soil pH distant from the band decreased compared to the initial soil pH, not only for slurry-amended soils, but also for the mineral fertilizer treatments (0P and 20P). Hence, the decrease in soil pH could not be attributed solely to the application of acidified slurry, but also to mineralization of N and nitrification during the experiment. On the sandy soil the 0P and 20P treatments had the lowest soil pH at all sampling times both below and distant from the fertilizer band. This could be due to the limited inherent capacity of this soil to buffer the acidification arising from nitrification of the added NH_4^+ combined with the absence of buffering slurry compounds (Sommer and Husted 1995), which are expected to be present in the slurry treatments. At the low pH we observed in this soil sorption of P with Fe and Al containing minerals would be expected (Lindsay et al. 1989), which could reduce the P availability in the reference treatments on the sandy soil. However, the P concentration in plant tissues at 45 DAS was significantly higher in the 20P treatment compared to the 0P treatment, which indicate that the plants were able to take up P also at this pH level. Application of untreated slurry to the sandy soil resulted in higher soil pH l below the band than both the initial slurry pH and the initial soil pH. This may be due to oxidation of slurry volatile fatty acids as described by Sørensen (1998) and buffering compounds in slurry (Sommer and Husted 1995) combined with the low

inherent buffer capacity of the sandy soil. The highest PU was observed in treatments with strongly acidified slurry on the sandy soil. The expected lower soil pH induced by the strongly acidified slurry itself and the abovementioned acidifying processes could probably prevent Ca added with the slurry to precipitate with P from soil and/or slurry, which could increase the P availability. Moreover soil pH also controls the P speciation (Hinsinger 2001). As soil pH decreases, the concentration of $H_2PO_4^-$ in the soil solution increases and $H_2PO_4^-$ is the substrate of the proton-coupled phosphate symporter in the plasma membrane (White 2006). Higher concentration of $H_2PO_4^-$ might therefore promote a higher P uptake, explaining the highest PU in the treatments with strongly acidified slurry.

In conclusion, the concentration of WEP_i in the slurry increased at both slurry acidification levels, but since the PU was significantly higher in treatments with strongly acidified slurry compared to moderately acidified slurry on the sandy soil, the increase of PU was probably not solely due to the higher content of dissolved P in the slurry, but rather to the stronger acidification of the soil and the indirect effect of this on properties related to soil P retention and release and therefore also to plant availability.

Effects of slurry application time

Application time had no effect on PU on either soil type (Table 4). Early application of moderately acidified slurry in a narrow band increased the concentration of WEP on the sandy soil, but it was not reflected in the DM yield. The lacking effect of application time could be explained by sorption to soil surfaces or immobilization of slurry P, which may occur rapidly, i.e. within the first few days, making the differences between application of slurry two and 30 days before sowing negligible. Apparently, early application of slurry did not provide a better root growth environment in the narrow fertilizer band as hypothesized. On the contrary, early application of untreated slurry on the sandy loam showed no positive effect on DM yield compared to 0P, which could be due to the low WEP_i concentration in untreated slurry and the continuing poor growing conditions in the narrow manure band, irrespective of how early cattle slurry was applied. DM yields and PU as high as or higher than the 20P treatment were obtainable if strongly acidified slurry was applied in a narrow band on the sandy soil no matter the application time. Consequently, application

of strongly acidified slurry in a narrow band and sowing do not have to be carried out at the same time, which might facilitate an earlier start of the slurry spreading season.

Practical perspectives

Injection of cattle slurry by tines at 24 cm distance before ploughing without considering the exact position of the following maize sowing is common practice in Denmark today. By this technique the slurry is still present in subsurface bands in soil after ploughing. Today GPS techniques allow positioning of seed rows precisely over previously applied slurry bands (Schröder et al. 2015), but the slurry has to be injected after ploughing/harrowing and before sowing. The present results indicate that this could preferably be done by placement by broad-banding under maize rows or by narrow-banding of acidified slurry on sandy soils. However, it will be necessary to find ways to handle compressed wheel tracks from slurry tankers especially on heavy soil types to establish a proper seed bed. Furthermore the results obtained here have to be verified under field conditions.

Conclusion

Application of slurry by placement in a broad band under a maize row increased maize P concentration at the five-leaf stage and increased the maize DM yield at the seven-leaf stage, irrespective of the acidification level and soil type. Thus, application of slurry in a broad band may provide a general better nutrient availability compared to injection of untreated slurry in a narrow band. Slurry acidification increased the concentration of dissolved inorganic P. However, only strongly acidified slurry increased DM yield and PU at the seven-leaf stage on the sandy soil compared to moderately acidified and untreated slurry, whereas strongly acidified slurry did not increase maize yield on the sandy loam. The results indicate that it was not the higher concentration of dissolved inorganic P in acidified slurry *per se* that improved DM yield and PU on the sandy soil, but rather an impact on soil pH and hence pH-sensitive processes related to P availability. We did not find an additive effect of combining strongly acidified slurry with broad band application on maize yield in the early growing stages. Application of cattle slurry by narrow-band

injection 30 days before sowing did not improve the maize growth response compared to narrow-band injection two days before sowing.

We conclude that mineral P starter fertilizer could potentially be replaced by broad-band injection of cattle slurry under maize rows or, on coarse sandy soil, by application of strongly acidified slurry injected in either narrow or broad bands. In this way the use of mineral P starter fertilizer might be obviated, and the P surpluses of silage maize cropping on dairy farms can be reduced.

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