

ARTICLE

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Damage to the primary root in response to cattle slurry placed near seed may compromise early growth of corn

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Abstract

Placement of cattle slurry below the row could potentially replace mineral phosphorus (P) starter fertilizer in corn (*Zea mays*) production, but a concentrated slurry layer near the seed may also restrict root growth. This study was designed to assess how the distance between seed and layer-banded slurry affected initial growth of corn. In a pot experiment with corn on a coarse sandy soil, nitrogen-labeled (¹⁵N) cattle slurry was placed 1.5, 5, 8.5, or 12 cm below the seed, and responses on early root growth, shoot biomass, and nutrient uptake were studied. Soil chemical properties near the slurry band were determined in unplanted soil. Placement of slurry 1.5 cm below the seed damaged the primary root, which subsequently reduced shoot biomass and N uptake. Shoot P uptake remained unaffected by slurry placement depth. The ¹⁵N assay revealed that plants were able to take up N from the slurry band despite damage to the primary root. The shoot biomass was higher in the inorganic N and P treatment than in the slurry treatments. Within a few centimeters above the slurry band, the soil was characterized by high moisture and high concentrations of ammonium and nitrite 21 and 35 d after slurry application, which may have caused the damages of the primary root. We conclude that placement of slurry near the seed can damage the primary root of corn. To prevent root injuries, banded slurry should be placed at least 5 cm below the seed.

1 | INTRODUCTION

Animal manure is the largest resource of recyclable phosphorus (P) in Europe (Ott & Rechberger, 2012). Thus, improved utilization of P in manure is a key step towards improved sustainability of P management in agriculture in order to avoid soil P accumulation and to accommodate the challenge

of depleting finite global reserves of rock phosphate (Withers et al., 2015).

In corn (*Zea mays* L.) production, precision placement of mineral P fertilizer near the seed at sowing is common practice in regions with temperatures being suboptimal for corn growth (Schröder, Ten Holte, & Brouwer, 1997). Sufficient P supply during early growth of corn is of great importance in order to support optimum crop yield (Barry & Miller, 1989). Row-injection of cattle slurry close to the row is a promising option to ensure sufficient P uptake by young corn plants (Bittman et al., 2012; Pedersen, Rubæk, & Sørensen, 2017; Schröder et al., 2015). A targeted P supply to young corn plants via precision placement of cattle slurry could potentially obviate the use of mineral P starter fertilizer based

Abbreviations: DAS, days after sowing; DM, dry matter; EC, electrical conductivity; NP, inorganic nitrogen and phosphorus fertilizer; Slu, slurry; TON, total oxidized nitrogen; UNF, unfertilized; WHC, water holding capacity; WEP_i, reactive water-extractable phosphorus; WEP_t, total water-extractable phosphorus; WEP_u, unreactive water-extractable phosphorus.

on rock phosphate, thereby contributing to development of circular economies (EC, 2014), and at the same time minimizing soil P accumulation on intensive livestock farms.

Precision placement of fertilizers close to the seeds allows early contact between roots and fertilizers and thereby utilization of nutrients from early growth stages (Nkebiwe, Weinmann, Bar-Tal, & Müller, 2016). This is especially important for plant uptake of immobile nutrients in soil, such as P (Grant, Flaten, Tomasiewicz, & Sheppard, 2001), and for corn seedlings that are impeded by low soil temperatures (Richner, Soldati, & Stamp, 1996). Moreover, placement of P reduces the contact with soil and therefore, sorption of P to soil particles may be minimized compared to even incorporation of fertilizers in soil (Grant et al., 2001). The plant may also benefit from fertilizer placement, since roots are able to respond to heterogeneous nutrient concentration by increasing the root growth in the enriched nutrient zone referred to as “compensatory adjustments” (Bingham & Bengough, 2003; Drew, 1975).

Root growth and establishment of a root system during early growth is important for water and nutrient acquisition throughout the growing season (Lynch, 1995; Ma, Tang, Rengel, & Shen, 2013). The root system of corn has a unique architecture and consists of three root types formed at different development stages (Hochholdinger, 2009): The primary root and a variable number of seminal roots compose the embryonic root system, which constitutes the major portion of the root system during the first 2 wk of growth. The postembryonic roots (nodal roots), which develop later, are shoot born and organized in whorls (Hochholdinger, 2009). However, it is unclear how the different root types respond to cattle slurry placed in a concentrated layer, and how this in turn affects nutrient supply of the young corn plants.

Enrichment with simple inorganic P compounds can affect root growth, as demonstrated by Drew (1975), showing that patches enriched with a phosphate solution stimulate lateral root growth in barley (*Hordeum vulgare* L.) plants. More recently Ma et al. (2013) found that banding of P and ammonium (NH_4^+) as mono- or di-ammonium phosphate improved nutrient uptake and root proliferation in corn on a calcareous soil. However, only few studies have attempted to study root growth responses to a localized supply of more complex P sources such as slurry. In a study with liquid beef manure, Sawyer and Hoeft (1990) reported restricted corn root growth in the manure zone. Especially high concentrations of NH_4^+ combined with high pH, high concentrations of nitrite (NO_2^-) and reducing conditions resulted in toxic conditions. In contrast, Bittman et al. (2012) did not find any adverse effects associated with placed dairy sludge on seedling germination or corn growth, when liquid manure was injected in 13- to 15-cm depth with the lateral distance from the slurry band to the seed row varying from 5 to 15 cm. However, it remains unknown how the vertical distance from

Core Ideas

- Placement of slurry can improve nutrient utilization but may also damage roots.
- Slurry placed near the corn seed restricted growth of the primary root.
- Concentrations of ammonium and nitrite were high in soil near the slurry band.
- Cattle slurry placed at least 5 cm below the corn seed prevented root injuries.

the seed to the slurry band influences corn growth during the early growth stages. This is of particular importance to study, since broad-banded slurry placed below the corn seed seems as a promising injection technique (Pedersen et al., 2017).

In the present paper, we studied how different depths of slurry placement affected corn dry matter yield, N and P uptake, root morphology, and soil chemical properties. Specifically we examined if a short vertical distance from seed to a slurry band can compromise any positive effects of placed slurry on corn growth.

2 | MATERIALS AND METHODS

A pot experiment with corn on a coarse sandy soil was conducted in a climate-controlled chamber organized as a full-factorial design with four replicates. The treatments consisted of four slurry treatments applied in four different placement depths (1.5, 5, 8.5, and 12 cm below the seed), an unfertilized treatment (UNF) and an inorganic nitrogen (N) and P fertilized treatment (NP; Table 1). Soil chemical transformations after slurry application were studied simultaneously in pots without corn, where the slurry was placed at 8-cm depth.

2.1 | Pot preparation and fertilization

Soil for the experiment was collected in October 2018 from the topsoil layer (5–15 cm) at Jyndevad Experimental Station, Southern Denmark. The soil was a coarse sand with 3% clay (<2 μm), 2% silt (2–20 μm), 3% coarse silt (20–63 μm), 89% sand (63 μm to 2 mm), 1.69% carbon, and 0.12% N and classifies as a Orthic Haplohumod. The previous crop was faba bean (*Vicia faba* L), and the soil is a common soil type of Danish agricultural land with corn cropping. The soil had a water holding capacity (WHC) under pot conditions of 24 g of water per 100 g of dry soil defined after Kirkham (2004). Initially, the soil contained 0.04 mg NH_4^+ -N and 15.0 mg NO_3^- -N kg^{-1} dry soil and had a pH (CaCl_2) of 5.0. The soil had an Olsen-P content of 40 mg P kg^{-1} soil.

TABLE 1 Treatment overview of the pot experiment with corn. Inorganic nitrogen (N) and phosphorus (P) fertilizer and cattle slurry were placed in a layer covering the whole pot area

Treatment abbreviation	Fertilization	Distance from soil surface to fertilizer band	Distance from seed to fertilizer band
		cm	cm
UNF	No fertilizer	-	-
NP	Placed inorganic NP fertilizer	8	5
Slu-1.5	Placed slurry	4.5	1.5
Slu-5	Placed slurry	8	5
Slu-8.5	Placed slurry	11.5	8.5
Slu-12	Placed slurry	15	12

The soil was sieved (5 mm), mixed, and carefully packed into cylindrical pots (103-mm inner diameter, 27.5-cm total height). A total of 2,428 g dry soil was added per pot (height of soil in pots was 23.8 cm) to a final bulk density of 1.23 g cm⁻³. The soil was divided into two portions of relevant sizes to allow packing of bottom soil layers to heights of 19.3, 15.8, 12.3, or 8.8 cm.

Fertilizers (cattle slurry and NP) were applied on top of the bottom soil layer 3 d before pre-germination of the seeds. Cattle slurry originating from dairy cows was collected in October 2018 from a storage tank at Research Centre Foulum, Aarhus University. The slurry was well mixed in a tank before sampling and the collected slurry was stored at 2°C. The cattle slurry contained 0.16% NH₄⁺-N, 0.40% total N, and 0.06% total P. Slurry was applied at a P application rate of 10 kg P ha⁻¹ based on a plant density of 90,000 plants ha⁻¹, assuming placement of the whole amount of cattle slurry to each plant. This resulted in 111 mg P, 280 mg NH₄⁺-N, and 741 mg total N pot⁻¹ applied via 187 g fresh slurry. The amount of slurry corresponded to a 2.2-cm thick slurry band covering the whole pot area.

To study the plant uptake of N from the slurry band, ¹⁵N-labeled NH₄⁺-N was added as ammonium sulphate ([NH₄]₂SO₄; 60 atom% ¹⁵N) dissolved in water. For each pot, 6.25 ml of this solution (9.7 mg NH₄⁺-N pot⁻¹) was carefully mixed with 187-g slurry before application to the pot to ensure homogenous labelling of the NH₄⁺-N pool of the slurry. The addition of the highly enriched ¹⁵N solution to the slurry resulted in an enrichment at 1.86 atom% ¹⁵N of the total NH₄⁺-N applied with the fertilizers.

The inorganic fertilizer in the NP treatment was applied to the soil layer with a height of 15.8 cm. The NP treatment received 280 mg NH₄⁺-N pot⁻¹ as (NH₄)₂SO₄ and 111 mg P pot⁻¹ as KH₂PO₄ dissolved and mixed in 50 ml of demineralized water pot⁻¹. To this solution, 6.25 ml of the ¹⁵N-labeled NH₄⁺-N solution was also mixed before application to the whole surface area of the bottom soil layer.

The remaining soil portion was then packed above the slurry equaling heights of 4.5, 8.0, 11.5, or 15 cm, respec-

tively. The NP treatment was covered by an 8.0-cm thick layer of soil to mimic the typical placement depth of inorganic NP starter fertilizers in corn cropping.

Micronutrients were applied to all pots with corn by later surface irrigation 17 d after sowing (DAS) at a rate per pot of 2.8 mg Mn, 2.1 mg Zn, 0.4 mg B, 1.2 mg Cu, 0.02 mg Co, and 0.5 mg Mo. Additional nutrients K and S (as K₂SO₄) were surface-applied 20 DAS at a rate per pot of 140 mg K and 57 mg S.

For studies on changes in soil chemical properties near the slurry band, pots without plants were prepared, where a 2.2 cm thick slurry layer was placed 8 cm below the soil surface. A nylon mesh (8-mm mesh size) was placed below and above the slurry band in order to be able to identify the slurry band when sampling the soil.

2.2 | Sowing, climate conditions and irrigation

Before sowing, the soil moisture was adjusted to 63% of the WHC. Three d after slurry application, corn seeds of an early developing corn hybrid (cultivar Conclusion, FAO 190, Limagrain) with an average weight of 306 mg were pre-germinated on moist filter paper for 48 h at 20°C and then transplanted in the pots (i.e., 5 d after slurry application) at 3-cm depth with the pedicle end facing down.

The pots were placed in a greenhouse during the first 4 d of growth and then moved to a climate-controlled chamber. The chamber had a daily average temperature of 15°C with a daily amplitude from 11 to 19°C for the first 13 d, a mean temperature increase of 0.1°C day⁻¹ after 13 d, and a relative mean air humidity of 75%. The plants were grown under the same settings as in Pedersen, Sørensen, Rasmussen, Withers, and Rubæk (2019) with 16 h photoperiods with light intensities ranging from 170 to 1060 μmol photons m⁻² s⁻¹ to mimic Danish corn growing conditions in spring. The moisture content was adjusted to 63% of WHC with demineralized water during the first 35 d of growth and then up to 67% of WHC until the last harvest. To minimize

the positional effect in the growth chamber, the pots were re-randomized every second day.

2.3 | Soil and plant measurements

The corn plants were harvested by cutting the culm 0.5 cm above the soil surface 24, 39, and 55 d after sowing corresponding to the two-, three- and five-leaf stage (V2, V3 and V5), respectively. The shoots were oven-dried at 60°C to constant weight (min 48 h) for determination of dry matter (DM) and ground to a fine powder in a ball-mill prior to analyses.

After harvest of the corn shoots, the intact soil column was pushed out of the pots with a hydraulic pusher, and the intact root system was removed from the soil column and washed immediately after sampling. To catch any treatment induced changes in root morphology, the roots were separated into primary, seminal, and nodal roots defined after Hochholdinger (2009). Seed and mesocotyl were sampled together. Fresh weight of each root part was recorded, and the roots were preserved in 3% acetic acid at −20°C until root scanning. At the last harvest date at V5, soil above and below the slurry band was also collected for determination of inorganic N in soil.

To study the soil environment near to the slurry band, soil was sampled from the unplanted pots at 21, 35, and 55 d after slurry application by pushing the soil column out of the pot with a hydraulic pusher. The soil above the slurry band was sampled by slicing the intact soil column with a knife in layers 0–0.5, 0.5–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3.5, 3.5–4.5, 4.5–5.5, and 5.5–8 cm above the slurry band. A slice was sampled 4.5–5.5 cm below the band as reference. At the last sampling date, 55 d after application, additional soil samples were collected at a distance of 0–1 and 1–4.5 cm below the slurry band. Before sampling, the pots were irrigated to 63% of the WHC. The soil samples were kept at 2°C until analyses.

2.4 | Analytical methods

Total NH_4^+ –N in slurry was measured by flow colorimetry (Sommer, Kjellerup, & Kristjansen, 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, and P in the digestate was determined colorimetrically using the molybdate blue method (ISO, 2004) after Murphy and Riley (1962). Slurry DM was determined by drying at 80°C for 24 h.

Nitrogen concentration and ^{15}N enrichment of shoots were determined with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europe 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). Phosphorus

concentrations in shoots at V3 and V5 were determined by digesting 300 mg DM in 3 ml H_2O_2 (9.7 M) and 6 mL HNO_3 (14.3 M) using Teflon-coated vessels and a pressurized microwave oven (Anton Paar GmbH, Graz, Austria), with the P concentration in the diluted digest being determined by ICP-OES (Thermo Fisher Scientific, Waltham, MA). In case of material less than 300 mg, a minimum of 100 mg was digested. Shoot P concentrations were not determined at V2 due to too little shoot material.

For estimation of root morphological features, the roots were thawed and stained before scanning (Richner, Liedgens, Bürgi, Soldati, & Stamp, 2000). Staining was done with a solution consisting of 0.5 g “Neutral red” dissolved in 100 ml of 96% ethanol and 900 ml of demineralized water. The roots were soaked in the solution for 24 h. The roots were scanned and imaged using an Epson XL 10000 professional scanner and the software WinRHIZO at a resolution of 600 dpi to determine total root length (cm) and specific root length (length of roots g^{-1} root dry matter yield). After scanning, the roots were washed and dried at 70°C for 48 h for dry weight determination.

Soil pH was measured by glass electrode in 0.01 M CaCl_2 suspensions (1:2.5, w/w). Electrical conductivity (EC) in a soil-water mixture was measured after shaking 5 g fresh soil in 45 ml of demineralized water for 1 h at 20°C.

Approximately 10 g of fresh soil was mixed with 1 M KCl immediately after sampling and shaken for 30 min. Concentrations of NH_4^+ –N and total oxidized N (TON, nitrate-N [NO_3^- –N] + NO_2^- –N) in filtered extracts were determined by flow colorimetry (Autoanalyzer III, Bran + Luebbe GmbH, Norderstedt, Germany). Concentration of NO_2^- –N in the filtered extracts was determined colorimetrically after Keeney and Nelson (1982). Concentrations of NO_3^- –N were determined as the difference between TON and NO_2^- –N.

Dissolved reactive water-extractable P (WEP_i) and total water-extractable P (WEP_t) in soil at the final soil sampling were measured by extracting 5 g of fresh soil (sieved through 4-mm sieve) in 50 ml of deionized water (shaken end-over-end) for 1 h at 20°C followed by centrifugation for 10 min at 20°C with a relative centrifugal force at $1831 \times g$. Reactive water-extractable P (WEP_i) concentrations were determined in the supernatant by spectrophotometry on the centrifuged sample using the molybdic blue method (ISO, 2004) modified after Murphy and Riley (1962). The WEP_t in the soil 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 fraction of unreactive water-extractable P (WEP_u) was determined as the difference between WEP_t and WEP_i .

The soil was oven-dried at 105°C for 24 h for determination of dry matter, and soil analyses are expressed on an oven-dry basis.

2.5 | Calculations and statistical analysis

Percent of N in plant deriving from $\text{NH}_4^+\text{-N}$ in fertilizer (NdfNH_4) was calculated as:

$$\text{NdfNH}_4 = \frac{{}^{15}\text{N}_{\text{excess plant}}}{{}^{15}\text{N}_{\text{excess fertilizer}}} 100$$

where ${}^{15}\text{N}_{\text{excess plant}}$ was calculated as the difference between atom% ${}^{15}\text{N}$ in the fertilized plant tissue and the atom% ${}^{15}\text{N}$ of plant tissue in unfertilized control treatment, and ${}^{15}\text{N}_{\text{excess fertilizer}}$ was the atom% ${}^{15}\text{N}$ of the added $\text{NH}_4^+\text{-N}$ in slurry or inorganic N-fertilizer minus the natural atom% ${}^{15}\text{N}$ fraction measured in unfertilized plants.

All statistical analyses were performed using R version 3.4.1 (R Development Core Team, 2015). For each harvest date, the effect of fertilization on DM yield, root growth, and nutrient uptake was analyzed using a one-way analysis of variance (ANOVA) with treatment as a categorical variable. When the treatment effect was found to be significant, the differences between treatments within each sampling date were analyzed by the Tukey's honestly significant difference (HSD) using estimated marginal means from the R-package *emmeans*. The assumption of homogeneity of variance and normality of residuals was verified using plot of residuals against fitted values and histogram of the residuals. Data were log-transformed in cases where homoscedasticity of the residuals was not obtained (length of the seminal roots at V5, shoot N concentration at V3, shoot NU at V2 and P uptake per root length at V3). Significance was declared at the $P \leq .05$ level of probability.

3 | RESULTS

3.1 | Shoot and root biomass

At the first harvest date at V2, the shoot DM yield in the slurry (Slu)-1.5 treatment was lower than in the other slurry treatments, and in the subsequent growing stages, the shoot DM yield in the Slu-1.5 treatment remained low compared to the other slurry treatments (although not always significant; Figure 1). At V5, the Slu-12 treatment had a significantly higher shoot DM yield than the Slu-1.5 treatment. At V3 and V5, there was a significant positive response of applying inorganic NP fertilizers, and the shoot DM yields were significantly higher in the NP treatment than in the slurry treatments (Figure 1).

During the entire experimental period, the DM of the primary root was significantly lower in the Slu-1.5 treatment than in the other treatments (Figure 1). At V3 and V5, the DM yield of the nodal roots were significantly higher in NP treatment than the other treatments, whereas the DM yield

of the nodal roots was not affected by the slurry placement depth among the slurry treatments. The shoot/root ratios at V5 were similar among the fertilized treatments, whereas the unfertilized control had a lower shoot/root ratio (Table 2).

3.2 | Nitrogen and phosphorus uptake in shoot

The shoot N concentration decreased substantially from V2 to V5 in the UNF and NP treatments, whereas the shoot N concentrations at V5 remained high in the slurry treatments and averaged 4.38%. At V5, the NP treatment had the highest shoot N uptake, and among the slurry treatments, the N uptake increased with increasing slurry placement depth (Table 2).

At V2 and V3, the Slu-12 treatment had a lower percentage of N in shoot derived from $\text{NH}_4^+\text{-N}$ in slurry than the other slurry treatments (Figure 2). At V5, the percentage of shoot N derived from $\text{NH}_4^+\text{-N}$ in slurry was not different among the slurry treatments and averaged 53%.

The shoot P uptake did not differ among slurry treatments at V3 and V5, whereas the shoot P uptake in the NP treatment was significantly higher ($+16 \text{ mg pot}^{-1}$) than the slurry treatments. The unfertilized control had the lowest shoot P uptake (Table 2).

The shoot N/P ratio at V5 in the slurry treatments ranged from 13 to 16, whereas the NP treatment had a significantly lower shoot N/P ratio of 8 (Table 2).

3.3 | Root development

The growth of the primary root was restricted in the Slu-1.5 treatment, and the length of the primary root did not increase from V3 to V5 in this particular treatment (Table 3). At V2 and V3, placement of slurry at 5-, 8.5-, or 12-cm depth below the seed resulted in similar lengths of the primary root. At V5, the length of the primary root increased with increasing distance between seed and slurry, and the greatest length of the primary root was observed, when the slurry was placed 12 cm below the seed (Slu-12). Application of inorganic NP fertilizer stimulated elongation of the primary root at V2 and V3 compared to the UNF treatment, whereas the length of the primary root at V5 did not differ between the unfertilized and inorganic fertilized treatments.

During the experimental period, the seminal roots were shorter in the Slu-1.5 treatment than in other slurry treatments, although not always significant. In turn, the length of the seminal roots increased substantially from V2 to V5 in the Slu-1.5 treatment, indicating that the growth of the seminal roots was not as restricted as the growth of the primary root, when the slurry was placed very close to the seed. At V5, the NP treatment had the greatest length of the seminal roots (although not statistically significant), as well as the highest

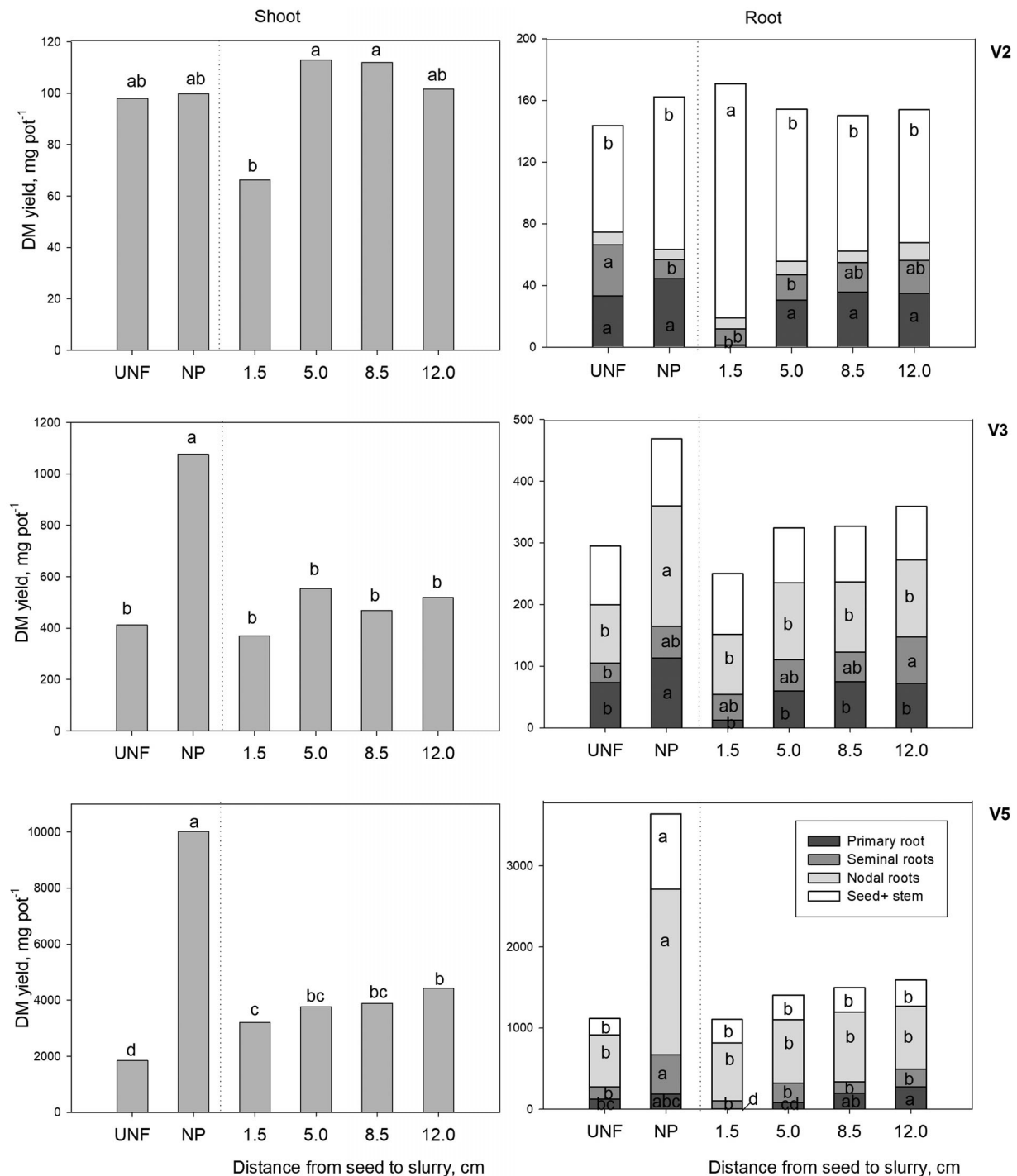


FIGURE 1 Shoot and root dry matter (DM) yield for the slurry treatments with different slurry placement depths and unfertilized (UNF) and inorganic nitrogen and phosphorus fertilized (NP) reference treatments at two-, three- and five-leaf stage (V2, V3 and V5) respectively. Different letters denote statistically significant differences within shoot DM yield and within each root type for each harvest date (Tukey, $P < .05$). There was no treatment effect on nodal root DM yields at V2 and on seed and stem DM yields at V3

DM yield of the seminal roots, resulting in the lowest specific root length (Table 3).

At V2, the length of the nodal roots did not differ among the treatments, and the nodal roots constituted a minor fraction of the total root length (Table 3). Rapid growth of

the nodal roots was apparent from V3 to V5, and the nodal roots constituted the major part of the total root system at the final harvest. The length of the nodal roots did not differ significantly among the slurry treatments, whereas an increase in DM yield and enhanced elongation of the nodal roots was

TABLE 2 Corn shoot phosphorus (P) and nitrogen (N) concentrations, shoot P and N uptake and N/P ratio in shoot for the treatments at the two-, three- and five-leaf stage (V2, V3 and V5). Different letters within each column for each harvest date denote significant differences among treatments (Tukey's HSD, $P < .05$). UNF, unfertilized reference treatment; NP, inorganic N and P fertilizer reference treatment; Slu, slurry treatments with a distance from seed to slurry band of 1.5, 5, 8.5, and 12 cm, respectively

Treatment	P shoot concentration % of dry matter			P shoot uptake mg pot ⁻¹			N shoot concentration % of dry matter			N shoot uptake mg pot ⁻¹			N/P shoot ratio			Shoot/root ratio		
	V3	V5		V3	V5		V2	V3	V5	V2	V3	V5	V3	V5		V2	V3	V5
UNF	0.21b	0.22c		0.85b	4.1c	4.23d	4.23d	3.40c	1.98b	4.17ab	13.8c	36.4d	16.2b	9.0c		0.68ab	1.38b	1.65b
NP	0.61a	0.29b		6.49a	29.2a	5.91a	5.76a	5.76a	2.27b	5.90ab	62.0a	227a	9.6c	7.8d		0.63ab	2.32a	2.76a
Slu-1.5	0.27b	0.35a		0.97b	11.3b	5.76a	4.72b	4.69a	4.69a	3.79b	17.4bc	150c	17.9ab	13.3b		0.39b	1.47b	2.91a
Slu-5	0.24b	0.28b		1.35b	10.7b	5.45ab	4.51b	4.35a	4.35a	6.16a	25.0b	164bc	18.6a	15.3a		0.73a	1.71b	2.71a
Slu-8.5	0.24b	0.28b		1.14b	10.9b	4.94bc	4.59b	4.29a	4.29a	5.55ab	21.5bc	165bc	19.0a	15.2a		0.76a	1.42b	2.59a
Slu-12	0.23b	0.26b		1.19b	11.6b	4.79c	4.38b	4.19a	4.19a	4.85ab	22.7bc	185b	19.2a	16.0a		0.66ab	1.46b	2.79a

evident in the NP treatment compared to the other treatments (Table 3).

The P uptake per root length at V5 was significantly higher in the Slu-1.5 treatment than in the Slu-8.5 and Slu-12 treatments (Table 4). The N uptake per root length was also higher in the Slu-1.5 treatment than in the Slu-8.5 and Slu-12 treatments at V2 and V3 (Table 4).

3.4 | Soil environment near the slurry band without plants

Concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ in soil were extremely high above the slurry band at the first sampling date, 21 d after slurry application (Figure 3ab). The concentration of $\text{NH}_4^+\text{-N}$ decreased with increasing distances to the slurry band. The $\text{NH}_4^+\text{-N}$ concentration in soil decreased rapidly with time, and 55 d after slurry application it was as low as the $\text{NH}_4^+\text{-N}$ concentration in soil before slurry application.

The concentrations of $\text{NO}_2^-\text{-N}$ in soil near the slurry band were high 21 and 35 d after slurry application indicating nitrification of $\text{NH}_4^+\text{-N}$ from the slurry but had decreased dramatically 55 d after application. At this date, the $\text{NO}_2^-\text{-N}$ concentration was not affected by the distance to the slurry band (Figure 3b). At the first sampling date, the $\text{NO}_3^-\text{-N}$ concentration was highest below the slurry band (67 mg $\text{NO}_3^-\text{-N}$ kg⁻¹ dry soil) and ranged from 40 to 64 mg $\text{NO}_3^-\text{-N}$ kg⁻¹ dry soil above the slurry band. At the second and third sampling date, the $\text{NO}_3^-\text{-N}$ concentration in soil increased markedly, especially near the slurry band and near the soil surface (Figure 3c).

Elevated soil pH levels were found near the slurry band 21 d after slurry application (Figure 3d), and the pH in soil decreased with increasing distances to the slurry band. The soil pH level decreased with time and was even lower than the original pH level 5.5–8.5 cm above the slurry band. The EC followed the same pattern as the soil pH across the distance-to-slurry gradient (Figure 3e), except near the soil surface (8.5 cm above the slurry band) at 35 and 55 d after application, where the EC increased. The EC remained at high levels during the experimental period. High moisture contents were found near the slurry band throughout the study period (Figure 3f).

The amount of WEP_i was clearly affected by the distance to the slurry band (Figure 4). The highest concentration of WEP_i was found 0–1 cm above the slurry band, and WEP_i decreased significantly only a few centimeters above the slurry band (Figure 4). The amount of WEP_u was also affected by the distance to the slurry band, but the concentrations of WEP_u were much lower than WEP_i .

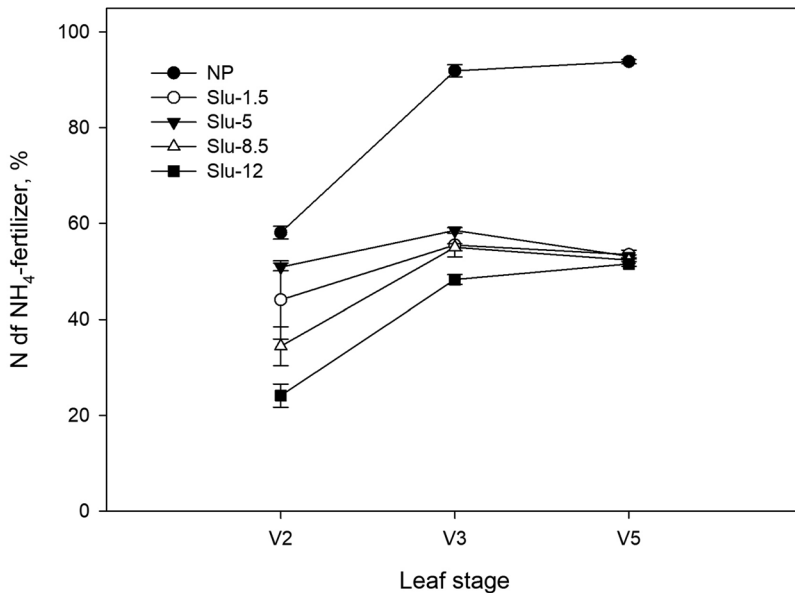


FIGURE 2 Percentage of nitrogen (N) in shoot derived from labeled NH_4^+ -fertilizer (N df NH_4^+ -fertilizer) in the inorganic NP fertilizer reference treatment (NP) and in the cattle slurry treatments for each slurry placement depth in relation to leaf stage. Error bars represent the standard deviation ($n = 4$). Slu, placed slurry treatments with a distance from seed to slurry band of 1.5, 5, 8.5, and 12 cm, respectively

TABLE 3 Root length and specific root length for each root type (primary, seminal and nodal roots) for the treatments at two-, three- and five-leaf stage (V2, V3 and V5) respectively. Different letters within each column for each root type denote significant differences among fertilizer treatments (Tukey's HSD, $P < .05$). ns, not significant; UNF, unfertilized reference treatment; NP, inorganic NP fertilizer reference treatment. Slu, placed slurry treatments with a distance from seed to slurry band of 1.5, 5, 8.5, and 12 cm, respectively

	Treatment	Root length			Specific root length		
		cm			m g^{-1} dry matter		
		V2	V3	V5	V2	V3	V5
Primary root	UNF	255b	905b	2,349ab	77a	129ns	204ns
	NP	436a	1,539a	2,113ab	101a	141ns	115ns
	Slu-1.5	3.00c	90.0c	81c	23b	92ns	283ns
	Slu-5	257b	943b	1,320bc	84a	159ns	162ns
	Slu-8.5	360ab	913b	2,849ab	103a	128ns	146ns
	Slu-12	360ab	899b	3,468a	104a	124ns	128ns
Seminal roots	UNF	232a	456bc	2,548ns	70ab	150ns	176ab
	NP	120ab	786ab	4,276ns	107a	164ns	86c
	Slu-1.5	55.6b	392c	1,884ns	52b	113ns	190a
	Slu-5	181ab	770abc	2,988ns	111a	151ns	106bc
	Slu-8.5	194a	673abc	2,229ns	103a	143ns	160abc
	Slu-12	193a	899a	2,851ns	91ab	122ns	138abc
Nodal roots	UNF	15ns	454ab	5,900ab	19ns	51ns	92a
	NP	15ns	709a	7,486a	25ns	35ns	37c
	Slu-1.5	13ns	326b	4,364b	18ns	34ns	63b
	Slu-5	15ns	444ab	4,678b	18ns	36ns	59b
	Slu-8.5	14ns	405ab	5,410ab	19ns	36ns	63b
	Slu-12	22ns	621ab	4,405b	20ns	49ns	57b

4 | DISCUSSION

4.1 | Root morphology, biomass and nutrient uptake related to placement depth

The growth of the primary root was clearly restricted, when the slurry was placed 1.5 cm below the seed, and the primary

root did not recover during the experiment, that is, until V5. The embryonic root system (primary and seminal roots) constituted the major part of the root system at V2 as also described by Hochholdinger (2009). Ahmed, Zarebanadkouki, Kaestner, and Carminati (2016) found that the main function of the primary and seminal roots of 2-wk-old corn plants was to transport water to the shoot, whereas absorption

TABLE 4 Phosphorus (P) and nitrogen (N) shoot uptakes per root length for the treatments at the two-, three- and five-leaf stage (V2, V3 and V5). Different letters within each column for each harvest date denote significant differences among treatments (Tukey's HSD, $P < .05$). UNF, unfertilized reference treatment; NP, inorganic NP fertilizer reference treatment; Slu, placed slurry treatments with a distance from seed to slurry band of 1.5, 5, 8.5, and 12 cm, respectively

Treatment	P uptake per root length		N uptake per root length		
	mg m ⁻¹		mg m ⁻¹		
	V3	V5	V2	V3	V5
UNF	0.047d	0.038d	0.83b	0.76b	0.35c
NP	0.217a	0.212a	1.06b	2.08a	1.65ab
Slu-1.5	0.122b	0.181ab	6.20a	2.17a	2.41a
Slu-5	0.063c	0.140bc	1.36b	1.17b	2.15ab
Slu-8.5	0.057cd	0.105c	1.00b	1.08b	1.59b
Slu-12	0.049cd	0.108c	0.85b	0.95b	1.73ab

of water from the soil took place in the lateral roots of the seminal roots. The restricted growth of the primary root in the present study could therefore limit the ability of the plant to transport water to the shoot. This was also reflected in a significantly lower shoot DM yield in the Slu-1.5 treatment. The restricted growth of the seminal roots at V2 and V3 in the Slu-1.5 treatment could potentially also stunt the growth of young corn plant, since the formation of seminal roots plays an important role in the uptake of immobile nutrient such as P by increasing soil exploration (Zhu, Mickelson, S.M., & Lynch, 2006). Surprisingly, the shoot P uptake at V3 and V5 was not affected by the slurry placement depth despite reduced growth of the seminal roots at V3, indicating that the plants were able to compensate for the restricted P uptake through the primary and seminal roots.

The nodal roots constituted the greatest portion of the total root biomass at V3 and V5, and the slurry placement depth did not affect the growth of the nodal roots at these leaf stages. Ahmed et al. (2018) found that for 5-wk-old corn plants water was mainly taken up by nodal roots. In line with this, Robert et al. (2012) found that crown roots (nodal roots formed below ground) were more vital for young corn plants than the primary root, when they studied the effect of excision of the crown and primary roots, respectively. Moreover, the crown roots were the primary sink for newly fixed CO₂ and contained higher amounts of free amino acids, sugar, and starch than the primary root, which shows that newly developed belowground tissues have the highest value for the plant (Robert et al., 2012).

At V5, the plants in the Slu-1.5 treatment had a significantly lower DM yield than the Slu-12 treatment, despite similar growth of the nodal roots in the slurry treatments. This suggests that the young corn plants were not able to fully recover in subsequent growth stages after significant root injuries of the primary root at V2 induced by slurry applied 5 d before transplanting, even though the nodal roots take over functions such as water and nutrient uptake after the first weeks of growth.

The ¹⁵N assay revealed that shoot N uptake from NH₄⁺-N in the slurry band depended on the slurry placement depth. We recognize that addition of highly enriched ¹⁵NH₄⁺ to the slurry does not ensure labelling of the organic N fraction in the slurry. To ensure this, feeding with ¹⁵N labeled fodder to the animals is needed (Sørensen & Jensen, 1998). However, by using the current approach it is possible to compare the plants' ability to take up N from the highly concentrated slurry band among the treatments with different slurry placement depths. For the treatment with a slurry placement depth of 1.5 cm from the seed, the percentage of N in shoot derived from NH₄⁺-N in the slurry band was rather constant from V2 to V5, indicating that the roots were able to benefit from NH₄⁺-N in the slurry band despite restricted root growth. For the treatment with a slurry placement depth of 12 cm, the plant was not able to take up similar proportions of NH₄⁺-N in slurry at V2 as the other slurry treatments, indicating that the root reached the slurry band later in this treatment. Even though the roots reached the slurry band and utilized the nutrients later in the Slu-12 treatment, this treatment had the highest DM yield and shoot N uptake at V5 among the slurry treatments. This suggests that damages to the primary roots were more important for corn growth than a delayed uptake of NH₄⁺-N from slurry due to increased slurry placement depth. However, Pedersen et al. (2019; unpublished data) found in a field study with corn that placing the slurry at 17-cm depth (approximately 13 cm below the seed) was too far from the young roots in order to ensure sufficient nutrient uptake from the slurry band and caused reduced yield.

The lower proportion of N in shoot derived from the slurry-NH₄⁺ than from the inorganic fertilizer-NH₄⁺ could be due to uptake of mineralized unlabeled organic N in slurry, (Sørensen & Jensen, 1998). Moreover, microbial immobilization of slurry NH₄⁺ (Sørensen & Jensen, 1995) and losses of N via denitrification induced by the NO₂⁻ accumulation (Figure 3b), may further explain the lower amount of N in shoot derived from NH₄⁺-slurry and the lower N recovery

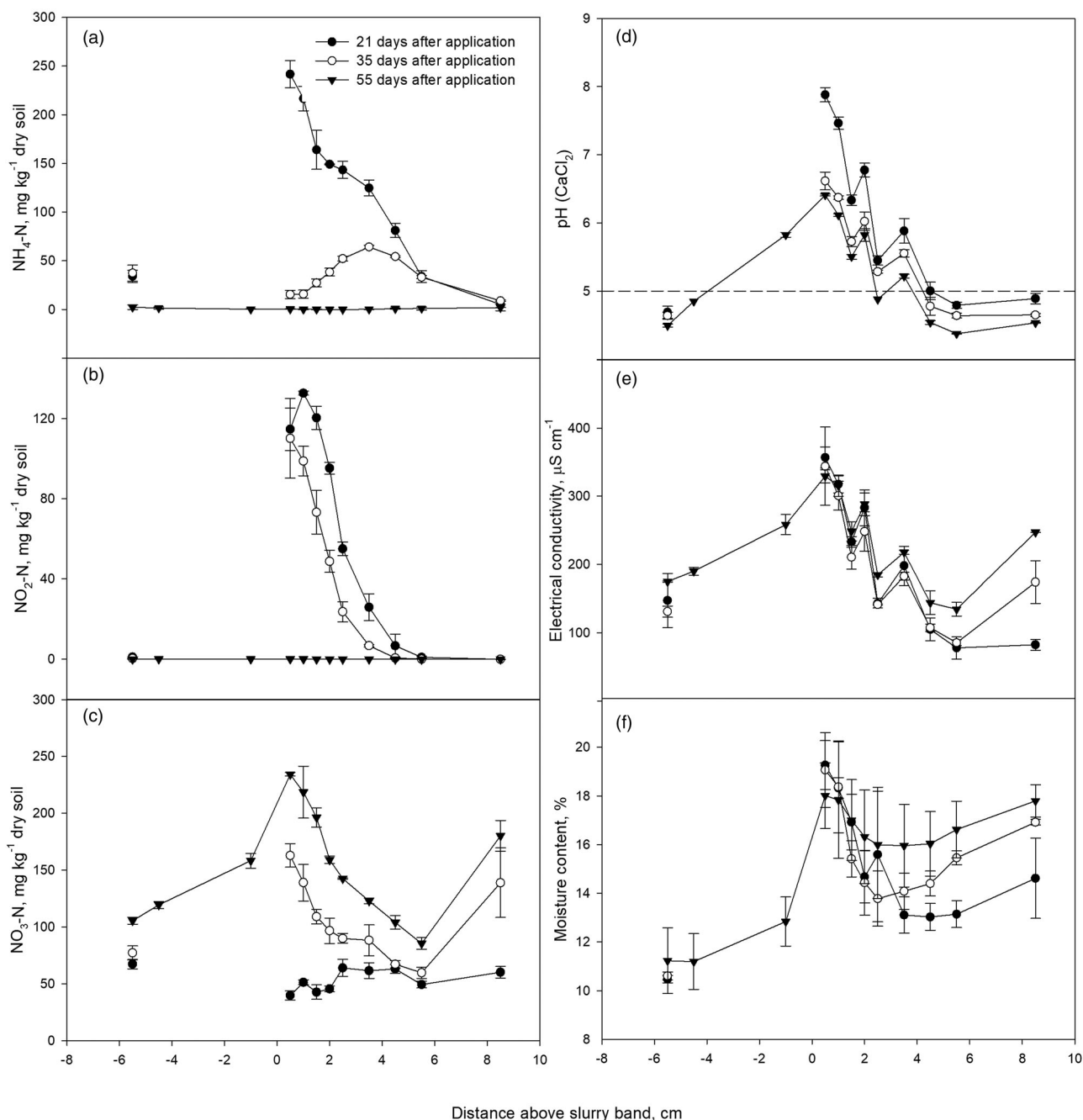


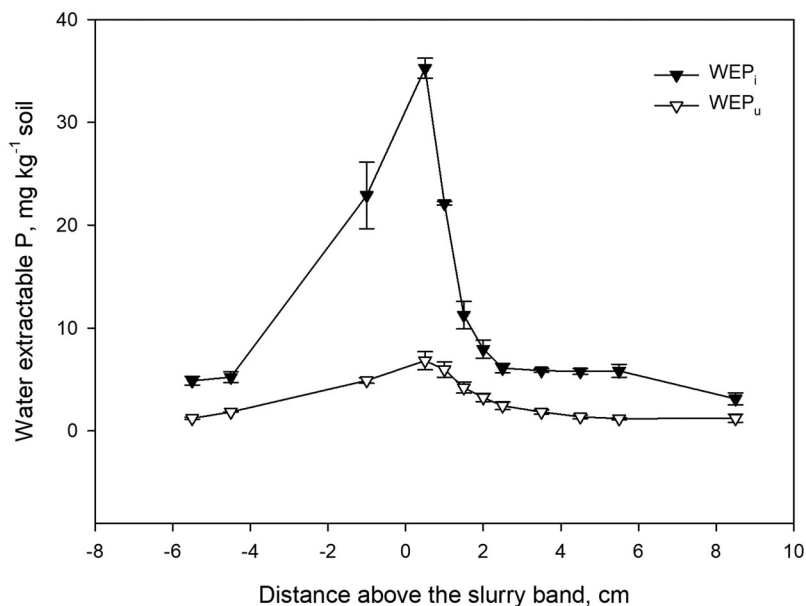
FIGURE 3 a) Ammonium-N ($\text{NH}_4^+\text{-N}$), b) nitrite-N ($\text{NO}_2^-\text{-N}$), c) nitrate ($\text{NO}_3^-\text{-N}$), d) pH (CaCl_2), e) electrical conductivity, and f) moisture content in soil without plants as affected by the distance to the slurry band at the three sampling dates (21, 35, and 55 d after slurry application). The dashed line at d) indicates the initial pH level. Error bars represent the standard deviation ($n = 3$). Note that positive and negative values on the x-axis denote the soil sampling distances above and below the slurry band, respectively

rate of NH_4^+ fertilizer by the corn plants in the slurry treatments than in the NP treatment (Supplemental Table S1).

Interestingly, the shoot P uptake at V5 was unaffected by the slurry placement depth. The greater P uptake per root length in the treatment where slurry was placed 1.5 cm below the seed may indicate a physiological response to the nutrient-rich slurry band (Hodge, 2004) in order to maintain sufficient P uptake, despite restricted root growth in this treatment.

Application of inorganic NP fertilizer in the NP treatment did not increase the shoot DM yield at V2 compared to the UNF control, thus reaffirming that seed N and P reserves dominate the nutrient supply during the first weeks of growth (Nadeem et al., 2011). Corn plants grown without fertilizer had a low shoot/root ratio at V5 compared to the other treatments. Allocation of resources to root growth and elongation on the expense of shoot growth is most likely a

FIGURE 4 Dissolved water-extractable reactive P (WEP_i) and unreactive P (WEP_u) in soil without plants as affected by the distance to the slurry band 55 d after slurry application. Error bars represent the standard deviation ($n = 3$). Note that positive and negative values on the x-axis denote the soil sampling distances above and below the slurry band, respectively



response to the limited availability of N and P in order to increase soil exploration (Zhu & Lynch, 2004).

4.2 | Changes in soil chemical properties near the slurry band

High concentrations of NH_4^+ -N near the slurry band 3 wk after slurry application were expected, since high NH_4^+ -N concentrations were originally present in the cattle slurry. Restricted root growth caused by the combination of high moisture content, high pH, and accumulation of NH_4^+ -N near the slurry band is in accordance with previous studies (Sawyer & Hoef, 1990). In particular, NH_4^+ has been shown to inhibit the growth of primary roots in *Arabidopsis thaliana* (Liu et al., 2013).

From 21 to 35 d after slurry application, the NH_4^+ -N content in soil decreased due to nitrification, and thus the potential toxic effect of NH_4^+ was reduced to its less toxic form of NO_3^- . However, the intermediate step in the nitrification process, where NO_2^- accumulates, reached its maximum at the first and second sampling date. Toxicity of NO_2^- on plant growth have mainly been observed under acid conditions (Bingham, Chapman, & Pugh, 1954). Lee (1979) stresses that the NO_2^- anion itself is not toxic, but the toxicity of NO_2^- is due to free nitrous acid, which is present in the equilibrium with NO_2^- , and therefore nitrous acid is dominating under acid conditions. Soil pH decreased from 21 to 35 d after application, and hereby the toxicity of nitrous acid may have increased 35 d after application. Whether early damage of the primary root observed in the present study primarily was ascribed to high concentrations of NH_4^+ -N or NO_2^- -N remains uncertain.

The elevated soil pH near the slurry band 21 d after slurry application could be due the presence of carbonates and other buffering compounds in slurry, and due to microbial decomposition and CO_2 release during the transformation of organic acids and organic N in slurry to NH_4^+ -N (Schmitt, Sawyer, & Hoef, 1992; Sommer & Husted, 1995). High concentrations of NH_4^+ -N and elevated pH in soil near the slurry band may have caused high concentrations of free ammonia, which strongly inhibits *Nitrobacter*, which oxidizes NO_2^- to NO_3^- (Lee, 1979; Smith, 1964). Furthermore, Vadivelu, Yuan, Fux, and Keller (2006) found that free nitrous acid inhibits the *Nitrobacter* metabolism. These mechanisms may explain the temporary accumulation of NO_2^- -N.

It is important to stress that the inorganic N concentration measured in soil without plants near the slurry band was likely higher in unplanted soil where there is no removal of N through plant uptake. In the slurry treatments, the shoot N uptake averaged 5, 22, and 166 mg N at V2, V3, and V5, respectively. The total NH_4^+ -N input in slurry was 290 mg, suggesting that high concentrations of inorganic N in soil were also present in planted soil, especially at V2 and V3.

The amount of WEP_i increased significantly in soil near the slurry band only (Figure 4), reaffirming the low mobility of P in soil, and that P remained near the site of fertilizer placement even 55 d after application. It also underlines that the roots have to grow near the slurry band, before they can benefit from the increased amounts of WEP_i , derived from the placed slurry.

The slurry placement depth did not affect the rapid growth of the nodal roots from V3 to V5, suggesting that conditions were less toxic at these growth stages, and that toxic conditions only dominated during the first 24 DAS (= 27 d

after slurry application), where the embryonic root system develops.

4.3 | Challenges by replacing inorganic starter fertilizers with placed slurry

Dry matter yields at V3 and at V5 were higher in treatments applied with inorganic NP than slurry despite equal NH_4^+ -N and P application rates. This may have several explanations. First, the inorganic fertilizers were applied dissolved in water which infiltrates in the soil more easily than slurry. This might result in better conditions for root growth. In line with this, observations at irrigation showed a much quicker water infiltration at the surface in the NP treatment than in the slurry treatments, indicating that the slurry formed a barrier for both water and roots in the present pot study. Second, formation of other compounds such as organic acids and methane under low oxygen concentrations near the slurry band could further inhibit growth (Lynch, 1980; Sawyer & Hoeft, 1990; Schmitt et al., 1992), whereas such conditions were unlikely in the NP treatment due higher infiltration capacity. Within a few days, the concentration of organic acids near the slurry band will decrease, since organic acids are an easily decomposable carbon source for microorganisms in soil (Kirchmann & Lundvall, 1993), and hereby the time span from slurry application to sowing might affect the potential toxic effect of placed slurry. Third, the distance from seed to inorganic NP fertilizer was 5 cm, which allowed the root to reach the fertilizer earlier than in the treatments with a slurry placement depth of 8.5 and 12 cm. In treatments with a slurry placement depth of 1.5 and 5 cm, the benefits of a shorter distance from seed to slurry were overruled by unfavorable conditions near the slurry band. Lastly, labile organic carbon contained in the applied slurry could affect the soil microbial processes that may cause P and N immobilization (Brod et al., 2016), which could reduce growth in the slurry treatments.

The yield reduction in slurry treatments compared to the NP treatment measured in pots with firm boundaries and restricted soil volume might be much less pronounced under field conditions where roots easily can explore soil compartments unaffected by the concentrated slurry band. Another important point is that shoot N concentrations, and thus N/P ratios below 10 were observed in the NP treatment at V5, which according to Güsewell (2004), suggested that biomass production had become N-limited in this treatment at this growth stage. At the same time, the concentrations of NO_3^- -N in planted soil at V5 were much lower in the inorganic NP treatment than in the slurry treatments (Supplemental Table S2). It is therefore possible that the corn plants in the slurry treatment at subsequent growth stages would catch up as they gain access to not yet utilized slurry N, while the N supply in the NP reference would be N-limited.

This would be in accordance to Pedersen et al. (2017) who demonstrated that the positive effect of broad-banded slurry on corn biomass only became evident at the seven-leaf stage. Altogether, this illustrates clearly that a complex nutrient source such as slurry can be less plant available than inorganic N and P fertilizers at initial growth stages, but also that the slurry can supply the corn plants with nutrients for a longer period during early growth. To further improve the initial fertilizer value of slurry, slurry processing technologies such as acidification could be applied (Pedersen et al., 2017).

5 | CONCLUSION

In this pot experiment, we found substantial injuries of the primary and seminal roots at V2 by placing the slurry 1.5 cm below the seed. The primary root was not able to recover in subsequent growing stages, and likewise the shoot biomass was reduced when slurry was applied at this depth. The shoot P uptake at V3 and V5 remained unaffected by slurry placement depth indicating that the young corn plants were partly able to compensate for early damage of the primary root, whereas the shoot N uptake at V5 decreased when slurry was placed too close to the seed. At V3 and V5, plants applied with inorganic N and P fertilizer had a higher dry matter yield than the slurry treatments.

Within a few centimeters from the slurry band, the soil was characterized by accumulation of NH_4^+ and NO_2^- and elevated levels of moisture content and pH 3 wk after slurry application. These soil chemical conditions could explain the restricted root growth of the primary root when slurry was placed very close to the seed. The nodal roots, which developed later and constituted the major portion of the root system at V5, were not affected by slurry placement depth, concurrent with the less toxic environment near the slurry band at this growth stage, where NH_4^+ -N and NO_2^- -N were oxidized to NO_3^- -N.

Placement of complex P sources such as cattle slurry can restrict growth of the primary root, if the slurry is placed 1.5 cm below the seed. To prevent root injuries, banded slurry should be placed 5–12 cm below the seed.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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