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RESEARCH ARTICLE

THE MINERAL NITROGEN DISTRIBUTION FROM THE COMBINED FREE-RANGE PIG FARMING AND ENERGY CROP PRODUCTION SYSTEM

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ABSTRACT

Keeping pigs in the outdoor pasture convey a high risk of environmental pollution through nitrate leaching. Integrating grassland based free-range pigs with energy crops has been proposed as an alternative approach to reduce pollution. This study investigated the N_{min} distribution and potential NO_3 -N leaching at various soil depths (0-25, 25-50, 50-100 cm) and distances (0.5, 2.5, 4.5, 6.5 and 9.5) from willow trees in the lactating sows' paddocks. The results observed the highest levels of N_{min} close to the huts and adjacent to feed troughs and the lowest N_{min} levels close to the willow trees with a 1 m soil depth. For soil water analysis, the nitrate leaching as expected was the highest near the huts with an average of 37 mg NO_3 -N/liter followed by 28 Mg NO_3 -N/liter at 6.5 m with the lowest levels close to willow trees. The lowest NO_3 -N leaching around willow zone could be subjected to high water and nutrients uptake by trees. The 9.5 m close to feeders had the low leaching which could be due to low NO_3 -N as NH_4 -N dominated with 90% of the total N_{min} with about 79% of this being in topsoil. Therefore, with a long growing season and deep root system of energy crops, the paddock design should maximize the trees uptake potential near this zone as pigs are known to have high excretion activities near shelter zones. As a result, a substantial N loss through nitrate leaching could be minimized.

KEYWORDS

Nitrate leaching, Free-range farming, Energy crops, Mineral nitrogen, Animal welfare.

1. Introduction

Free-range pig production is typically comprised of pregnant and lactating sows with piglets being grazing outside, roaming and resting around the pasture by day and sleep in small huts during night time (Horsted et al, 2012; Webb et al, 2014; Williams et al, 2005). The EU regulation requires free-range pigs to have permanent access to pasture during summer for at least 150 days a year even though some farmers tend to keep the pigs even longer. Weaning for piglets according to the regulation is done at 40 days since farrowing even though there are country-specific conditions that elongate the weaning age (Directive, 2008). With the indoors systems for weaners, which include a small outdoor running space, they will be fed until reaching the slaughter weight (Kongsted & Hermansen, 2005). In some farming systems, depending on national standards, farm-specific objectives, and local environment, different combinations of both outdoor and indoor settings can be practiced (Vieuille et al, 2003). Like in other EU countries, the presence of grazing areas in Danish free-range pig production has raised concerns about possible environmental impacts including increased ammonia volatilization (Sommer & Hutchings, 2001), denitrification (Petersen et al, 2001) and high nitrate leaching (Eriksen, 2001). The high N and P surplus from the urine and defecations have the environmental implication of increased leaching rate which may lead to contamination of groundwater. This has negative health impacts on humans (Nie et al, 2019; Williams et al, 2000) but also affects the aquatic ecosystems through eutrophication (Honeyman, 2005; Quintern &

Sundrum, 2006). The N loss in the free-range system is not distributed equally over the grassland as high N loss rates are more pronounced in hotspot areas such as near the huts, shelters and feeders compared to the rest of the field (Andresen, 2000). Apart from excess N and P loading problems to the environment, the free-range organic pigs have been associated with higher piglet mortality rate compared to indoors conventional pigs (Bilkei, 1995), management challenges due to seasonal weather fluctuations (Honeyman, 2005) and maintenance of the grassland cover (Vieuille et al, 2003). The combination of high nutrient loss plus higher piglet mortality rates in organic pig farming has led to lower N and P efficiencies (Nielsen & Kristensen, 2005).

Integrating free-range pig farming with selected energy crops particularly willow (*Salix spp.*), poplar (*Populus spp.*) and miscanthus has been a proposed approach to improve animal welfare by providing shelter and protection in adverse weather conditions (Horsted et al, 2012). Also, the trees with their high water and N uptake may reduce nutrients losses to the environment as well as improving agricultural diversity important for the ecosystem services. With the average nitrate leaching in Danish agricultural land being used to be 70 kg N/ha, perennial energy crops such as willow and miscanthus have shown promising results of reducing between 40 - 65 kg N/ha (Jørgensen et al, 2005). With deep and the permanent root system, the root zone for willow, for instance, can be as deep as 1.3 m when well established even though the root depth could vary with soil type, willow clone type, nitrogen source, and management

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(Mortensen et al, 1998; Smith et al, 2013). Additionally, willow is known to be tolerant to high plant density and waterlogging conditions as well as the coppicing ability (Mortensen et al, 1998; Sevel et al, 2014; Volk et al, 2006). Therefore, with the established energy crops grown along paddocks' boundaries in the study site, the investigation was done to quantify the distribution of mineral nitrogen ($N_{\rm min}$) in the soil for both nitrate (NO_3-N) and ammonium (NH_4-N) at various soil depths (0-25, 25-50, 50-100 cm) and distances (0.5, 2.5, 4.5, 6.5 and 9.5) from trees. Additionally, the quantification of NO_3-N leaching at each distance point was done using ceramic suction cups to understand the potential leaching of NO_3-N in this pig farming system.

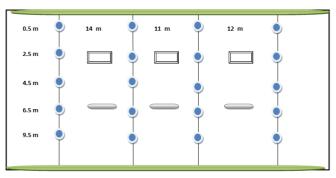
2. MATERIALS AND METHODS

2.1 Site description

The experiment was carried out in a free-range organic pig farm at Hovborgvej Brorup, Region of Southern Denmark (Ulvehøjvej 1, 6650 Brørup) located at 55° 34' 35" N, 8° 59' 30" E. This farm is among the two biggest organic pig farms in Denmark with established energy crops in the pig paddocks (Kongsted, 2015). The willow together with poplar trees have been established back in 2009 in a 1-hectare area of grassland that is used for lactating sows with the willow not been harvested at least by the time this study was carried out (Kongsted, 2015).

2.2 Experiment layout

In order to investigate nitrate leaching which in Denmark normally occurs between September and March (Blicher-Mathiesen et al, 2014), the ceramic suction cups were installed in autumn of 2014 (late October) in the paddocks where the pigs had been removed out 4 weeks before the installation started. The electric fence that marks the end of the paddock was between the two willows rows located on each side of the paddock. Four measurement rows of 10 meters long from the willow were established with five suction cups in each row at increasing distances of 0.5, 2.5, 4.5, 6.5 and 9.5 meters from the willow as seen in *Figure 1*. Due to the nature of willow roots which can go as deep as 1.3 m from the surface, the drilling machine made a hole of 1.5 m depth from the surface. Additionally, a 30 ml solution of *Silica Flour Millisil* M 6.1 was applied at the bottom before inserting the cup so as ensure good contact between the suction cup and soil. The sampling and vacuum control tubes from the five suction cups in each row were connected in one vacuum control chamber or also called a sampling chamber.



Legend:

Feeding troughs Suction cups Huts Willow trees

Figure 1: An experimental layout showing four measurement rows, each with five suction cups established against the rows of willow trees on each side of the paddock. The grazing area in the middle of the paddock each side of the willow rows was not covered with grass and was mainly dominated by mud during installation rather than grass.

2.3 Soil Sampling

At a distance of 1.5 m on each side of each suction cup installed, the soil drill was used to take soil samples at three depth levels of 0 - 25 cm, 25 - 50 cm and 50 - 100 cm from the soil surface. The samples were used to characterize both physical and chemical soil properties including soil texture, total carbon, phosphorus, mineral-N (NO $_3^-$ and NH $_4^+$) and soil pH. Therefore for all the soil sampling units and at all depth levels, there were a total of 120 samples.

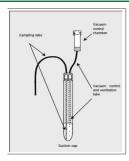


Figure 2: A sketch illustrating a typical suction cup and its components



Figure 3: The suction cups used in the experiment



Figure 4: A vacuum control chamber as seen after sampling and vacuum control tubes of five suction cups in one measurement row are already connected

2.4 Soil mineral N (Nitrate-N and Ammonium-N) analysis

For mineral N analysis (NO3- and NH4+), samples collected were stored at temperature -20 C to avoid the volatilization of ammonium which is triggered as the temperature increases. One day before the analysis, the samples were taken off the freezer, followed by weighing them and then mix with Potassium Chloride (1 M KCl) before being taken to a shaker for the solution and soil particles to thoroughly mix. The soil samples were then centrifuged for 5 minutes at 3000 rpm (rounds per minute). Using Spectrophotometry method, the samples were analyzed for Ammonium-N and Nitrate-N using the procedures outlined by Best, (1976). In the Autoanalyser machine, there were separate tubes for both nitrate and ammonium that pass through the pre-mounted analyzer membrane. Hydrazine was used to convert nitrite into nitrate under Copper catalyst (CuSO42-). The nitrate was then reacted with sulphur amide and ethylenediamine (C₂H₄(NH₂)₂) to an azo dye (which can be range from brown-orange-pink colour depending on the NO₃- concentration) while the actual concentration was determined using a spectrophotometer at 520 nm. For analysis of ammonium-N concentration, the ammonium reacts with salicylate (C₇H₆O₃) and sodium dichloroisocyanurate under the catalyst Sodium nitroprusside Na₂[Fe(CN)₅NO] to form a pale green or emerald solution before being determined by the spectrophotometer at 660 nm. The separate concentration readings for nitrate and ammonium from the spectrophotometer were eventually being displayed in the connected computer.

2.5 Soil Water Sampling and Analysis

Nitrate leaching was determined using the NO_3 -N concentration in the soil water that was sampled once every 2 weeks since the installation of cups with the first measurement being on 7th November 2014 until 5th March 2015. A suction of 70 kPa was applied 2 days before taking samples which

made soil water slowly penetrate the suction cup. After collection of samples for 9 times, water samples were sent to a private and independent environmental laboratory called "AnalyTech Miljølaboratorium A/S" for analysis of NO3-N concentrations in soil water. Using the UV spectrophotometer method as applied by Navone (1964), the wavelength of 220 nm was applied to obtain the nitrate levels in the sample. However, with the presence of dissolved organic matter in the sample which could also be absorbed in 220, the UV of 275 was used where only nitrate cannot be absorbed and the difference between the two wavelengths gives the approximate nitrate levels in the soil water. To avoid or minimize the interference effect of some suspended materials such as hydroxide and carbonates, simple filtration together with additional hydrochloric acid is normally used. Other reagents used with this method include distilled water that is nitrate-free.

3. STATISTICAL ANALYSIS

For N_{min} analysis, the two way factorial ANOVA design with the main effects of distance and depths was used. The distance consisted of five levels (0.5, 2.5, 4.5, 6.5 and 9.5 meters) while the depth had three levels (0-25, 25-50 and 50-100 cm). The four replications were represented as four measurement rows. Whenever there was a significant difference (when the F-test ($P \le 0.05$), the Tukey test was used to point out which pair of concentration means differed significantly. For soil water NO_3 -N analysis, the two way factorial ANOVA design was also used with the main effect with this analysis being distance from willow and time of sampling ANOVA was used to test whether there was an interaction of time of sampling and distance from willow on the nitrate leaching potential or whether the nitrate concentrations were affected by the time of sampling or the distance from willow trees.

4. RESULTS

4.1 Nitrate-N distribution at various soils depths and distances from willow

With the NO_3 -N distribution in the lactating sow paddocks, statistical analysis found a significant interaction effect of the depth and distance (df=8, p<0.05). There was also a strong significant difference in NO_3 -N distribution due to distance at various points from the willow trees (df = 4, p<0.001) while analysis found no effect due to the depth variations (df=2, p>0.05). The NO_3 -N distribution tended to increase from 0.5 m before reached peak levels at 4.5 m and then sharply decreased to the last distance point (Figure 5). At the distance 4.5 m from the willow, NO_3 -N concentration was significantly higher at all the soil depths compared to other distance points.

4.2 Ammonium-N distribution with soil depths and distances variations

The statistical analysis also showed the interaction effect of depth and distance on the distribution of NH₄-N in the pigs' paddocks (df = 8, p< 0.05). There was also a strong significant influence of both distances (df = 4, p<0.001) and depth (df = 2, p<0.001). The NH₄-N from willow to 4.5 m was statistically not different at all the three soil depths even though a large proportion of NH₄-N was found in topsoil. NH₄-N was however significantly higher at 6.5 and 9.5 m distance with 70.5 and 90.6 Kg N/ha respectively which were 3 times higher compared to the NH₄-N in the first 4.5 m from the willow trees (Figure 6). In all the distance points from willow, the topsoil (0 - 25 cm) contributed between 73 to 87% of the total NH₄-N in the whole soil profile. The distances 6.5 and 9.5 m are where the feeders were located in the summer and early autumn before the experiment commence as seen in Figure 6.

$4.3\,$ Total mineral-N distribution at different soil depths and distance from the willow

When considering the total average $N_{\rm min}$ for the four measurement rows, the statistical analysis did not find the interactive effect of the two main factors on $N_{\rm min}$ distribution (df=8, p>0.05). There was however strong statistical significance due to the main factors depth variations (df=2, p<0.001) and distance (df=4, p<0.001). From the willow up to 9.5 m distance, the $N_{\rm min}$ showed a significant increasing trend for the topsoil (0-25 cm) with the lowest levels of 25.4 Kg N/ha at 0.5 m and the highest of 72.6 Kg N/ha at 9.5m. The constituents of this $N_{\rm min}$ differed with distances. For instance, the highest contribution of NO_3 -N in the topsoil (0-25 cm) was found at 4.5 m with about 64% of $N_{\rm min}$ while 90 and 98% of NH₄-N accounted for total mineral N at 6.5 and 9.5 m respectively. For the other lower soil profiles, there was an increasing trend for mineral N up to 4.5 m while at 6.5 and 9.5 m the mineral N seemed to decrease.

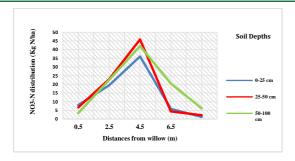


Figure 5: Nitrate-N distribution at different soil depths and distance from the willow

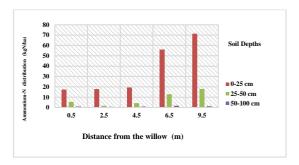


Figure 6: Ammonium-N distribution at different soil depths and distance from the willow

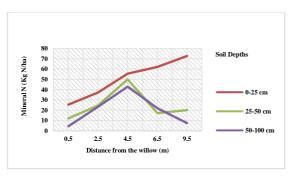


Figure 7: Mineral -N distribution at different soil depths and distances from willow

Table 1: ANOVA output summarizing the significance levels existed on the soil samples (NH₄-N, NO₃-N and Mineral N) and in the soil water (NO₃-N)

water (1103 11)				
Variables	Main Factors & Interaction	Degrees of Freedom	P-value	Significance
NO3-N concentration in soil	Distance: Depth	8	0.0206	*
	Distance	4	8.952e-09	***
	Depth	2	0.1709	NS
NH4-N concentration in soil	Distance: Depth	8	0.01832	*
	Distance	4	1.798e-08	***
	Depth	2	< 2.2e-16	***
Nmin concentration in soil	Distance: Depth	8	0.06761	NS
	Distance	4	0.0002116	***
	Depth	2	< 2e-16	***
NO3-N in Soil water	Distance: Sampling Time	32	0.9012	NS
	Distance	4	0.001523	**
	Sampling Time	8	0.939754	NS

Legend: NS - Not significant, * - Significance level Significance codes:

NS	P > 0.05
*	P < 0.05
**	P < 0.01
***	P < 0.001

4.4 Nitrate-N concentration in the soil water

Even though there was an interaction effect of distance from the willow and sampling time on the NO₃-N concentration in the soil water, the statistical analysis didn't find them significant (df=32, p>0.05). The NO₃-N concentration level was only found to be significantly affected by the distance variations (df=4, p<0.01) while NO₃-N variation at each distance due to time measurement was not statistically significant (df=4, p>0.05) as summarized in Table 1. With variations due to distances, the Tukey test found only significant difference of NO₃-N concentration at 4.5 and 6.5 distances when they were compared to the closest measurement point to the willow (0.5 m) and at 9.5 m. The other variations with distances were not statistically different. The average NO₃-N in soil water didn't show high variations with sampling dates throughout the experiment where the highest and the lowest average levels were 24 and 18 mg NO₃-N/liter, respectively. Unlike sampling dates, distance variations revealed high NO₃-N differences during the sampling period. For example, 4.5 m recorded the highest average level of 37 mg NO₃-N/liter for all sampling dates even though the highest concentration was 57 mg NO₃-N/liter on 18th Dec 2014 (fourth sampling). Unlike other distances, the NO₃-N at 9.5 had the lowest average difference between the highest and lowest levels with 6 mg NO₃-N/liter while 4.5 m had the biggest average difference with 45 mg NO₃-N/liter.

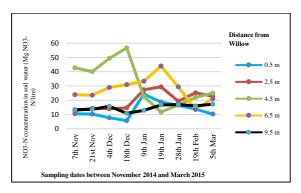


Figure 8: The average NO3-N concentrations in the soil water at different sampling periods and different distance points

5. DISCUSSION

5.1 Spatial Mineral N distribution in the paddocks

Several studies have documented the heterogeneity in the spatial distribution of N_{min} in the outdoor pig paddocks (Horsted et al., 2012; Kongsted & Hermansen, 2005). The defecation behavior and hence nutrients distribution has most been influenced by the spatial allocation of features such as feeding troughs, huts (Webb et al., 2014) and perennial trees within paddocks (Horsted et al., 2012). In the semi-natural environment, where there are diverse environmental and social stimuli, Stolba & Wood-Gush (1989) studied that pigs use mostly wooded and bushes areas for their shelter compared to other areas in the paddock. If available, pigs tend to find the shelter especially for temperature amelioration and where there is less stress from wind velocity (Stolba & Wood-Gush, 1989). This would, however, results in increased excretory behavior and hence nutrients loading close to these shelter zones as reported by an earlier study by Horsted et al., (2012) in free-range pigs with willow and miscanthus that reported high excretory behavior near the willow zones. From this study (Horsted et al., 2012), the pigs spent 54% of their activities for resting near the trees and about 49% of the excretion behavior close to the willow zone.

The current study observed an increasing trend for mineral-N from willow up to 4.5 m, which was where the huts located. Compared to the closest measurement to the willow with 42 Kg N/ha, the $N_{\rm min}$ at 4.5 m was threefold with 149 Kg N/ha (with about 84% being NO₃-N). At further distances of 6.5 and 9.5 m, even though the $N_{\rm min}$ wasn't as higher as at 4.5 distance, there were about two-fold $N_{\rm min}$ as compared to the willow zone (0.5 m). The significantly low $N_{\rm min}$ in the willow zone as found in our experiment could be explained by two main reasons. Firstly, may be due to higher N demand by the willow as $N_{\rm min}$ was increasing as moving away from the trees from 42 Kg N/ha at 0.5 m and reached peak levels of 149 Kg N/ha at 4.5 m before having a nearly uniform $N_{\rm min}$ at 6.5 and 9.5 m with 100 Kg N/ha. Willow with their fast biomass growth, long growing season and deep root system carry a potential for a significant $N_{\rm min}$ uptake (Dimitriou et al, 2012; Uffe Jørgensen et al., 2005; Sevel et al., 2014). Previous studies have reported the energy crops to have a potential of reducing 40 - 65 Kg

N/ha of NO₃-N that could be easily lost by leaching in sandy soil. With the willow in a study area being about 6 years old (Kongsted, 2015), the root system would be expected to be well established up to few metres horizontally away from the trees and this could reduce the N_{min} concentration from around the trees up to 4.5 m distance in the study site.

Also, the second possible explanation for low N_{min} close to the willow than what we expected was due to spatial allocation of trees, huts, and feeding and water troughs. The distance between huts and feeders was only about 4 metres apart and which was nearly the same distance from the huts to the trees. The experiment by Horsted et al., (2012) reported high excretion activities close to the willow, with the willow zone located between the huts and feeders while in our current study the hut was located between the willow trees and feed & water troughs. Unlike our experimental paddocks, the study by Horsted et al., (2012) was in particular complex with different zones within a single paddock which include zones of grass, willow, miscanthus plus willow + poplar for both small and large paddocks. The grazing area for pigs in our study area had little grass cover for the sows and this have made pigs to depend most of their daily diet from the imported feed. This might be the reason for higher levels of N_{min} between the hut and feeding troughs where pigs could spend most of their time. High NH₄-N concentration at 6.5 and 9.5 which were about 71 and 91 % of total N_{min} respectively, reflect the high urination hotspots as the two distance being close to the feed& water troughs. The pigs' urine that is mainly in the form of urea could rapidly change into NH_4 -N before being oxidized overtime when favourable conditions of nitrifications are available (Salomon et al, 2007). This has hence resulted in higher NH₄-N levels that were about 3 and 4 times more at 6.5 and 9.5 m respectively as compared to NH₄-N close to the willow. Eriksen, (2001) in the outdoor pig farming found the highest N_{min} in the feeding area particularly at the 0 - 40cm soil depth which was similar to our experiment.

5.2 Nitrate leaching from the paddocks

Nitrate leaching from the outdoor paddocks is associated with excess NO $_3$ N that could result in eutrophication. While most NH $_4$ -N would be attached to soil particles, the NO $_3$ -N is highly soluble to water and the high concentration in the soil with an increased percolation especially from autumn and winter could increase its loss into groundwater. NH $_4$ -N with time is mineralized into NO $_3$ -N which could either be denitrified, taken by crops or being added to the NO $_3$ -N pool which is prone to leaching.

When the soil sampling was done back in the autumn of 2014, the nitrate-N in the soil was on average the highest at 4.5 m for all the soil depths compared to other distance points (Figure 5). This has prevailed in the first four soil water samplings between 7th November and 18th with 4.5 m having the highest levels than other distances that range between 40 to 57 mg NO₃-N/liter. Also, the high leaching rates from late December to late January for all distance points (with exception of 4.5 m) were a result of precipitation and melting snow since the evapotranspiration during this period was insignificant. The higher early percolation in autumn and early winter resulted in lower NO₃-N from mid-January. The existed nitrate-N during the soil sampling, however, could have been accumulated from previous production season through the mineralized organic-N and ammonium-N. This means the distribution might possible not accounted only for NO₃-N from the sows kept in the paddock during spring and summer of 2014.

The 2.5 m distance from willow which was somehow close to the sow's huts also had secondly highest NO₃-N from soil samples apart from 4.5 m. Unlike for 4.5 m, the 2.5 m didn't reveal higher leaching as recorded from soil water sampling. This could due to NO₃-N uptake by extended roots of these perennial crops close to 2.5 m. The 0.5 m distance which had the second-lowest NO₃-N from the soil samples prevailed the lowest leaching. The lowest nitrate leaching at 0.5 m and unexpectedly highly reduced nitrate-N in soil water at 2.5 m favor our hypothesis which expected low leaching near willow zones. This could, however, be the result of both low excretion activities and the high N uptake by willow roots even though the clear-cut influence of two could be difficult to be established as an analysis for sow's excretion behavior wasn't conducted. On the distances which were close to the feeders (i.e. 6.5 and 9.5 m), most of N_{min} was dominated by NH₄-N particularly at 9.5 m which made us anticipate low leaching rates. With mineralization rate being insignificant during periods of low soil temperatures, most of the NH₄- N was assumed to be adsorbed by soil particles and this has resulted in low leaching rates at 9.5 m. However, contrasting a 9.5 m distance which had only a NO₃-N total of 9.6 Kg N/ha through a 1 m soil column, the 6.5 m had 30.7 kg NO₃-N/ha with the latter having about two-thirds of the amount (20.4 Kg N/ha) in the 50 - 100 cm soil layer. The high NO₃-N in the lower soil at 6.5 could be the reason for higher leaching more than at 9.5 m. This has made the 6.5 m to have the

second-highest leaching next to 4.5 m with an average of 28 mg/liter throughout the soil water sampling where the highest leaching at this distance was at 44 mg NO $_3$ -N/liter in mid-January and lowest levels came a month after. The mineralization rate of nitrification depends mainly on soil temperature and moisture.

The rate of nitrifier activities (of genera *Nitrosomonas* and *Nitrobacter*) responsible for mineralization decreases with low temperatures. From our experiment's soil temperature which has progressively been decreasing from an average of 13° C in October to the lowest levels of 1° C in February suggests some nitrification might have still been taking place up to autumn. The activity rate of nitrifiers is insignificant at temperatures below 5° C while the rate increases with temperature and the significant N mineralization can be achieved from 15° C. The soil moisture content may however influence the nitrifiers' activity rate and so is the N mineralization. Therefore, these high NH₄-N levels remained in the grassland especially near feeders will be mineralized and being available from spring and summer.

6. CONCLUSION

The spatial allocation of features such as feeding and water troughs, huts and trees along boundaries within sows' paddocks has been reported in earlier studies to influence on the activities and defecation behavior of pigs. In the semi-natural environment, pigs tend to spend most of their time close to the bushy and woody zone especially for regulating body temperature and wind velocity which in turn can create an uneven distribution of N_{min} . In the current study, however, N_{min} levels were instead lower at closer distances to the trees with the high levels found near the huts and feeders. Apart from the known high water and N_{min} uptake due to the fast biomass growth, long growing season and deep root system by willow, the other possible explanation for this finding was the influence spatial allocation of feeders, willow and huts. Pigs were assumed to spend most of their activities between the huts and the feeders since the grass cover was the insignificant feed source during autumn and they almost entirely depended their feed intake from the feeders. In addition to improved paddock design, other management options that could help to reduce nutrient loss and ensure even distribution of N_{min} include frequent reallocation of feeders and huts as this reduces the enormous loss from hotspot areas as well as improving grassland cover.

CONFLICT OF INTEREST

There is no conflict of interest from the authors

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