

Influence of pre-crop and root architecture on the mobilization of non-exchangeable NH_4^+

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ABSTRACT

It is well established that non-exchangeable NH_4^+ is an important N source for plants. However, release from clay minerals only occurs when the NH_4^+ concentration of the mineral environment declines below a certain level. This may be conditioned by the growing plant. Although root development differs depending on plant species, the influence of root architecture on the mobilization of non-exchangeable NH_4^+ is still open to question. We found that mobilization of non-exchangeable NH_4^+ was higher under oilseed rape as compared to barley; oilseed rape even has allorhize and fescue homorhize roots. This observation could be proved by labeling soil samples with $^{15}\text{NH}_4^+$, which were incubated in soil holders under oilseed rape and barley. The higher mobilization of non-exchangeable NH_4^+ by oilseed rape is therefore assumed to be caused by higher release of H^+ , displacing interlayer NH_4^+ . Although allorhize roots from pre-crops create more stable biopores with an increasing diameter and living roots of the subsequent crop enter these biopores, we could not find an influence of the pre-crop on the mobilization of non-exchangeable NH_4^+ .

Keywords: ammonium; fixation; availability; roots; biopores

Contents of non-exchangeable $\text{NH}_4\text{-N}$ of arable soils cover a wide range. For example in Germany they vary between 70 and 270 mg/kg (Scherer and Mengel 1979), in Italy between 175 and 184 mg/kg (Nannipieri et al. 1999) and in Canada between 57 and 367 mg/kg soil (Doram and Evans 1983). The main factor affecting the content of non-exchangeable NH_4^+ is the clay fraction and among the clay minerals the NH_4^+ fixing capacity of vermiculite is greatest, while the ability of montmorillonites to fix NH_4^+ under wet conditions is negligible. 1:1 clay minerals are not able to bind NH_4^+ ions in the interlayers (Sowden et al. 1978).

Although no constant differences were found between topsoils and subsoils with respect to the content of non-exchangeable NH_4^+ , as a percentage of total N this N fraction increases with depth in the profile (Nette and Resch 1992) and reaches values of 31.5% (as mean) in 20–40 cm soil depth as compared to 20.8% in 0–20 cm soil depth (Elmaci

et al. 2002). Moyano and Gallardo (1988) found that even up to 80% may be reached in subsoils. Enrichment of non-exchangeable NH_4^+ in subsoils is assumed to be the result of downward movement of soluble organic matter in the soil profile, especially in biopores created by earthworms (Beuters 2014), its subsequent mineralization to NH_4^+ and fixation in interlayer sites of clay minerals (Soon 1998).

It is well established that ammonium fixation and release from clay minerals can play a crucial role for the N nutrition of plants (Scherer and Mengel 1986, Steffens and Sparks 1999, Nieder et al. 2011). However, the availability of this N fraction can vary considerably. Extremely low availability was reported by Mohammed (1979). However, in pot experiments with perennial ryegrass, grown under greenhouse conditions, Dou and Steffens (1995) found that between 90% and 95% of recently fixed NH_4^+ were mobilized during a growing period of 14 weeks, while under field conditions 66%

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of recently fixed NH_4^+ was released in 86 days (Kowalenko 1978). Mengel and Scherer (1981) also observed a depletion of this N fraction in deeper soil layers, which occurred later in the growing season as compared to the topsoils. Also Soon (1998) reported that the content of non-exchangeable NH_4^+ changed throughout the growing season in all depths but tended to be higher in deeper soil layers. However, as compared to topsoils the information of the dynamics of non-exchangeable NH_4^+ in subsoils is still scarce and more attention should be paid to the subsoils.

As the release of non-exchangeable NH_4^+ depends on the concentration gradient of NH_4^+ between the interlayers of clay minerals and the external solution, it may be concluded that the release of non-exchangeable NH_4^+ only occurs when the NH_4^+ concentration of the mineral environment declines below a certain level (Scherer 1993). This may be conditioned by the ability of the roots favoring the diffusion of NH_4^+ ions out of the clay mineral interlayers. According to Mengel and Scherer (1981), investigating the dynamics of non-exchangeable NH_4^+ throughout the growing season in a Fluvisol, the content of this N fraction declined in the upper 60 cm from February until May, while in the soil layer 60–90 cm depletion occurred from May to July. This was in accordance with the root growth of spring oats. The influence of growing plants on the mobilization of this N fraction was confirmed by Scherer (1987). In experiments under field conditions with ^{15}N -labeled soil most of the non-exchangeable $^{15}\text{NH}_4^+$ was mobilized in the cropped plots while only 15% was released in the fallow plots. Using special pots, where a part of the root system was in direct contact with the soil also Mengel et al. (1990) demonstrated the influence of the roots on the mobilization of non-exchangeable NH_4^+ .

It could be even demonstrated that growing plants are a prerequisite for the mobilization of non-exchangeable NH_4^+ , investigations about the impact of the root architecture on the release of this N fraction are not available. For this reason the objective of the present research is to provide evidence whether the release of non-exchangeable NH_4^+ is influenced by the type of the plant roots, i.e. homogeneous distributed root systems (homorhize) as compared to allorhize distributed root systems. Furthermore the influence of the pre-crop on the availability of non-exchangeable NH_4^+ was investigated.

MATERIAL AND METHODS

Experiment 1. The experiment was conducted on a haplic luvisol of the Research Station Klein-Altendorf, University of Bonn (Germany) (50°37'8.5"N, 6°59'24.5"E). Soil characteristics are shown in Table 1. In 2010 the influence of the pre-crop alfalfa (*Medicago sativa* L.; allorhize roots) and fescue (*Festuca arundinacea* SCHREB.; homorhize roots), respectively, followed by mallow (*Malva sylvestris* L.; allorhize roots) and in 2011 the influence of the root system (homorhize roots: oilseed rape (*Brassica napus* L.); allorhize roots: barley (*Hordeum vulgare* L.)) after the pre-crop spring wheat (*Triticum aestivum* L., homorhize roots) on the dynamics of non-exchangeable NH_4^+ were investigated. Soil samples were taken from the soil layers 0–45, 45–75, 75–105 cm in monthly intervals from March until August 2010 and March until July 2011, respectively, with an auger.

Experiment 2. The soil (soil characteristics see Table 1) was removed from the same site as experiment 1 in 2010 in a depth of 45–75 cm after the harvest of wheat, because at this time the interlayers of the clay minerals were exhausted. This was necessary, because in the next step the interlayers were refilled by ^{15}N -labeled NH_4^+ . Ten soil holders were placed in the field in a depth of 60 cm horizontally underneath barley (*Hordeum vulgare* L.) and oilseed rape (*Brassica napus* L.), respectively, on April 6th 2011 and removed in autumn 2011 after the harvest of barley and oilseed rape. After removing soil holders were stored at -18°C . Before analysis the soil monoliths were cut with a knife into slices. The four outer slices – from the surface of both sides to the center – were 2 mm thick, the following slice of each side was 4 mm and the center slice was 6 mm thick. Afterwards soil samples were dried at 40°C in a cabinet dryer and passed through a sieve (< 0.1 mm).

Preparation of the soil samples. About 5 kg soil (≤ 2 mm) were mixed with $^{15}\text{NH}_4\text{Cl}$ solution

Table 1. Soil characteristics

Soil depth (cm)	$\text{pH}_{\text{CaCl}_2}$	C_{org}	N_t	Sand (%)	Silt (%)	Clay (%)
0–45	6.7	0.73	0.079	6.5	75.5	17.5
45–75	6.9	0.42	0.051	4.0	69.0	27.0
75–105	7.0	0.25	0.034	4.5	67.5	27.5

C_{org} – organic carbon; N_t – total N

(96.5 at. % ^{15}N , soil:solution ratio = 1:1) that contained 120 mg $^{15}\text{N/L}$. The soil suspension was incubated in a dryer at 45°C for 36 h. Afterwards the exchangeable $^{15}\text{NH}_4^+$ was eliminated by 0.5 mol/L CaCl_2 solution. CaCl_2 was used instead of KCl for the removal of the exchangeable NH_4^+ to avoid a strong contraction of the clay mineral interlayers by K^+ . After thorough washing with deionized water the soil was dried at 30°C, crushed and passed through a sieve (2 mm).

Soil holder. The soil holders consisted of a hard-PVC-ring with 81 mm interior diameter and 30 mm high (Figure 1). Each side of the ring was covered by a nylon net (mesh 1 μm) that allowed solute fluxes in and out of the soil holder while roots and mycorrhiza could not penetrate into the soil. The labeled soil was placed in soil holders (200 g per soil holder) before incubation in the field under growing plants.

Determination of total and ^{15}N -labeled non-exchangeable NH_4^+ (according to Beuters and Scherer 2012). Transfer 0.5 g of the soil sample into a 100 mL beaker, add 10 mL of freshly prepared KOBBr solution, shake it well and incubate for about 2 h to remove organic compounds, in between shake the mixture once again. Add 60 mL of distilled H_2O , heat on a heating plate and when boiling continue for exactly 5 min. After cooling down over night suck of the clear supernatant and refuse it. Dry the soil sample in the beaker in a cabinet dryer at 60°C for 20 h. Homogenize the dried soil sample well with a glass stirrer and transfer 30 mg into a tin cup. Determine the content of non-exchangeable NH_4^+ with an elemental analyser with a thermal conductivity detector (TCD). The decomposition of the clay minerals containing the non-exchangeable NH_4^+ is reached by the dynamic spontaneous combustion by 1100°C.

After pretreating the soil samples as mentioned above non-exchangeable $^{15}\text{NH}_4^+$ was determined using a mass spectrometer (GC-MS 'ANCA SL-2020', PDZ Europa Ltd., Crewe, UK).

RESULTS AND DISCUSSION

In general the spatial accessibility of plant nutrients is higher in the tilled layer of the topsoil. In the subsoil preferential root growth takes mainly place in biopores because roots follow pathways of the least resistance, such as channels previously created by roots of the pre-crop (Jassogne

et al. 2009). Therefore nutrient acquisition from the subsoil is a function of the crop sequence because extended biopore systems are related to crop specific root systems (Stewart et al. 1999). For this reason we investigated the influence of the pre-crop alfalfa with an allorhizous root system and fescue with a homorhizous root system on the mobilization of non-exchangeable NH_4^+ by mallow. In all soil layers the content of non-exchangeable NH_4^+ stayed on the same level from March until June, followed by a statistically not significant decrease until July and a refixation of NH_4^+ ions until August (Figure 2). Generally, dicotyledons like alfalfa are assumed to create more stable biopores than monocotyledons like fescue (Materchera et al. 1993) and living roots growing in biopores contact the pore wall and potentially deplete nutrients (Jones et al. 2004). However, we could not detect a significant influence of the pre-crop on the mobilization of non-exchangeable NH_4^+ . We assume that in the deeper soil layers the effect of the pre-crop was too short to generate differences in the number and size of biopores between alfalfa and fescue. However, it should be emphasized that the content of non-exchangeable NH_4^+ was higher by tendency under the N_2 -fixing alfalfa in March. Therefore it may be speculated that a part of the biologically fixed nitrogen was bound in the interlayers of clay mineral.

In a further experiment we compared the influence of the root system of plants (barley with a homorhizous root system; oilseed rape with an allorhizous root system (tap roots)) on the mobilization of non-exchangeable NH_4^+ on plots with the pre-crop spring wheat. The release of non-exchangeable NH_4^+ is an exchange and diffusion process and diffusion of NH_4^+ out of the clay mineral interlayers will occur when the NH_4^+ concentration

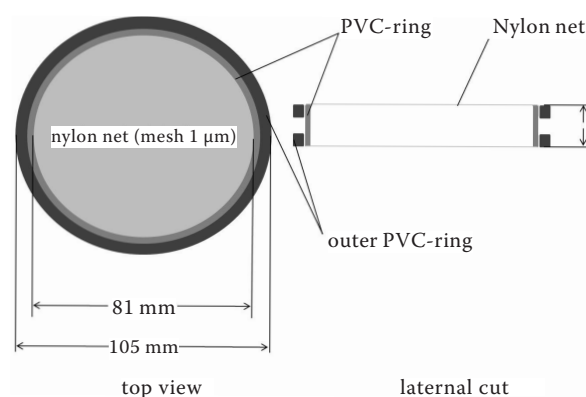


Figure 1. Design of a soil holder

of the soil solution is low. This may be conditioned by growing plants. Therefore root growth, leading to increased root contact, is an important factor determining the mobilization and uptake of less mobile nutrients like non-exchangeable NH_4^+ (Gahoonia et al. 1997). Independent of the crop we found a decrease in the content of non-exchangeable NH_4^+ during the growing season 2011 until May and June, respectively (Figure 3). However, it should be pointed out that the depletion of this N fraction was higher under oilseed rape as compared to barley. Confirming the results of Mengel and Scherer (1981) the depleted clay minerals were partially refilled with NH_4^+ later in the growing season. At the end of the growing

period N uptake by plants ceases while the process of ammonification continues providing NH_4^+ ions for refixation (Scherer 1993). In total, the pattern of the non-exchangeable NH_4^+ content during the growth period provides evidence that the formerly so-called fixed NH_4^+ is not a static fraction but seems to be much involved in the N dynamic of soils.

Caused by the refixation of NH_4^+ ions even under growing plants the net release of non-exchangeable NH_4^+ may be underestimated. For this reason soil samples were taken in autumn from the subsoil and treated with $^{15}\text{NH}_4^+$ in the laboratory as mentioned above. After the preparation of the soil with $^{15}\text{NH}_4^+$ the total content of non-exchangeable NH_4^+ soil increased to 217.2 mg N/kg soil containing

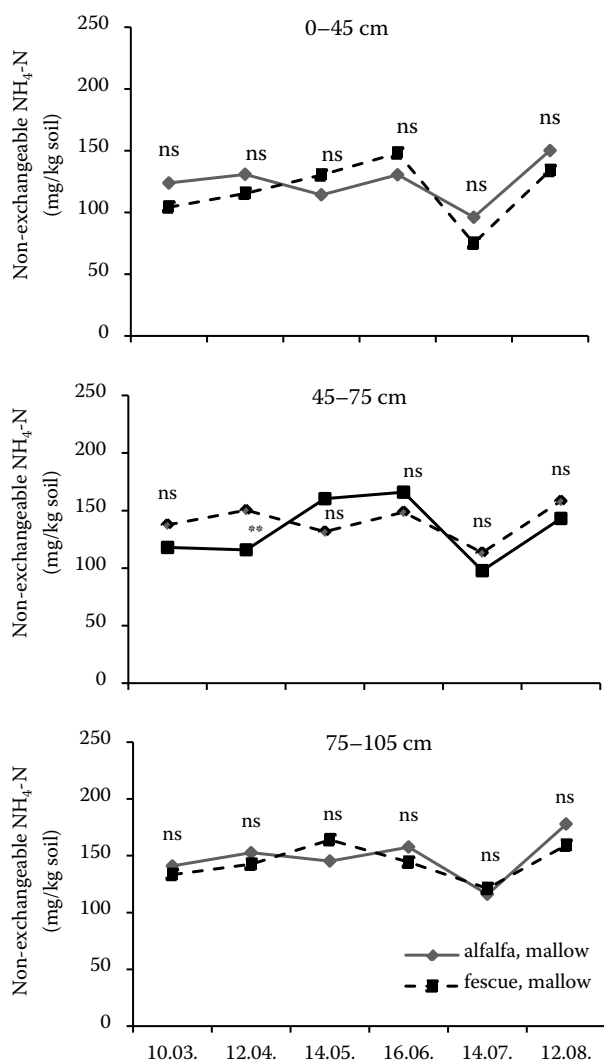


Figure 2. Content of non-exchangeable $\text{NH}_4\text{-N}$ throughout the growing season 2010; current crop: mallow (*Malva sylvestris* L.); pre-crops: alfalfa (*Medicago sativa* L.) and fescue (*Festuca arundinacea* SCHREB.). ANOVA, Tukey's-test ($\alpha = 0.05$): * $P \leq 0.05$; ** $P \leq 0.01$

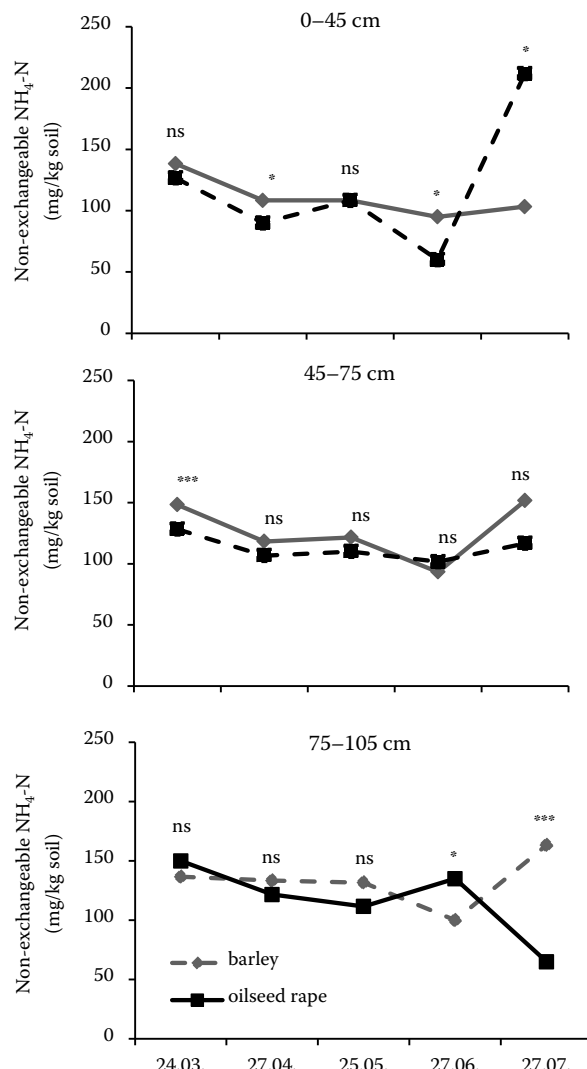


Figure 3. Content of non-exchangeable $\text{NH}_4\text{-N}$ throughout the growing season 2011; current crops: oilseed rape (*Brassica napus* L.) and barley (*Hordeum vulgare* L.); pre-crop spring wheat (*Triticum aestivum* L.). ANOVA, Tukey's-test ($\alpha = 0.05$): * $P \leq 0.05$; *** $P \leq 0.001$



Figure 4. Root accumulation around the soil holders

35.2 mg ¹⁵N-labeled non-exchangeable NH₄-N. Independent of the plant species at harvest we found an accumulation of roots in the soil, where the soil holders were placed (Figure 4) and that both sides of the soil holders were covered with roots. During an experimental period of time of 4 month significant amounts of ¹⁵N-labeled non-exchangeable NH₄⁺ were mobilized (Figure 5a).

Because cereals have a homorhizous root system with a frequently higher rooting density, favoring the use of less mobile nutrients as compared to allorhizous root systems of dicots like oilseed rape, as in the previously described experiment we expected a higher mobilization under barley. However, surprisingly the mobilization rate was again significantly higher under oilseed rape and

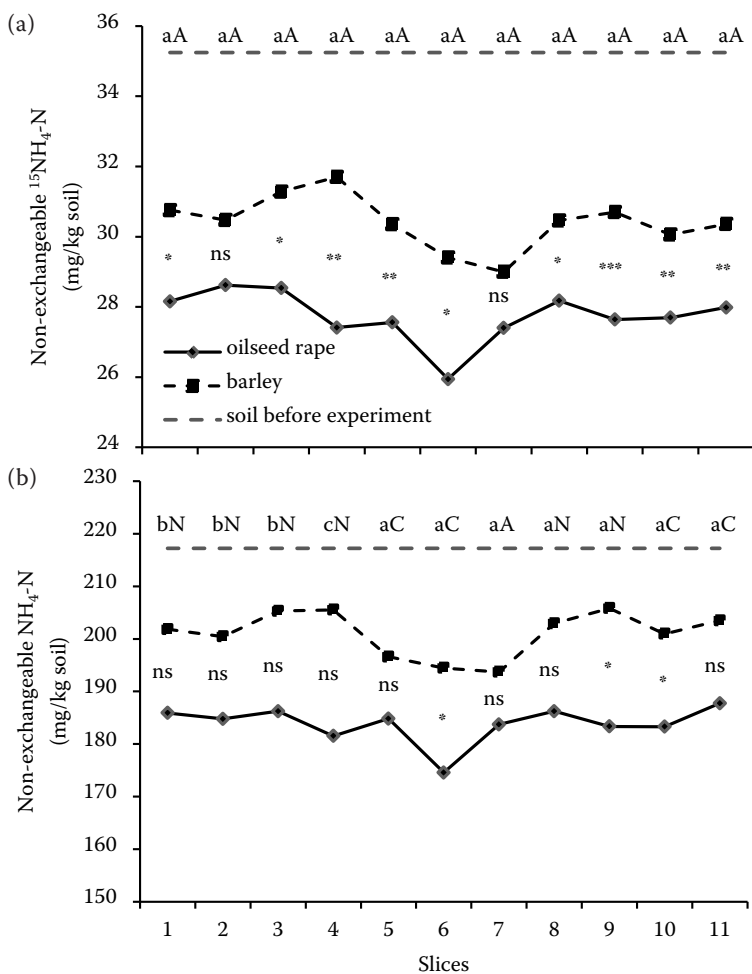


Figure 5. Content of non-exchangeable ¹⁵NH₄-N (a) and total non-exchangeable NH₄-N (b) in the soil of the soil holders in different distances to the gauze. ANOVA, Tukey's-test ($\alpha = 0.05$), a or A – significant difference between soil at the start of the experiment and soil under oilseed rape or barley. Difference between oilseed rape and barley: ns – not significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

amounted to 19% while under barley 11% of the ^{15}N -labeled non-exchangeable NH_4^+ was released. Under both crops also significant amounts of N were released from the pool of total non-exchangeable NH_4^+ (Figure 5b). On average, they amounted to 16.2 mg under barley and 33.9 mg non-exchangeable $\text{NH}_4\text{-N/kg}$ under oilseed rape. Even the mean difference between both plant species amounted to 17.2 mg non-exchangeable $\text{NH}_4\text{-N/kg}$, the difference was not always significant.

Taking the higher N demand of oilseed rape as compared to barley into consideration it may be speculated that the higher N uptake of oilseed rape favored the dilution of the NH_4^+ concentration of the soil solution, thus promoting the diffusion of NH_4^+ ions out of the interlayers. Furthermore plant roots can also affect the release of non-exchangeable NH_4^+ indirectly by releasing protons. Because it is common to observe that rhizosphere pH decreases when ammonium fertilizers are used (Ortas and Rowell 2005), it may also be assumed that the higher ammonium uptake of oilseed rape from the pool of non-exchangeable NH_4^+ resulted in a higher net H^+ release per unit root weight. The relatively high replacing power of H^+ is an important factor in the release of non-exchangeable NH_4^+ , because H^+ leaves the crystal lattice in an expanded state and thus renders the non-exchangeable NH_4^+ more accessible for replacing cations (Scherer and Ahrens 1996). For this reason it is assumed that the pH decrease may be the reason of the higher mobilization capacity of this N fraction by oilseed rape. According to Newman (1969) H^+ ions may enter the wedge zone of the clay minerals, displace specifically bound cations and thus favor the release of non-exchangeable NH_4^+ .

Furthermore the different amounts of K^+ taken up by oilseed rape and barley must be taken into consideration, being higher with oilseed rape. A higher uptake K^+ ions by roots may reduce the K^+ concentration in the soil solution and therefore diminish the blocking effect of K^+ on the release of NH_4^+ (Welch and Scott 1960), resulting in a higher mobilization of non-exchangeable NH_4^+ .

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