

# Modification of the standard method for determination of non-exchangeable $\text{NH}_4\text{-N}$ in soil

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## ABSTRACT

It is well accepted that non-exchangeable  $\text{NH}_4\text{-N}$  plays an important role in the N dynamics of arable soils. However the widely-used Silva/Bremner method for determining this N fraction is very time consuming and the use of the hazardous hydrofluoric acid (HF) is indispensable. In the modification the use of HF is avoided and the quantity of analysed samples per unit of time could be increased by a factor of 2.5. After pretreating soil samples with KOBBr to destroy soil organic matter soil samples are dried and the content of non-exchangeable  $\text{NH}_4\text{-N}$  is measured using a CNS analyzer. The results of the analysis of 3 out of 4 different soils show no significant differences between both methods.

**Keywords:** fixed ammonium; nitrogen; clay minerals; hydrofluoric acid; KOBBr

Depending on the type and amount of clay minerals soils are able to bind ammonium ions ( $\text{NH}_4^+$ ) in such a manner that they cannot be easily replaced by other cations. This process was already observed by McBeth (1917), who defined the unrecovered part of the added ammonium as 'fixed ammonium'. Osborne (1976) defined the fixation of ammonium as 'the adsorption or absorption of ammonium ions by the mineral or organic fraction of the soil in a manner that they are relatively unexchangeable by the usual methods of cation exchange'. To distinguish between  $\text{NH}_4^+$  which is bound organically or as  $\text{NH}_4\text{-phosphate}$  (Frye and Hutcheson 1981). Osborne (1976) suggested to use 'intercalary  $\text{NH}_4^+$ ' and Mengel and Scherer (1981) to use 'non-exchangeable  $\text{NH}_4^+$ ' for the  $\text{NH}_4^+$  ions which are recovered from clay minerals by digestion of the soil with HF and HCl.

The quantities of non-exchangeable  $\text{NH}_4^+$  in soils can be considerable. According to Scherer and Mengel (1979) representative arable soils of the western part of Germany contain up to 3000 kg non-exchangeable  $\text{NH}_4\text{-N/ha}$  in the rooting zone. Assuming that only a part of this  $\text{NH}_4^+$  is available to plants, it may contribute substantially to the N nutrition of plants. Indeed, Mengel and Scherer (1981), who determined the content of non-exchangeable  $\text{NH}_4^+$  five times throughout the growing season in a farmer's field,

found a depletion of this N fraction in the upper soil layers in the early growing stage, while in the deeper layer  $\text{NH}_4^+$  was depleted when the roots penetrated in this layer. That means non-exchangeable  $\text{NH}_4^+$  is not a static fraction, but may be involved in the N dynamics of soils, which was also shown by Lu et al. (2010) as well as by other authors.

Ammonium fixation can play a crucial role for the efficiency of fertilizer N (Scherer and Mengel 1986, Dou and Steffens 1995, Steffens and Sparks 1999). Especially in soils with a high  $\text{NH}_4^+$  fixation capacity a part of the  $\text{NH}_4^+$  supplied with  $\text{NH}_4^+$ -forming or  $\text{NH}_4^+$ -containing fertilizers may be bound in the interlayers of clay minerals. Because after penetration of the  $\text{NH}_4^+$  ions into the clay mineral interlayers they are excluded from nitrification (Guo et al. 1983) and thus are protected against leaching. The pool of non-exchangeable  $\text{NH}_4^+$  can thus function as a kind of buffer which contributes to reduction of N emissions from soils on the one hand and provides mineral N to crops on the other hand (Nieder et al. 2011).

In the past, several methods to analyze the content of non-exchangeable  $\text{NH}_4\text{-N}$  in soils were developed. They differ in the pretreatment of the soil samples to destroy soil organic matter as well as in the disintegration of the clay minerals before determining non-exchangeable  $\text{NH}_4\text{-N}$ . Barshad

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(1951) described the duplicate method for the determination this N fraction in soils. The soil sample is distilled with NaOH and a duplicate sample is distilled with KOH. The difference between both distillations is defined as non-exchangeable  $\text{NH}_4\text{-N}$ . According to Mogilevkina (1964) non-exchangeable  $\text{NH}_4\text{-N}$  is determined after heating the soil sample at  $400^\circ\text{C}$  for 24 to 72 h, depending on the organic matter content followed by a Kjeldahl procedure. Other methods use hydrofluoric acid (HF) to destroy the lattice structure of the clay minerals after pretreating the soil sample to eliminate soil organic matter (Rodrigues 1954, Bremner 1959, Silva and Bremner 1966). The method developed by Silva and Bremner (1966) is the most accepted method, because of the lack of apparent defects (Scherer 1986).

However the method to analyze this N fraction includes the use of the harmful HF, which is a highly corrosive acid and therefore dissolving glass. Furthermore aqueous HF is a contact poison which will be resorbed by the skin immediately. So the fluoride ions can enter the blood circuit and are able to disturb the Ca and Mg metabolism hence it could cause multiple organ failure. Burns are initially painless, but if they are as large as a palm of hand they are generally lethal. Therefore the aim of the present investigation was to improve the method to determine non-exchangeable  $\text{NH}_4^+$  in such a way that the accurateness of the results of the method of Silva and Bremner (1966) is guaranteed by avoiding the use of HF.

## MATERIAL AND METHODS

**Soil samples.** Four soils from Germany with different clay contents were chosen to compare the modified method with the method of Silva and Bremner: Luvisol (1) derived from Loess from Meckenheim (subsoil 30–75 cm); Cambisol (2) from Vinxel (subsoil 65–75 cm); Cambisol (3) from Uedorf (subsoil 30–75 cm) and Alfisol (4) from Gießen (topsoil 0–30 cm). Physicochemical characteristics of these soils are presented in Table 1. Soil samples were air dried and ground to pass a 1 mm sieve.

Table 1. Physicochemical characteristics of the soils

Soil No.	Clay (%)	Silt (%)	Sand (%)	$C_{\text{org}}$ (%)	$N_{\text{tot}}$ (%)
1	23.9	71.4	4.8	0.42	0.04
2	49.9	41.2	9.3	0.20	0.04
3	3.4	11.0	85.7	0.23	0.04
4	30.4	59.0	6.5	4.10	2.78

**Reagents.** (1) Potassium hypobromite solution (KOB<sub>r</sub>): add 15 mL bromine (Br) slowly with constant stirring to 500 mL 2 mol/L potassium-hydroxide-solution (KOH). Cool the KOH in an ice bath during the addition of Br. Solution must be prepared immediately before use.

(2) 2 mol/L potassium hydroxide (KOH) solution: dissolve 120 g of KOH in about 800 mL of water in a 1000 mL volumetric flask and bring the cooled solution up to volume.

(3) 0.5 mol/L potassium chloride (KCl) solution: dissolve 186 g of KCl in 5000 mL of water.

(4) Hydrofluoric acid – hydrochloric acid solution (5 mol/L HF – 1 mol/L HCl): Place about 200 mL of water in a polypropylene volumetric flask, add 250 mL of HF (40%) and 84 mL of HCl (37%) and dilute the solution with water to the 1000 mL mark.

**Standard method (Silva and Bremner 1966):** Treat 1 g of the soil sample with 20 mL fresh prepared alkaline KOB<sub>r</sub> solution in a 250 mL beaker. Shake it well and incubate it for about 2 h to remove organic compounds, in between shake the mixture once again. After that add 60 mL of high-purity water, heat it on a heating plate until it starts boiling and boil for 5 min exactly. Cool it down over night. Suck of the clear supernatant liquid and refuse it.

Transfer the complete soil residues with 0.5 mol/L KCl into 250 mL polyethylene centrifuge tubes, fill it up to 40 mL with 0.5 mol/L KCl and shake it for about 5 min. Then centrifuge it ( $1.100 \times g$ ) for 10 min. Subsequently suck of the clear supernatant liquid, add 40 mL KCl again and repeat the washing three times overall. Add 20 mL HF-HCl-solution after the third suction and shake it for 24 h on an overhead shaker to destroy the clay minerals. The dissolved  $\text{NH}_4^+$  is determined by steam distillation (5 min) after adding 50 mL of sodium hydroxide solution (30%). Catch the distillate in boric acid (4%). Finally titrate it with 0.005 mol/L HCl. For the distillation and titration part the automatic apparatus Vapodest 5 from Gerhardt (Königswinter, Germany) was used.

**Modified method.** Transfer 0.5 g of the soil sample into a 100 mL beaker, add 10 mL of freshly

prepared KOBr solution and proceed like described above. After cooling down over night suck of the clear supernatant and refuse it. Dry the soil sample in the beaker after the KOBr treatment in a cabinet dryer at 60°C for 20 h. Homogenise the soil sample well with a glass stirrer and transfer 30 mg into a tin cup. Determine the content of non-exchangeable  $\text{NH}_4\text{-N}$  with an elemental analyser with a thermal conductivity detector (TCD) to analyse the residual N content. The decomposition of the clay minerals containing the non-exchangeable  $\text{NH}_4\text{-N}$  is reached by the dynamic spontaneous combustion by 1100°C.

**Statistics.** Statistical analyses were performed with IBM SPSS Statistics (version 20, Chicago, USA). All measured results were first characterized by classical descriptive statistics (means and standard deviation). The data of the comparison of the original (Silva and Bremner 1966) and the modified method for the analysis of non-exchangeable  $\text{NH}_4\text{-N}$  in soils and the result of the influence of the graininess of the soil were first proved by Kolmogorov-Smirnov-test (K-S test) to test the distribution for normality, followed by a *t*-test ( $\alpha = 5\%$ ), which includes a Levene's test to assess the equality of variances.

## RESULTS AND DISCUSSION

Before we introduced the modified method in our laboratory to analyze soil samples from field experiments, we compared the results of the modified method with the results of the Silva-Bremner method. Therefore we used 4 soils with different clay contents ranging from 3.4% to 49.9% (Table 1). Using the Silva-Bremner method we found between 81.7 mg and 242.3 mg non-exchangeable  $\text{NH}_4\text{-N/kg}$  soil, while using the modified method the content ranged between 91.0 mg and 240.0 mg non-exchangeable  $\text{NH}_4\text{-N/kg}$  soil (Figure 1). It should be pointed out that each value of both method is the mean of 21 replications (soil 1 to soil 3) and 38 replications (soil 4), respectively. According to Figure 1 the content of non-exchangeable  $\text{NH}_4\text{-N}$ , analyzed according to both methods, is not statistically significant in the soils with the higher clay content (soils 1, 2 and 4), while with soil 3 with the low clay content the modified method resulted in a significantly higher content of this N fraction. This difference could derive from the limit of determination of our CNS-analyzer (EuroEA 3000, HEKAtech, Wegberg, Germany) which is able to detect a minimum of 0.01% N (100 mg N/kg soil).

But soil 3 contains medial only 0.009% N (90 mg non-exchangeable  $\text{NH}_4\text{-N/kg}$  soil). Accordingly the modified method should not be used for soils with an expected low value of non-exchangeable  $\text{NH}_4\text{-N}$ , assessable from low clay content. However, it should be pointed out, that in the case of a CNS-analyzer with a more sensitive determination limit ( $< 0.01\%$  N) the modified method will provide reliable results. Another possibility could be to enrich the soil sample with a defined quantity of N to reach the determination limit of the CNS-analyzer.

Apart from avoiding the use of HF, a further advantage of the modified method is time saving. Per time unit the number of soil samples analyzed is increased by a factor of 2.5. The reason is that the time consuming washing part is omitted, also the distillation and titration is lapsed (6–8 min per sample). Additionally, time could be saved when samples labeled with  $^{15}\text{N}$  are analyzed. In special investigations the interlayers of clay minerals may be labeled with  $^{15}\text{NH}_4\text{-N}$  to distinguish between native non-exchangeable  $\text{NH}_4\text{-N}$  and  $\text{NH}_4\text{-N}$  fixed after the application of ammonium containing or ammonium forming fertilizers or plant residues and organic matter (Dou and Steffens 1995). To determine the share of  $^{15}\text{NH}_4\text{-N}$  from total non-exchangeable  $\text{NH}_4\text{-N}$  a time consuming process succeeds after the Silva/Bremner method. The titrated distillates of soil digestions must be acidified with HCl and evaporated until dryness. In these samples  $^{15}\text{N}$  is determined by emission spectrometry (Dou and Steffens 1995). Using our modified method, dried

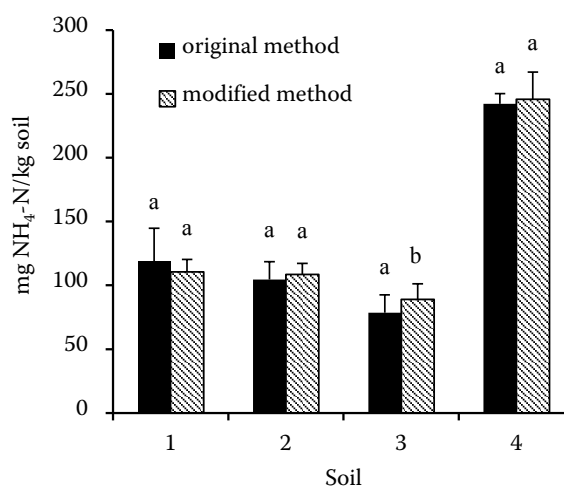


Figure 1. Comparison of the original (Silva and Bremner 1966) and the modified method for the determination of non-exchangeable  $\text{NH}_4\text{-N}$ . Error bars: SD. Soil 1:  $P = 0.205$ ,  $n = 21$ ; soil 2:  $P = 0.264$ ,  $n = 21$ ; soil 3:  $P = 0.013$ ,  $n = 21$ ; soil 4:  $P = 0.347$ ,  $n = 38$ . Values with the same letter are not significantly different

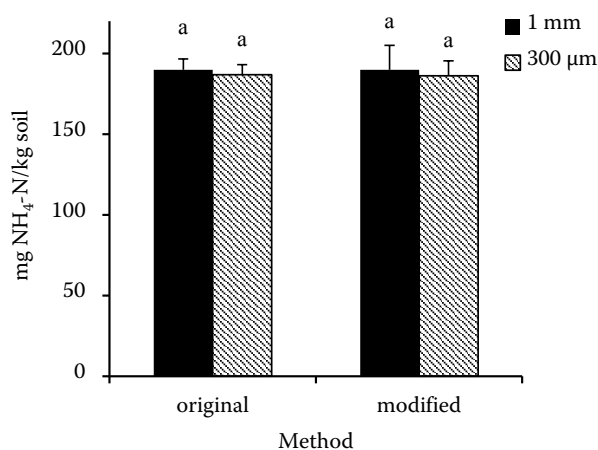


Figure 2. Influence of particle size of soil No. 1 on the concentration of non-exchangeable  $\text{NH}_4\text{-N}$ . Error bars: SD. Original method  $P = 0.520$ ,  $n = 4$  replications; modified method  $P = 0.645$ . Values with the same letter are not significant different

soil samples can immediately be weighed into tin cups, followed by the use of a mass spectrometer.

A prerequisite for the use of a CNS-analyzer or a mass spectrometer is that soil samples are very homogenous, because the initial weight is only 30 mg. However, according to the Silva/Bremner method soil samples must pass only a 1 mm sieve. For this reason we ground aliquots of soil No. 1 to pass a 1 mm sieve and a 300 µm sieve, respectively. It should be pointed out that in this experiment the natural content of non-exchangeable  $\text{NH}_4\text{-N}$  of soil No. 1 was increased by treating the soil with  $\text{NH}_4\text{Cl}$  solution, containing 120 mg N per 1000 mL. As shown in Figure 2 we could not find any significant differences between both particle sizes. Therefore to save time we recommend using of the same particle size as recommended in the original method.

In total, the modified method is time saving, avoids the use of hazardous HF and the results of soils with a content of non-exchangeable  $\text{NH}_4\text{-N}$  higher than 100 mg/kg soil do not differ from the Silva/Bremner method.

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