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Is KCl a reliable extractant of $^{15}NH_4^+$ added to coastal marine sediments?

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Abstract. The release of NH_4^+ and ^{15}N-labelled NH_4^+ by one-step KCl extraction was assessed in different types of coastal marine sediments. KCl was efficient to extract NH_4^+ from sandy sediments and less efficient in silt sediments, where an extended extraction period was required for obtaining a maximum NH_4^+ yield. Extraction at 0 or 20 °C had only a little effect on the efficiency of KCl. KCl gave always non complete recovery of $^{15}NH_4^+$ in silt sediments. However, the added label could be fully recovered by addition of 80 $\mu mol \cdot cm^{-3}$ exogenous NH_4^+ prior to KCl, or when NaCl or ASW replaced KCl. $^{15}NH_4^+$ was added to non-biological silt sediment, which was incubated at 0 °C up to 16 hours, to see the effect of physical processes on the partition of $^{15}NH_4$ among porewater (29–49%) exchangeable (9–30%) and non-extractable, organic bound pools (24–42%). Total ^{15}N recovery was approximately 100%. KCl failed to remove $^{15}NH_4$ which entered to unknown, bound pools in sediment. Only shortly after addition of ^{15}N (0.1 h), the extraction period resulted in significantly different ^{15}N recoveries ($P < 0.05$) in KCl extractable NH_4^+, 17% versus 9% of label was recovered after 1 min or 60 min extraction of sediment, respectively. Two hours of incubation time were required for complete equilibrium of $^{15}NH_4^+$ among porewater, exchangeable and organic bound pools. Sediments (silt) to which $^{15}NH_4^+$ has been added in order to measure NH_4^+ turn-over and KCl is used as extractant, should be incubated for at least 2 hours, before taking a zero-time sample.

Introduction

The predominant nitrogen species in O_2-limited sediments is NH_4^+. There are possible fates of NH_4^+, such as it remaining in a dissolved form in the interstitial water, joining the exchangeable pool or becoming fixed as non-exchangeable NH_4^+. KCl has been the most used technique to quantify the pool of exchangeable NH_4^+ in marine sediments (Mackin & Aller 1984) and soils (Bremner 1965). There are variations in the use of the technique with regard to the degree of agitation (Haines et al. 1977), the molarity of KCl solution (Bremner 1965), the extraction period (Simon & Kennedy 1987). Recently, some authors have pointed out problems associated with the use of KCl, as it seems unclear whether this reagent is unable to quantitatively extract exchangeable nitrogen species, or if other nitrogen forms are being extracted as well (Brodrick et al. 1987). On the other hand, it was observed that a one-step KCl extraction did not recover all $^{15}NH_4^+$ added to marine sediments, which may underestimate calculated NH_4^+ turn-over rates in these

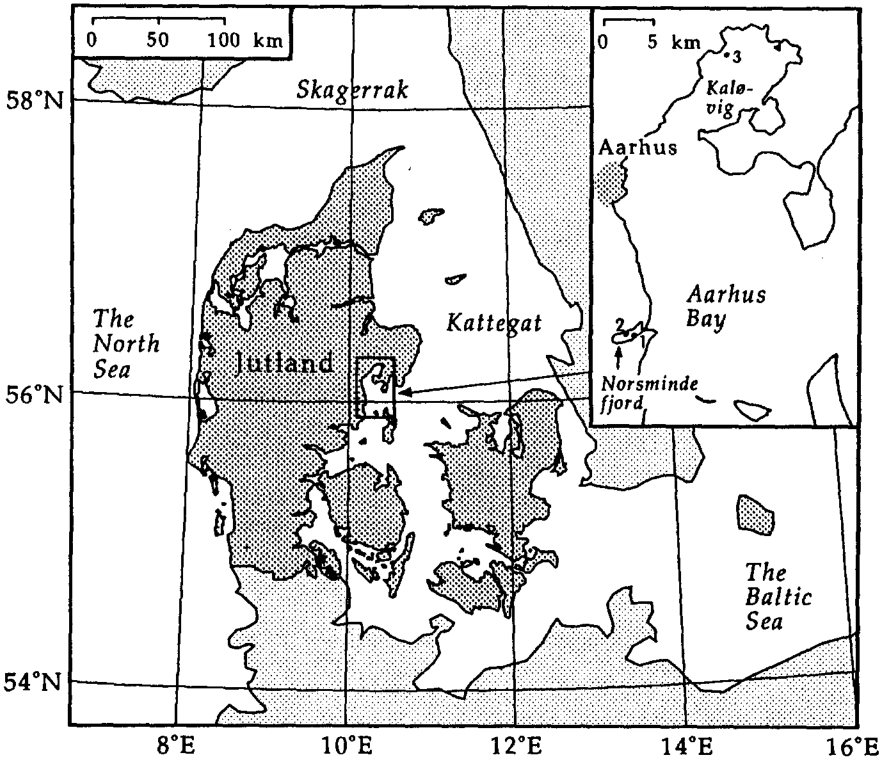

Fig. 1. Map of Jutland (Denmark), showing location of Stations 1 & 2 (Norsminde fjord), and Station 3 (Kaløvig).

environments (Laima, unpublished). A possible fate of some added $^{15}NH_4^+$ could be the entry to sediment pools from which it could not be extracted by KCL (Laima 1993).

This communication reports my attempt to improve the effectiveness of one-step KCl extraction, to quantify the exchangeable NH_4^+ pool and to recover all $^{15}NH_4^+$ added to marine sediments. Assays were carried out to determine: 1 – Effect of sediment type, extraction period and temperature on the KCl extraction of NH_4^+; 2 – Comparison of KCl with NaCl and seawater in the extraction of $^{15}NH_4^+$; 3 – Effect of exogenous NH_4^+ additions on ^{15}N recovery; 4 – Effect of KCl on the incorporation of $^{15}NH_4^+$ into sediment pools.

Sampling and methods

Sites

Sediments were collected at the east coast of Jutland, Denmark (Fig. 1). Two stations (Stns 1 & 2) were situated at Norsminde fjord, a small estuary of 1.9 km^2 with a narrow entrance to the sea (Jørgensen & Sørensen 1985). The irregular water exchange produces a fluctuation of salinity levels in this fjord (Muus 1967). The sediment from Stn. 1 was sandy (1% of organic matter or OM), until a depth of 7–8 cm. A fine textured sand/silt sediment (2.5% of OM) dominated at Stn. 2. Stn. 3 was located at KaløVig, a small bay situated 20 Km north of Aarhus. The sediment was a fine silt (20% of OM). Sediments were sampled using plexiglass coring tubes (3.6 cm i.d., 15 cm long), and transported quickly to the laboratory.

Characterization of sediments

In the laboratory, batches of sediment from the 0–4 cm stratum were sieved through a 4 mm mesh to remove shells and other particles, homogenized and stored at −20 °C until further processing. Organic content was determined as ignition loss of dried sediments at 500 °C, during 12 hours. The H_2O content was determined in weighed fractions of known volume which were dried to constant weight at 105 °C. Specific density was determined gravimetrically, in duplicate. The H_2O content and specific density data were used to convert the concentrations of extractable NH_4^+ in units of wet sediment, μmol·cm^{-3}.

Nitrification inhibitor

A dicyandiamide solution (McCarthy & Bremner 1989) was prepared at a concentration of 0.4 g/l deionized H_2O and applied at 100 ml kg^{-1} sediment. In the assays described below, dicyandiamide was applied at 15 μg g^{-1} wet sediment. Nitrification is inhibited at temperatures not exceeding 15 °C (Amberger 1989).

Effects of extraction period and temperature on the KCl extraction of NH_4^+

Sandy, silt/sand and silty sediments were weighed into 500-ml beakers, under a stream of N_2. 2*M* KCl was added at a sediment:extractant ratio of 1:1, vol:wt. Slurries were very well mixed and 10 ml volumes (n = 45) were dispensed anaerobically into weighed glass centrifuge tubes (100 by 14.5 mm i.d.), using an open-ended syringe. The tubes were closed with butyl stoppers, reweighed and one series was incubated in a ice-bath (0 °C) using a rotary shaker R010 (Gerhardt, Germany) adjusted to 80 strokes min^{-1}. The other series was incubated at 15 °C in the same way. At 0, 10, 40, 80, 160 and 200 min of exposure to KCl, samples were taken ($n = 3$) from each series and centrifuged at 3000 × g, for 5 min. Supernatants were collected and stored at −20 °C, until analysis of KCl extractable NH_4^+. The most suitable extraction parameters (extraction time, temperature) were used in the ^{15}N assays.

Comparison of KCl with NaCl and seawater in the extraction of added $^{15}NH_4^+$

The recovery of added $^{15}NH_4^+$ was assessed in three sediment types using KCl, NaCl and artificial seawater (ASW) as extractant solutions. Twenty ml of anaerobic, N_2-bubbled seawater containing $^{15}NH_4^+$ (99.7% ^{15}N, Amersham) and a nitrification inhibitor were added to sandy, sand/silt and fine silt sediments. Final concentrations of $^{15}NH_4^+$ were respectively, 0.6, 0.1 and 0.3 μmol·cm^{-3} of wet sediment. This corresponded to a 10% increase in KCl-extractable NH_4^+, as determined before the addition of label. Each sediment was then divided into 3 series, receiving, respectively, 2*M* KCl, 2*M* NaCl and ASW (prepared by dissolving 25 gr NaCl, 8 gr $MgSO_47H_2O$ and 0.035 gr $NaHCO_3$ in 1 l deionized H_2O). Final pH was 7.0 for all treatments. An extractant:sediment ratio of 1:1 was used as above. Slurries were shaken during 40 min (sand, sand/silt) or 160 min (silt) at 0 °C. Supernatants were collected and stored at −20 °C until analysis of total NH_4^+ (unlabelled plus ^{15}N-labelled NH_4^+) and of atom % $^{15}NH_4^+$.

Effect of exogenous NH_4^+ *on* ^{15}N *recovery in silt sediments*

Exogenous NH_4^+ was added prior to $^{15}N_4$ and KCl for occupation of available exchangeable sites in sediment. A non-biological silt sediment was dispensed anoxically into 10 ml glass tubes ($n = 42$), in two series. To one series were added 0, 2, 15, 30, 53, 75 and 150 μmoles NH_4^+·cm^{-3} in 1.0 ml. $^{15}NH_4^+$ (0.3 μmoles·cm^{-3} sediment) was then added. The other series was treated similarly and received 2*M* KCl as above. The slurries were extracted at 0 °C during 160 min. Centrifugation of slurries and collection of supernatants were done as before. The pools extracted are named NH_4^+-extractable and (NH_4^+ + K^+)-extractable.

Effect of KCl on the incorporation of $^{15}NH_4^+$ *into sediment pools*

A possible role of KCl on the disappearance of $^{15}NH_4^+$ added to the silt sediment (Stn. 3) was investigated, because one-step KCl gave a low recovery of label in exchangeable pools. Slurries from a non-biological silt sediment were labelled with $^{15}NH_4^+$ (0.3 μmol·cm^{-3} sediment) and were dispensed into 50 ml plastic tubes ($n = 21$), under a stream of N_2. The tubes were closed with butyl stoppers and gently shaken at 0 °C, as previously. At time intervals of 0.1, 1, 2, 4, 8, 12 and 16 h, samples ($n = 3$) were centrifuged at $3000 \times g$ during 5 min, and supernatants collected(P_1). The respective pellets were mixed with 2*M* KCl. The tubes were again closed under N_2. One series was shaken for 1 min at 0 °C, whereas the other series was shaken for 60 min, at the same temperature. Respective extracts contain the residual NH_4^+ in porewater plus NH_4^+ exchangeable with KCl (P_2). The remainder residue was dried (105 °C) and subjected to HF treatment and Kjeldahl digestion (Wang & Øien 1986), to obtain the fraction of non-exchangeable NH_4^+ (P_3).

Chemical analysis

NH_4^+ was analysed in suitable dilutions of extract (KCl, NaCl and seawater) or after diffusion of extracts (Kjeldahl), using a modification of the salicylate-hypochlorite method (Laima 1992). Sample diffusates were distilled prior to analysis, to ensure a normal colour development owing to NH_4^+. A diffusion chamber was used, which consisted of a 13 ml serum bottle with a butyl rubber stopper, through which was inserted a Pasteur pipette. The end of the Pasteur pipette was sealed and rounded; it extended 1 cm through the stopper. Saturated NaOH (2.0 ml) was added to the sample (extract or standard, 2.0 ml) to make it alkaline. The NH_3 diffused upwards and was trapped by a film of 2.5 mol l^{-1} H_2SO_4 which coated the round pipette bulb. The microdiffusion of NH_3 was enhanced by gentle shaking, and was complete within 2–3 days of diffusion time at 22 °C. The glass bulb was then detached, dropped into 2 ml of distilled water and the NH_4^+ content was determined. Concentrations were corrected for dilution and NH_4^+ from the dicyandiamide solution.

The ^{15}N atom % was analysed after microdiffusion of samples (Laima 1993), using an Isogas Mass Spectrometer (Middlewich, Cheshire, UK) linked to a microcomputer.

Results and discussion

Extraction of endogenous NH_4^+

The pool size of extractable NH_4^+ depended on the extraction period, temperature and sediment type (Fig. 2). KCl was efficient in sandy sediments and poorly efficient in silty sediments. The NH_4^+ was slowly extracted from silty sediments, the pool size increased and was constant during 40–160 min, and declining afterwards (Fig. 2C). This confirmed the suspicion that the length of incubation might influence the effect of KCl on NH_4^+ extractability (Simon & Kennedy 1987).

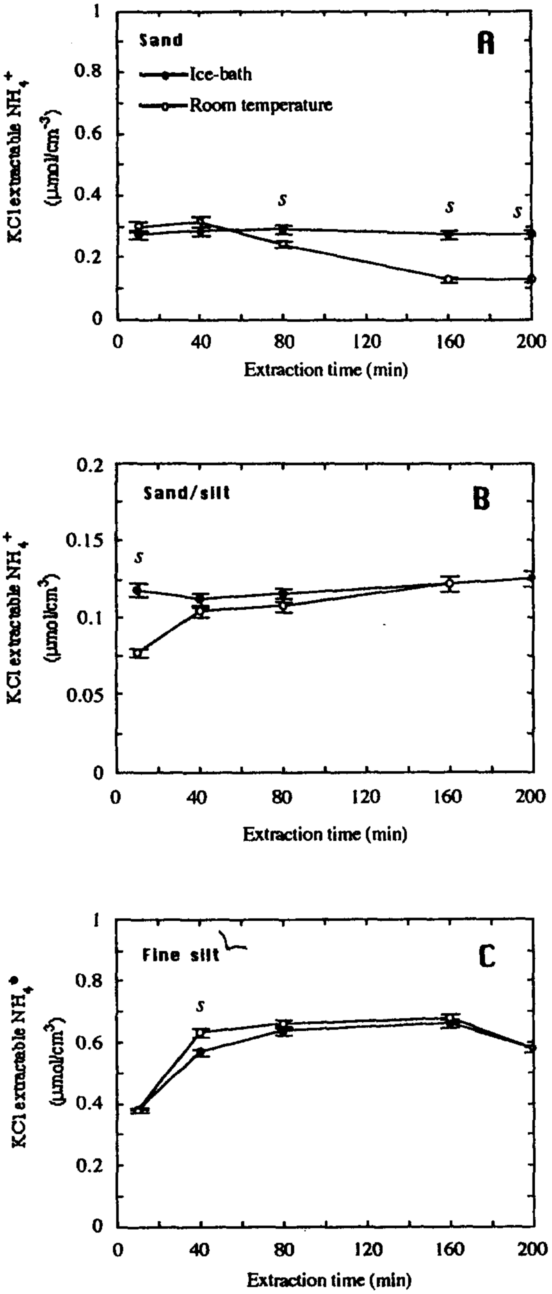

Fig. 2. Concentration of KCl-extractable NH_4^+ as a function of extraction period and temperature, in sandy (A), silt/sand (B), and silt sediment (C). Extractions were carried out at 0 °C (circles, filled) or at 15 °C (circles, unfilled), after addition of dicyandiamide (nitrification inhibitor) to sediment. s: statistically different ($P < 0.001$).

Table 1. Effect of extractant type (KCl, NaCl and ASW) on the concentration of NH_4^+ and recovery of added $^{15}NH_4^+$ from 3 sediment types ($n = 3$, each treatment). Statistics among treatments for ^{15}N recovery are also shown.

Station	Sediment type	Extraction period (min)	Extractant	Total extractable NH_4^+ ($\mu mol \cdot cm^{-3}$)	^{15}N recovery (%)	Statistics[3] *P*
1	Sand*	40	KCl[1]	6.3	98 ± 3	
		40	NaCl[1]	6.4	92 ± 2	< 0.01
		40	ASW[1]	6.4	97 ± 3	ns
2	Sand/silt**	40	KCl[1]	1.2	97 ± 11	
		40	NaCl[1]	1.2	90 ± 7	ns
		40	ASW[1]	1.2	85 ± 3	ns
3	Fine silt***	160	KCl[2]	3.2	20 ± 3	
		160	NaCl[2]	2.7	75 ± 1	< 0.001
		160	ASW[2]	2.8	91 ± 5	< 0.001

[1] Extraction period was 10 minutes at 0 °C. [2] Extraction period was 120 minutes at 0 °C. [3] Students's test was used to statistically compare the ^{15}N recovery data from NaCl or ASW extraction to KCl extraction. ns: non-significant at the 95% confidence level. Final concentration of added $^{15}NH_4^+$ was: *0.6, **0.1 and ***0.3 ($\mu mol \cdot cm^{-3}$).

The temperature had a significant effect on the extraction of NH_4^+ from sediment (Fig. 2A–C); Extraction at 0 °C gave the best results, as the pool size of NH_4^+ was larger and nearly constant with time (except in silt). At 20 °C, there was some evidence in one sediment, that NH_4^+ disappeared with time (Fig. 2A), likely caused by an increase of NH_4^+ fixation into particles (Nõmmik & Vahtras 1982), as there was no increase in NO_3^- concentration during the experiment. From these results, extractions of $^{15}NH_4^+$ labelled sediments were performed at 0 °C, using the best suitable extract period found for each sediment type.

Recovery of $^{15}NH_4^+$ by direct extraction

A comparison was made between 2*M* KCl, 2*M* NaCl and seawater itself in extracting NH_4^+ and added $^{15}NH_4^+$ from these sediments types, using the most suitable extracting parameters from non-labelling experiments. The recovery of added $^{15}NH_4^+$ was used to assess the efficiency of extraction. Variations among KCl, NaCl, or ASW-extractions were not statistically significant in sandy type sediments, where all extractants were equally efficient (Table 1). It was in silt sediment that a mostly significant variation among treatments was found ($P < 0.001$), with ASW giving an almost complete recovery of $^{15}NH_4^+$ (Table 1), KCl resulted in a poor recovery of $^{15}NH_4^+$ (20%), as compared to ASW or NaCl. This confirmed the suspicion, raised by assay 1, that K^+ might play some role in altering the availability of NH_4^+.

No correlation was found between the NH_4^+ pool displaced by different extractants and the ^{15}N recovery (Table 1). NaCl or ASW extractions gave better recovery of added $^{15}NH_4^+$ (75% or 91% vs 20%, Table 1) than did KCl, but KCl gave a higher total NH_4^+ extraction. A higher availability of unlabelled NH_4^+ to K^+ relative to Na^+ has been reported in soils (Sahrawat 1979) and sediments (Simon & Kennedy 1987). An important question may arise: Why did KCl-extractions give alow recovery of added $^{15}NH_4^+$ and a simultaneous high yield of total NH_4^+? At present, only two possible explanations are possible: (1) a differential disappearance of $^{15}NH_4^+$ in relation to unlabelled NH_4^+ has occurred during sediment extraction, that is an isotope effect must have occurred, the unlabelled NH_4^+ being more easy extractable by KCl than $^{15}NH_4^+$. This is an agreement with results shown in Table 1 and with previous work (Laima 1993). Another possibility is that the added ^{15}N-label was, in fact, not randomly distributed in sediment. This hypothesis has been discussed recently (Laima 1993). In any case, these results highlight the need to improve current methods to label, homogenize and extract $^{15}NH_4^+$ from sediments.

Effect of exogenous NH_4^+ on the recovery of $^{15}NH_4^+$

It is believed that the magnitude of competition of K^+ with NH_4^+ for fixation sites will depend on the amount of K^+ added, addition of K^+ prior or after addition of NH_4^+, mineral composition and the ratio between NH_4^+ and the K^+ (Nõmmik 1957). In assay no. 3, involving the addition of KCl to a sediment labelled with $^{15}NH_4^+$ and amended with exogenous NH_4^+ (Fig. 3), ratios of measured/added NH_4^+ in the NH_4^+-extractable pool were < 1 and about 1 in the (KCl + NH_4^+)-extractable pool (Fig. 3A–B). Thus, a loss of NH_4^+ from exchangeable pools were definitively prevented by the added K^+. This points to a depressive effect of the K^+ on the fixation of NH_4^+, as reported by Nõmmik (1957) for soil systems.

In controls (no NH_4^+ addition), about 40% of added ^{15}N was recovered in NH_4^+-extractable pool and only 20% in the (KCl + NH_4^+)-extractable pool (Fig. 3). This suggests that K^+ might have induced a differential disappearance of $^{15}NH_4^+$ in relation to $^{14}NH_4^+$, as the ratio NH_4^+ added/NH_4^+ measured was always 1 in the tested range (Fig. 2B). The increase of non-extractability of $^{15}NH_4^+$ in presence of KCl has been recently found (Laima 1993).

Extraction of $^{15}NH_4^+$ was enhanced by addition of exogenous NH_4^+ to the sediment (Fig. 3C & D). The ^{15}N recovery in NH_4^+-extractable and (NH_4^+ + K^+)-extractable pools was 100% at NH_4^+ concentrations >80 $\mu mol \cdot cm^{-3}$.

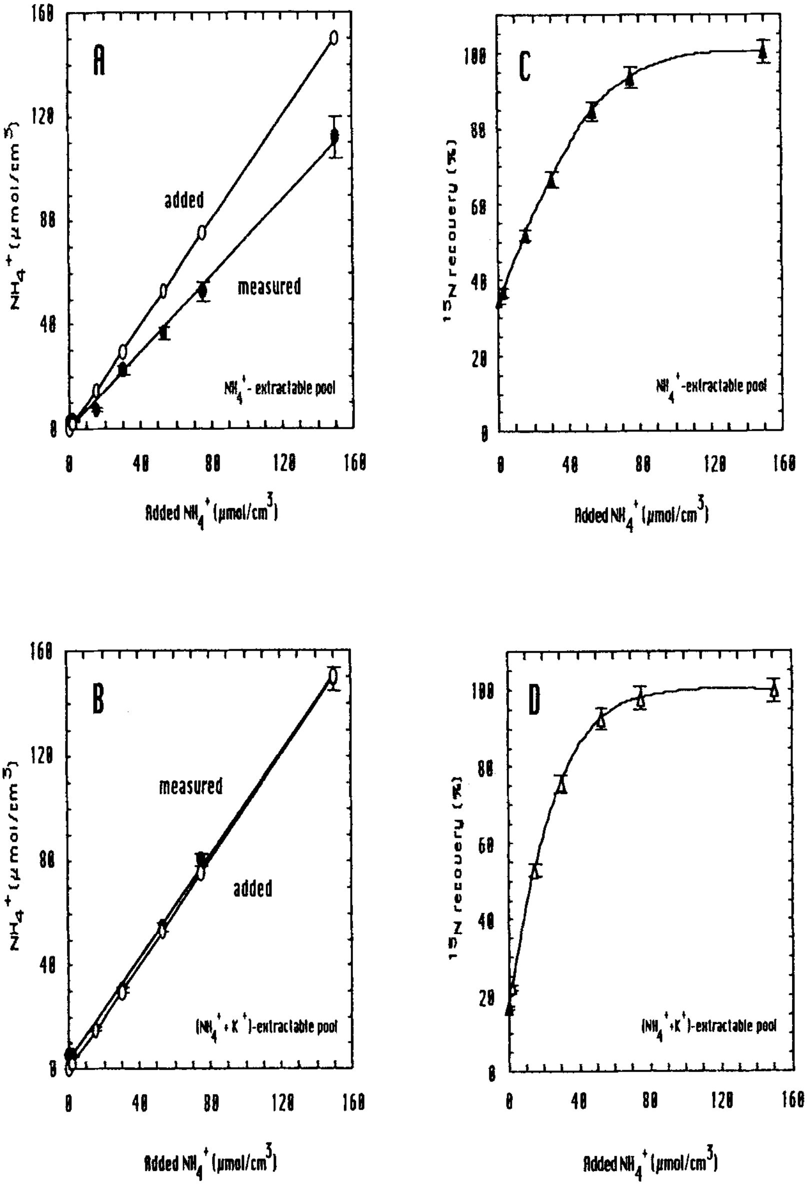

Fig. 3. The extractability of total NH_4^+ and of added $^{15}NH_4^+$ at different addition levels of exogenous NH_4^+ to a silt sediment. Concentrations of NH_4^+ (added, measured) vs added NH_4^+ are shown for the NH_4^+-extractable pool (A) and (NH_4^+ + K^+)-extractable pools (B). The ^{15}N recovery in these two pools is also shown (C & D, respectively).

KCl had a non significant effect ($P > 0.5$) on the recovery obtained in the NH_4^+-extractable pool, likely because exogenous NH_4^+ had successfully exchanged with $^{15}NH_4^+$ that was adsorbed to sediment at the moment of addition of KCl.

Fate of $^{15}NH_4^+$ into sediment pools

The fate of $^{15}NH_4^+$ was investigated in the following extracted NH_4^+ pools from a non-biological silt sediment: porewater (P_1), KCl-extractable with residual porewater (P_2) and non-exchangeable (P_3). After 0.1 h of exposure to $^{15}NH_4^+$ there had already been a rapid equilibrium of $^{15}NH_4^+$ between P_1 and P_2, about 50% of label was apparently lost from P_1 (Table 2). Different extraction periods of P_2, e.g. KCl_{1min} vs KCl_{60min} did not result in significantly different ^{15}N recoveries in sediment pools (Table 2). Only at the initial stages of incubation of the sediment ($t < 2$ h), the extraction period of the P_2 pool had resulted in significantly different recoveries of label ($P < 0.05$) in the KCl extracts: KCl_{1min} extractions gave an higher recovery of $^{15}NH_4^+$ as compared KCl_{60min} extractions: 17–20 vs 9–11%, respectively (Table 2). Azam et al. (1989) also reported low recoveries of added $^{15}NH_4^+$ in the KCl-extractable pool, during the initial stages of incubation. Not all the $^{15}NH_4^+$ that was apparently lost from P_1 did appear in P_2. At 0.1 h of incubation, over 20% of the initial label was found adsorbed to P_3 in a unknown form. The distribution of $^{15}NH_4^+$ was completed after 2 hours of incubation. After the equilibrium was achieved, 28% and 40% of the label was recovered from P_2 and P_3, respectively. The summed ^{15}N recovery in $P_1 + P_2 + P_3$ was essentially 100% (Table 2). Thus, about 40% of added ^{15}N was complexed to OM in a form non-recoverable by KCl, and that KCl itself induced the entry of label into these pools, before the steady-state among pools was attained. The label which entered into an non-exchangeable pool was not available for further exchange with K^+, suggesting that K^+ might have opened crystal lattices, into which some $^{15}NH_4^+$ (and probably some K^+) had entered.

Conclusion

The analysis of NH_4^+ and $^{15}NH_4^+$ extractable by KCl has shown the following points:

(1) KCl is efficient to remove non-labelled NH_4^+ from coastal marine sediments, although extraction conditions had some effect on yield. KCl extractions of non-biological sediments at 0 °C resulted in a expected, stable pool size of NH_4^+ in two from three sediment types tested.

(2) KCl is unable to remove all $^{15}NH_4^+$ added to organic, fine textured silt sediments. The 'disappeared' ^{15}N can be re-found in exchangeable pools, if suitable amounts of exogenous NH_4^+ are added prior to labelling and extrac-

Table 2. Total extractable NH_4^+ and recovery of added $^{15}NH_4^+$ into porewater (P_1), exchangeable pool (P_2) and non-exchangeable pools (P_3). P_2 was removed after 1 min (KCl_{1min}, open) and 60 min (KCl_{60min}, parenthesis) of extraction time at 0 °C. Averages of triplicates are shown. SEs were below 10% of mean values (not shown).

	Porewater (P_1)		Exchangeable (P_2)		Non-exchangeable (P_3)		Total ($P_1 + P_2 + P_3$)
Time (h)	NH_4^+ ($\mu mol \cdot cm^{-3}$)	^{15}N rec (%)	NH_4^+ ($\mu mol \cdot cm^{-3}$)	^{15}N rec (%)	NH_4^+ ($\mu mol \cdot cm^{-3}$)	^{15}N rec (%)	^{15}N rec (%)[3]
0.1	4.0	49	3.3 (3.1)	17 (09)[1]	46.2 (46.3)	20 (24)[1]	86 (90)
1	4.0	44	3.3 (3.2)	20 (11)[1]	46.2 (46.3)	26 (37)[1]	90 (92)
2	3.9	33	3.6 (3.3)	26 (28)[2]	46.2 (46.3)	41 (39)[2]	98 (97)
4	3.9	33	3.4 (3.4)	27 (28)[2]	46.2 (46.3)	39 (39)[2]	99 (98)
8	3.8	33	3.2 (3.4)	28 (28)[2]	46.2 (46.3)	39 (39)[2]	98 (97)
12	3.8	32	3.2 (3.4)	29 (28)[2]	46.2 (46.3)	32 (35)[2]	93 (95)
16	3.8	29	3.2 (3.4)	30 (28)[2]	46.2 (46.3)	39 (42)[2]	98 (99)

[1] ^{15}N recoveries are significantly different ta $P < 0.05$.

[2] ^{15}N recoveries are not significantly different at $P < 0.05$.

[3] Summed ^{15}N recoveries in $P_1 + P_2 + P_3$ pools are not significantly different at $P < 0.05$.

tion of sediments. This is a successful improvement of a standard technique which is utilized worldwide. The technique may be useful in the future, if the objective is to measure nitrification rates from ^{15}N discrimination kinetics. Because the original pool if inflated, it should not be used in studies of NH_4^+ turnover in anoxic sediments.

(3) KCl extractions resulted in a lack of correlation between the pool size of total NH_4^+ and the corresponding ^{15}N recovery. These observations can not be explained at present. Two major hypothesis are given: (1) KCl induces a differential disappearance of $^{15}NH_4^+$ in relation to unlabelled NH_4^+ and (2) the ^{15}N-label was not randomly distributed in sediment. Further experiments are need to assess the validity of these hypothesis.

(4) In non-steady state conditions, KCl influences the incorporation of ^{15}N into unknown, non-extractable pools. Sediments, to which $^{15}NH_4^+$ is added in order to measure $^{15}NH_4^+$-turnover after KCl extraction, should be incubated for at least 2 h, before taking a zero time ($t_{=0}$) sample. This is necessary for achievement of the steady state of label among different NH_4^+ pools in sediment. The suitable $t_{=0}$ may vary from sediment to sediment.

The problems that seem to be associated with KCl, particularly its failure to remove $^{15}NH_4^+$ adsorbed to organic bound pools from physical processes, suggest that KCl should be used with caution in silt sediment. An alternative, successful methodology using $Na_4P_2O_7$ has been developed to solubilize non-extractable $^{15}NH_4^+$ (Laima 1993). Other authors have also developed more powerful techniques than KCl, for example based on electro-ultracentrifigation (Fort et al. 1993).

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