Intracellular NH₄⁺ and NO₃⁻ pools associated with deposited phytoplankton in a marine sediment (Aarhus Bight, Denmark)

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ABSTRACT: Concentration profiles of NH₄⁺ and NO₃⁻ in pore water and particulate matter were determined at high spatial resolution (mm scale) in surface sediment from a coastal bay area (Aarhus Bight, Denmark) at 15 m depth during an annual cycle. Pore water pools of NH₄⁺ and NO₃⁻ were always considerably lower than particulate pools in the surface sediment. Particulate NH₄⁺ and NO₃⁻ were apparently intracellular pools in deposited microalgae and were extracted after freezing sediment samples in liquid N₂ (−196 °C). Pore water NH₄⁺ and most of the adsorbed (KCl-extractable) NH₄⁺ were also extracted by the freezing technique, and an estimate of the intracellular NH₄⁺ pool was obtained by difference. In the absence of an adsorbed NO₃⁻ pool, intracellular NO₃⁻ was determined by subtraction of the pore water pool from the liquid N₂-extractable pool. Highest concentrations of intracellular NH₄⁺ and NO₃⁻ pools were always observed in the upper 2 mm of sediment, declining sharply with depth. A distinct seasonal maximum for both pools, ca 200 nmol cm⁻³ at 0 to 2 mm depth, appeared after sedimentation of a phytoplankton bloom in early spring, and should be compared to a minimum of only 25 nmol cm⁻³ or less in fall and winter. The freeze-extraction technique is proposed for a reliable estimate of intracellular NH₄⁺ and NO₃⁻ pools in surface sediments rich in microalgae, and may thus be used as an indicator of sedimentation of phytoplankton blooms. The significance of intracellular pools for sediment nitrogen cycling is discussed.

INTRODUCTION

In marine sediments, NH₄⁺ appears both in the pore water and bound to clay minerals or organic matter. Adsorbed NH₄⁺ can be extracted in an exchange reaction with K⁺ ions (KCl-extractable or exchangeable NH₄⁺), but if NH₄⁺ is structurally incorporated into the interlayers of clay minerals (fixed NH₄⁺) the pool is extractable only by treatment with hot hydrofluoric acid (Bremner et al. 1967, Rosenfeld 1979). In contrast, adsorbed NO₃ pools are unlikely to exist in sediments, and in studies of NO₃ transformations attention has so far only been given to NO₃ dissolved in pore water.

Both monocultures and naturally occurring phytoplankton populations of marine microalgae have been shown to contain intracellular pools of NH₄⁺ and NO₃⁻ (Dortch et al. 1985 and references therein). For instance, pelagic diatoms such as Skeletonema costatum which often dominate the spring blooms of primary production in temperate coastal waters may internally contain considerable amounts of inorganic nitrogen (e.g. Dortch 1982, Thoresen et al. 1982, Dortch et al. 1984, Raimbault & Mingazinni 1987). Since mass sedimentation of a phytoplankton bloom in early spring, and should be compared to a minimum of only 25 nmol cm⁻³ or less in fall and winter. The freeze-extraction technique is proposed for a reliable estimate of intracellular NH₄⁺ and NO₃⁻ pools in surface sediments rich in microalgae, and may thus be used as an indicator of sedimentation of phytoplankton blooms. The significance of intracellular pools for sediment nitrogen cycling is discussed.

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The objective of the present study was to demonstrate and quantify the presence of particulate, intracellular pools of NH$_4^+$ and NO$_3^-$ in the surface sediment of a coastal bay area, characterized by extensive sedimentation after a phytoplankton bloom in spring. A freeze-extraction technique was developed for an assay of such intracellular inorganic nitrogen pools in sediment. During an annual cycle, undisturbed cores of the surface sediment were sectioned at mm-thick depth intervals and profiles of pore water, and adsorbed and intracellular NH$_4^+$ and NO$_3^-$ were measured. The study took place in parallel to an investigation of fluxes and concentration gradients of NH$_4^+$ and NO$_3^-$ at the sediment-water interface (Jensen et al. 1990).

MATERIALS AND METHODS

Study site and sampling. The investigation was carried out between September 1987 and August 1988 at a 15 m deep site in Aarhus Bight (Stn 16 of Jensen et al. 1988, 1990) in the southwestern Kattegat. Sampling was most intensive during spring, when cores were collected 2 to 4 times per month. The macro-infauna was relatively scarce; bivalves (Abra alba) and polychaetes (Nephtys spp.) were most abundant throughout the investigation period.

Undisturbed sediment samples were collected with a Haps bottom corer (Kanneworff & Nicolaisen 1973), from which a number of subcores were taken into Plexiglas tubes and carefully brought to the laboratory. Only cores with an undisturbed sediment surface were used. The cores were kept for a few hours in the dark at the in situ temperature before processing.

Sediment sectioning and chemical analysis. The overlying water was carefully removed from the sediment cores and a small subcore was taken into a 20 ml disposable syringe (i.d. 2 cm) with cut-off ends (Fig. 1). Each subcore was sectioned into 0–2, 2–4, 4–7, 7–10, 10–15, 15–20, 20–30 and 30–40 mm depth intervals by cutting with a razor blade. This allowed fine-sectioning of the core with only slight disturbance of the sediment surface.

Specific density (weight of 1 cm$^3$ fresh sediment) and water content (weight loss after 24 h at 105°C) were determined in duplicate for each depth interval. The sediment porosity, as determined by multiplication of specific density and water content, decreased from 0.89 at 0–2 mm depth to 0.80 at 30–40 mm depth.

The following techniques were used for extraction of

![Fig. 1. Flow chart of the procedures used for determination of NH$_4^+$ and NO$_3^-$ pools in the sediment. I: Centrifugation (pore water pool); II: KCl extraction, III: liquid N$_2$ extraction; IV: ethanol extraction.](image-url)
NH₄⁺ and NO₃⁻ pools in the sediment (Fig. 1). Pore water pool (I): Each segment was centrifuged for 5 min at 2900 × g and the supernatant frozen for later analysis. KCl-extractable pool (II): Each segment was placed in ca 2 volumes of 1 N KCl solution, shaken vigorously for 2 min and centrifuged at 1600 × g for 10 min. The supernatant was frozen for later analysis. Liquid nitrogen-extractable pool (III): Each segment was wrapped into a piece of PVC-film and quickly frozen in liquid N₂ (−196 °C). The segment was then thawed during a centrifugation at 2900 × g for 5 min and the supernatant frozen for later analysis. Ethanol-extractable pool (IV): Each segment was treated with 5 volumes of 96% ethanol, shaken vigorously for 1 min and centrifuged at 1600 × g for 10 min. The supernatant was transferred to a centrifuge tube and dried at 65 °C for 24 h. The dry matter was then dissolved in 50% n-pentanol, and the green chlorophyll pigments in the n-pentanol phase discarded. The water phase containing the NO₃ was frozen for later analysis. The samples were analyzed for NH₄⁺ (Solorzano 1969) and NO₃⁻ (Armstrong et al. 1967) using an autoanalyzer. All concentrations were determined in triplicate (3 segments from 3 different cores were used to obtain a concentration for a particular depth interval).

RESULTS

NH₄⁺ and NO₃⁻ pools in the sediment

An example of in situ profiles of dissolved and particulate NH₄⁺ and NO₃⁻ is shown in Fig. 2. On this particular sampling date (7 April 1988), a flocculent layer of deposited diatoms was apparent on the sediment surface indicating that sedimentation of the spring bloom had occurred immediately before the sampling occasion (Jensen et al. 1990). It should be noted that the pools extracted by KCl (II) and by freezing in liquid N₂ (III) include the NH₄⁺ and NO₃⁻ in pore water. The pore water pool of NH₄⁺ was smaller than the KCl-extractable pool at all depths (Fig. 2A). The 2 techniques obviously extracted 2 different pools of particulate NH₄⁺ in the surface sediment. Deeper in the sediment, at 20 to 40 mm depth, the KCl treatment always extracted slightly more NH₄⁺ than the freezing technique. This suggests that a relatively constant and large fraction of the KCl-extractable NH₄⁺ is also extracted by freezing. Similarly, Yamada et al. (1987) found that freezing and subsequent thawing release the exchangeable NH₄⁺ pool to the interstitial water. Both an exchangeable NH₄⁺ pool and an NH₄⁺ pool only extractable by freezing seemed to be present in the surface sediment, while the latter pool was unimportant in the deeper part of the sediment. As most of the KCl-extractable NH₄⁺ was also extracted by freezing, a surface-located pool of particulate, but not KCl-extractable, NH₄⁺ was represented by the liquid N₂-extractable pool minus the NH₄⁺ pool in the KCl extract. Based on the data from deeper fractions (Fig. 3A), this particulate NH₄⁺ pool could possibly be underestimated by up to 20% by the difference estimate, since not all exchangeable NH₄⁺ was extracted by freezing.
Unlike for NH₄⁺, there seemed to be a quantitative relationship between NO₃⁻ extracted by KCl and by freezing in liquid N₂ for the upper 2 mm (Fig. 3B). The KCl treatment thus extracted a small but relatively constant fraction (ca 25%) of the NO₃⁻ pool obtained by freezing in liquid N₂. Additional NO₃⁻ could not be extracted when freezing was followed by KCl extraction (data not shown). This indicates that the two techniques extracted the same NO₃⁻ pool throughout the sediment. Further evidence to support the presence of a particulate NO₃⁻ pool came from a comparison of sediment samples frozen in liquid N₂ and extracted in ethanol (IV), respectively. Samples originating from 0 to 2, 2 to 4 and 4 to 7 mm depth (cores collected in September and October 1987) showed essentially the same quantities of NO₃⁻ extracted by the two techniques (Fig. 4). Results indicate that the sediment contained a distinct particulate NO₃⁻ pool located at or close to the sediment-water interface. In the deeper sediment layers, none of the techniques could detect NO₃ beyond the pore water pool. The surface-located particulate NO₃⁻ pool was thus quantified by subtracting the NO₃⁻ pool in the pore water from the liquid N₂-extractable pool. The pool could be extracted either by freezing in liquid N₂ or by extraction in ethanol, but the former is clearly the simplest for routine analyses.

**Seasonal variation of NH₄⁺ and NO₃⁻ pools**

Seasonal patterns of dissolved and surface-located particulate NH₄⁺ and NO₃⁻ are shown by the isopleths in Figs. 5 and 6. The production and sedimentation period of the spring phytoplankton bloom is indicated by the shaded area; the length of this period is judged from the time course of NO₃⁻.

![Fig. 3. Concentrations of KCl-extractable (A) NH₄⁺ and (B) NO₃⁻ plotted against NH₄⁺ and NO₃⁻ concentrations obtained by liquid N₂-freezing (Stn 16, Aarhus Bight). Pore water contents of NH₄⁺ and NO₃⁻ are subtracted. Concentrations are means of triplicate determinations for (●) 0 to 2 mm depth and (▲) 20 to 40 mm depth made during a whole season. Broken lines indicate 1:1 ratio.](image1)

![Fig. 4. Concentrations of ethanol-extractable NO₃⁻ plotted against liquid N₂-extractable NO₃⁻ in sediment from Stn 16, Aarhus Bight (September and October 1987). Pore water contents are subtracted. Concentrations are means of triplicate determinations for (●) 0 to 2 mm depth, (▲) 2 to 4 mm depth and (▲) 4 to 7 mm depth. Broken line indicates 1:1 ratio.](image2)
Pore water NO₃, µM

Particulate NH₄, nmol cm⁻³

Particulate NO₃, nmol cm⁻³

Fig. 5. Iso-concentration (µM) diagram of pore water (A) NH₄⁺ and (B) NO₃ in Aarhus Bight sediment (Stn 16) during the investigation period (1987 to 1988). Shaded area indicates the production and sedimentation period of the spring phytoplankton bloom.

Fig. 6. Iso-concentration (nmol cm⁻³) diagram of particulate (intracellular) (A) NH₄⁺ and (B) NO₃ in Aarhus Bight sediment (Stn 16) during the investigation period (1987 to 1988). Shaded area as in Fig. 5.

consumption in the surface water and from visual inspection of the surface sediment (Jensen et al. 1990).

The pore water typically showed decreasing NH₄⁺ concentrations towards the sediment surface except for a short period after spring bloom sedimentation (Fig. 5A). The 100 µM isoline was found at 30 to 40 mm depth throughout the investigation period (data not shown). The low concentration of ca 10 µM at 0 to 2 mm depth was interrupted for 1 to 2 mo immediately after the sedimentation, when a peak of more than 50 µM was observed. There was a marked seasonal variation in the dissolved NO₃ pool (Fig. 5B). A maximum was found either in the uppermost 0 to 2 mm of the sediment or in the bottom water. During winter, bottom
water and pore water concentrations were typically high (up to 15 and 25 \( \mu M \) \( NO_3^- \), respectively), and also the penetration depth was high. After sedimentation of the spring bloom, both the concentration and the penetration depth of \( NO_3^- \) decreased and remained relatively low throughout the summer. From late summer, bottom water as well as pore water concentrations were very low (5 \( \mu M \) or less).

The surface-located particulate \( NH_4^+ \) pool was small during the winter (less than 25 nmol cm\(^{-3}\)), but increased considerably in the uppermost sediment after the spring sedimentation (Fig. 6A). The pool size was thus ca 200 nmol cm\(^{-3}\) in the upper 0 to 2 mm in April, but decreased rapidly with depth and was negligible below 10 mm. The pool gradually disappeared during the following 3 to 4 mo. The surface-located particulate \( NO_3^- \) pool also increased strongly after the spring sedimentation when concentrations above 200 nmol cm\(^{-3}\) were recorded in the 0 to 2 mm segment (Fig. 6B). The pool was confined to the upper 5 mm. A final depletion occurred in August as was the case for \( NH_4^+ \). The period following spring sedimentation was characterized by a considerable patchiness of deposited material (Jensen et al. 1988, 1990), and the variability of the particulate \( NH_4^+ \) and \( NO_3^- \) pools was generally high between cores. For instance, a few cores with dense layers of diatoms showed particulate pools (0 to 2 mm depth) of up to 250 and 400 nmol cm\(^{-3}\), respectively.

The seasonal pattern of depth-integrated particulate \( NH_4^+ \) and \( NO_3^- \) pools (0 to 7 mm depth) are shown in Fig. 7. The distinct increase of both pools after the sedimentation event resulted in a 5-fold higher total particulate \( NH_4^+ \) plus \( NO_3^- \) pool. The total pool was high during spring and early summer, although each of the contributing \( NH_4^+ \) and \( NO_3^- \) pools showed considerable oscillations during this period. The gradual decrease of the total pool during summer occurred at the expense of both \( NH_4^+ \) and \( NO_3^- \).

DISCUSSION

Extraction and origin of \( NH_4^+ \) and \( NO_3^- \) pools in the sediment

Besides being dissolved in the pore water, \( NH_4^+ \) may be bound to the sediment matrix in an exchangeable pool which can be extracted by cations such as \( K^+ \). The dynamic nature of the exchangeable \( NH_4^+ \) pool implies that it might play a significant role in sediment nitrogen cycling, for instance by retaining \( NH_4^+ \) otherwise available for diffusion flux (Blackburn & Henriksen 1983). Throughout the investigation period, the exchangeable \( NH_4^+ \) concentration generally increased with depth in the sediment, and the pool size (0 to 4 cm depth) ranged from a factor of ca 0.5 to 2 of the pore water pool showing no specific seasonal pattern (data not shown). The observed range is within the range reported for other marine sediments (Blackburn & Henriksen 1983, Mackin & Aller 1984).

A particulate \( NH_4^+ \) pool, extractable by freezing in liquid \( N_2 \) but not in \( KCl \), was found in the uppermost part of the sediment. The particulate pool was relatively large compared to the pore water and exchangeable pools and appeared immediately after the spring bloom sedimentation (Fig. 6A). Intracellular \( NH_4^+ \) pools have been described for planktonic diatoms, both in cultures and in situ (Dortch 1982, Thoresen et al. 1982, Dortch et al. 1984, Dortch et al. 1985), and Thoresen et al. (1982) found that treatment with liquid \( N_2 \) efficiently ruptures algal cells. Based on this evidence, we ascribe the surface-located particulate pool to an intracellular pool in the deposited diatoms. In the following we therefore refer to a pool of intracellular \( NH_4^+ \) in the surface sediment. Deeper in the sediment, the intracellular \( NH_4^+ \) pool appeared to be absent, since the freezing technique extracted a similar or slightly smaller amount of \( NH_4^+ \) than the \( KCl \) extraction (Fig. 3A; Yamada et al. 1987).

It was unlikely that the measured particulate \( NO_3^- \) originated from an exchangeable (adsorbed) pool, although non-specific binding of \( NO_3^- \) within an electrical double layer is theoretically possible (e.g. Wang et al. 1987). The affinity for such adsorption sites must be stronger for \( Cl^- \) than for \( NO_3^- \) ions, however, and marine sediments typically contain 1000-fold more \( Cl^- \) than \( NO_3^- \). The various treatments (I to IV) used in the present study extracted \( NO_3^- \) from a single particulate pool in the sediment, and the largest pool size was measured in the period immediately after bloom sedimentation (Fig. 6B).
This was in good agreement with the existence of a particulate pool associated with intracellular NO₃ in deposited microalgae. Diatoms such as *Skeletonema costatum* may be dominant in spring blooms of temperate coastal waters, can store large amounts of NO₃ in batch cultures (e.g. Dortch 1982, Raimbault & Mingazzini 1987), and Dortch et al. (1985) measured high in situ concentrations of intracellular NO₃ in natural assemblages of phytoplankton during a spring bloom. As for NH₄⁺, we shall therefore refer to an *intracellular NO₃* pool in the sediment. Treatments with liquid N₂ and ethanol extracted the same quantity of intracellular NO₃ (Fig. 4) after the algal cells were ruptured. Ethanol has not previously been used for extraction of intracellular NO₃, but we suppose that the microalgae were ruptured due to a dramatic change in osmotic pressure. The KC1 extraction was less efficient in releasing intracellular NO₃ (Fig. 3B). Garber (1984) found KC1-extractable NO₃ concentrations of about 50 nmol cm⁻³ in Narragansett Bay (RI, USA) sediment, but no detectable NO₃ in the pore water. However, the origin or significance of this KC1-extractable NO₃ pool was not discussed.

A drawback of the presented techniques is a weak distinction between KC1-extractable and intracellular NH₄⁺. However, the high intracellular NH₄⁺ concentrations measured at the very surface was not in serious error, but the intracellular NH₄⁺ pool at depth could be underestimated. Further refinement of the method for deeper layers is needed. Comparing various methods for determination of intracellular pools of inorganic nitrogen in microalgae, Thoresen et al. (1982) considered boiling water extraction as the most efficient. We used the freezing technique because it is easy, rapid and requires no addition of solutes.

In light of the present demonstration of particulate, intracellular NH₄⁺ and NO₃ pools in sediments, care has to be taken when determining NH₄⁺ and NO₃ concentrations in marine sediments, especially in those characterized by sedimentation of planktonic blooms or high densities of benthic diatoms. A KC1 extraction of surface sediment (Henriksen et al. 1980, Andersen et al. 1984, Jørgensen & Sørensen 1985, Jørgensen & Sørensen 1988) and a liquid N₂ freezing procedure for conservation purposes (Sørensen 1978) are not recommended if the samples are to be assayed for dissolved NO₃. The NO₃ concentration obtained may thus include an intracellular NO₃ pool and result in erroneous estimates of pore water NO₃. On the other hand, freezing in liquid N₂ may provide a satisfactory estimate of intracellular NH₄⁺ and NO₃ pools in sediments when combined with separate determination of NO₃ and NH₄⁺ in the pore water and in the KC1-extractable fraction. The freezing assay may also be useful as an indicator of sedimentation of phytoplankton blooms in shallow marine areas.

### Seasonal pattern of NH₄⁺ and NO₃ pools

The profiles shown in Fig. 5 represent the first seasonal pattern of pore water NH₄⁺ and NO₃ concentrations measured at mm-resolution in a marine surface sediment. The input of an easy-degradable pool of phytodetritus to the sediment in early spring resulted in distinct changes in the benthic NH₄⁺ and NO₃ flux at Stn 16 (Jensen et al. 1990). Thus, both the highly increased release of NH₄⁺ and decrease of NO₃ in the surficial pore water pool immediately after the spring sedimentation was apparently a result of increased mineralization and reduced O₂ penetration, which limited nitrification and increased NO₃ consumption (denitrification) (Jensen et al. 1988, 1990).

Sedimentation of the phytoplankton bloom in early spring represents a mass transfer of nitrogen from the water column to the sediment in the form of detrital nitrogen, but also as particulate, intracellular pools of NH₄⁺ and NO₃ in live diatoms. However, the role of the live fraction of the deposited diatoms in the sediment nitrogen cycle is poorly known. In shallow estuaries where light is sufficient, inorganic nitrogen assimilation and photosynthetic O₂ production by benthic diatoms profoundly affect the NH₄⁺ and NO₃ flux, nitrification and denitrification (Henriksen et al. 1980, Andersen et al. 1984, Granéli & Sundbäck 1985, Nowicki & Nixon 1985, Henriksen & Kemp 1988, Jørgensen & Sørensen 1988). Only a few studies have looked for such interactions in coastal, light-limited sediments at greater water depth, however. Diatoms, which sink out of the surface waters, may continue their assimilation in the often nutrient-rich bottom waters or at the sediment surface. It has been proposed that mass sinking of diatoms is a survival reaction when surface waters are depleted in nutrients (Smetacek 1985), and recent evidence suggests that mass flocculation of live diatoms is a widespread mechanism by which their sinking rate increases several-fold (Kranck & Milligan 1988, Alldredge & Gotschalk 1989). The sedimentation may represent a transition phase from a pelagic to a benthic stage in the life history cycle of such diatoms (Noji et al. 1986). Various investigations in light-limited Kattegat sediments (14 to 16 m depth) indicate that viable *Skeletonema costatum* cells deposited in early spring and benthic species developing in the summer are responsible for significant photosynthetic O₂ production (Sundbäck & Jonsson 1988), which even may exceed the sediment O₂ uptake (Granéli & Sundbäck 1986, Jørgensen & Revsbech 1989), and that micro-
Microscopic inspection of the Aarhus Bight sediment in April revealed that a large fraction of the deposited diatoms, notably *Skeletonema costatum*, was still viable. They may have continued assimilation of NH$_4^+$ and NO$_3^-$, thereby sustaining the large intracellular pools for 3 to 4 mo after the initial increase. The actual gradients and availability of NH$_4^+$ and NO$_3^-$ experienced by the diatoms at the very surface may be difficult to determine, but the presence of high levels of intracellular NH$_4^+$ and NO$_3^-$ indicated that the diatoms were not deficient in nitrogen during this period (Dorch et al. 1985). Whereas deposited planktonic diatoms are responsible for the initial increase of the intracellular pools at Stn 16 in April, populations of benthic diatoms may have contributed to the maintenance of the large pools throughout summer. Benthic species, e.g. of the genus *Nitzchia*, were in fact identified in the surface sediment although in smaller numbers than the pelagic species.

Later in summer, both intracellular pools decreased considerably (Figs. 6 and 7). However, the mechanism responsible for the decrease is not clear. Likely explanations include excretion or incorporation of the intracellular pools or microbial degradation of the microalgae. During fall when the intracellular pools almost disappeared, light conditions probably became further unfavourable for growth of the microphytobenthic community. Planktonic diatoms are able to survive for very long periods in the dark as vegetative or resting cells (Smayda & Mitchell-Innes 1974, Durbin 1978, Sicko-Goad et al. 1989), even in deep sediment strata (Wasmund 1989). It is not known whether NH$_4^+$ and NO$_3^-$ pools are maintained in the intracellular volume under such conditions, but it was suggested by Wasmund (1989) that microalgae buried in the sediment survive by drawing slowly on their internal reserves.

The present study demonstrates the existence of large, particulate pools of inorganic nitrogen which developed after extensive sedimentation of a spring phytoplankton bloom. The particulate NH$_4^+$ and NO$_3^-$ was ascribed to intracellular pools in deposited microalgae and possibly, to a lesser extent, in benthic diatoms. The role of these pools and of the possible microphytobenthic activity (i.e. nutrient assimilation and O$_2$ production) for the nitrogen cycling in light-limited, marine sediments is, however, not well understood.

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**LITERATURE CITED**


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