

# NH<sub>4</sub><sup>+</sup> turnover in intertidal sediments of Marennes-Oléron Bay (France): effect of sediment temperature

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**Abstract** – NH<sub>4</sub><sup>+</sup> turnover plays an important role in benthic compartment as source of nutrient for primary production. The intertidal mudflats of the Marennes-Oléron Bay are characterized by high fluctuations of sediment temperature that generally ranges from 10 to 30 °C during emersion periods. Influence of sediment temperature on NH<sub>4</sub><sup>+</sup> processes was investigated. Gross ammonification and NH<sub>4</sub><sup>+</sup> incorporation kinetics were studied using the <sup>15</sup>N isotope dilution method. The method was first adapted to these very fine sediments and applied to 2 upper sediment layers (0–2 and 2–4 cm) at different incubation times of up to 120 h. The effect of sediment temperature on gross ammonification and NH<sub>4</sub><sup>+</sup> incorporation rates was investigated at 10, 20 and 30 °C. There was a strong increase of both rates as a function of temperature, gross ammonification rates ranged from 0 to 17 µg NH<sub>4</sub><sup>+</sup>-N.g<sup>-1</sup> dw.d<sup>-1</sup> and NH<sub>4</sub><sup>+</sup> incorporation rates from 2 to 13 µg N.g<sup>-1</sup> dw.d<sup>-1</sup> between 10 and 30 °C. This study showed that short-term temperature variations during the emersion period should be taken into account in quantifying NH<sub>4</sub><sup>+</sup> turnover in intertidal sediments and that benthic regeneration of NH<sub>4</sub><sup>+</sup> significantly supports benthic and planktonic primary production in Marennes-Oléron Bay. It suggests also that there is no N-limitation for benthic primary production in this bay and that there is up to 44 % of NH<sub>4</sub><sup>+</sup> produced in surface sediments, which can diffuse to the water column and become available to phytoplankton.

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**Résumé – Régénération du NH<sub>4</sub><sup>+</sup> dans les sédiments intertidaux de la baie de Marennes-Oléron (France) : effet de la température du sédiment.** La régénération du NH<sub>4</sub><sup>+</sup> joue un rôle important dans le compartiment benthique comme source de nutriment pour la production primaire. Les vasières intertidales de la baie de Marennes-Oléron sont caractérisées par de fortes fluctuations de la température du sédiment généralement comprises entre 10 et 30 °C durant les périodes d'émersion. L'influence de la température du sédiment sur les processus du NH<sub>4</sub><sup>+</sup> a été examinée. Les cinétiques d'ammonification brute et d'incorporation du NH<sub>4</sub><sup>+</sup> ont été étudiées par la méthode de dilution isotopique au <sup>15</sup>N. Cette méthode a d'abord été adaptée sur ces sédiments très fins et appliquée à deux couches du sédiment (0–2 et 2–4 cm) pendant différents temps d'incubation jusqu'à 120 h. Tous les résultats indiquent clairement que les incubations de 24 h sont les plus fiables pour les calculs des taux. L'effet de la température du sédiment sur les taux bruts d'ammonification et d'incorporation du NH<sub>4</sub><sup>+</sup> a été étudié à 10, 20 et 30 °C dans le sédiment de surface. Il y avait une forte augmentation des deux taux en fonction de la température, les taux bruts d'ammonification varient de 0 à 17 µg N-NH<sub>4</sub><sup>+</sup>.g<sup>-1</sup> ss j<sup>-1</sup> et les taux d'incorporation

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du  $\text{NH}_4^+$  de 2 à 13  $\mu\text{g N.g}^{-1} \text{ss j}^{-1}$  entre 10 et 30 °C. Cette étude montre que les variations de température à court-terme durant l'émersion doivent être prises en compte dans l'estimation de la régénération du  $\text{NH}_4^+$  dans les sédiments intertidaux et que la régénération benthique du  $\text{NH}_4^+$  soutient significativement la production primaire benthique et planctonique dans la baie de Marennes-Oléron. Cela suggère aussi qu'il n'y a pas de limitation par l'azote pour la production primaire benthique dans cette baie et qu'il y a jusqu'à 44 % du  $\text{NH}_4^+$  produit dans les sédiments de surface, qui peut diffuser dans la colonne d'eau et devenir disponible pour le phytoplancton. © 2000 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

**$\text{NH}_4^+$  turnover / sediment temperature / intertidal sediments /  $^{15}\text{N}$**

**régénération du  $\text{NH}_4^+$  / température du sédiment / sédiments intertidaux /  $^{15}\text{N}$**

## 1. INTRODUCTION

The role of benthic regeneration in supporting the primary productivity of coastal waters is now well-established (Klump and Martens, 1983). The process of organic matter mineralization within the sediment returns  $\text{NH}_4^+$  back into the environment (Bowden, 1984), thus making it potentially available both for benthic and planktonic primary production. This ammonification process is controlled by a few key factors, such as temperature (Bowden, 1984; Thamdrup and Fleischer, 1998), oxygen depth penetration in the sediment (Henriksen et al., 1981), the nature of organic matter (Blackburn and Henriksen, 1983) and the physiological characteristics of microbial communities (Harder and Veldkamp, 1971). The effects of these environmental factors on  $\text{NH}_4^+$  production and incorporation by microbial communities must be quantified to establish an N-budget in coastal environments, where N is frequently considered as a limiting factor (Ryther and Dunstan, 1971). Some studies of N cycling have been devoted to intertidal sediments (Rocha, 1998; Trimmer et al., 1998) because these play an important role in primary productivity (Blanchard et al., 1997) and in the nutrient cycling (Feuillet-Girard et al., 1997) of littoral areas.

The intertidal environment is further characterized by large and rapid changes of the factors which might control N cycling. For example, sediment surface temperature is likely to influence  $\text{NH}_4^+$  production and incorporation rates since it varies at several time scales: seasonally, fortnightly due to the tidal cycle and hourly due to the emersion period (Guarini et al., 1997). It is therefore necessary to quantify the relationship between  $\text{NH}_4^+$  production, incorporation rates and temperature – as well as the other N cycle

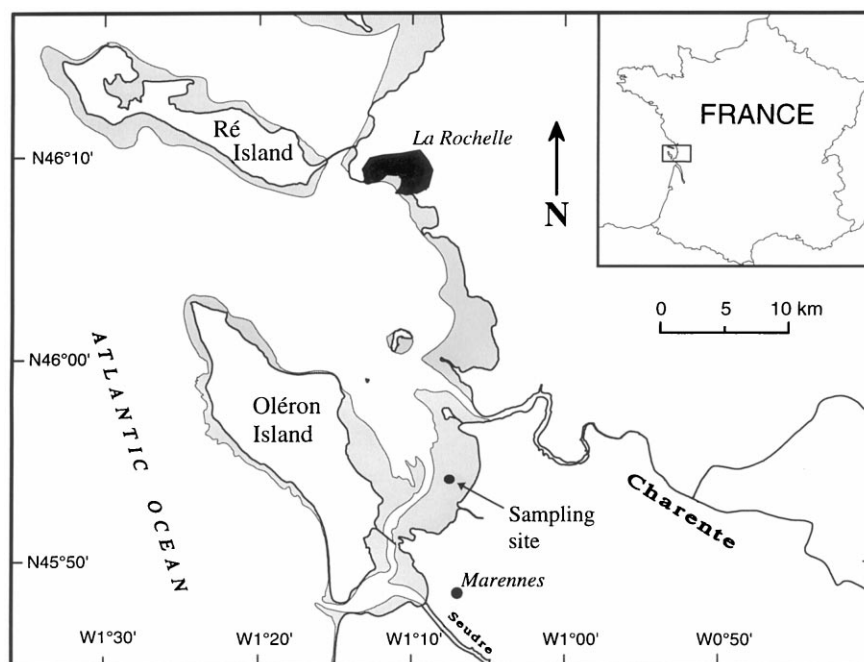
processes, including nitrification (Laima et al., 1999) – to be able to model the dynamics of  $\text{NH}_4^+$  in intertidal sediments and thus the contribution of benthic regeneration to coastal productivity.

This study aimed at quantifying, for the first time, gross  $\text{NH}_4^+$  production rates and  $\text{NH}_4^+$  microbial incorporation rates in the sediment of the Marennes-Oléron Bay so as to complement earlier studies of N cycling in this bay (Feuillet-Girard et al., 1988). Since the surface sediment is subjected to strong temperature fluctuations during the emersion period, we also studied the influence of sediment temperature variations on these rates. By using  $^{15}\text{N}$  isotope labelling techniques, which allow a precise quantification of  $\text{NH}_4^+$  turnover processes (Guiraud, 1984), this study was carried out in two steps: (i) to achieve reliable rate estimates, the  $^{15}\text{N}$  isotope dilution method was first adapted to the very fine grained sediments of the Marennes-Oléron Bay and (ii) the second step involved the short-term effect of temperature (range 10–30 °C) on the rates of gross  $\text{NH}_4^+$  production and incorporation.

## 2. MATERIALS AND METHODS

### 2.1. Study area characteristics

The Marennes-Oléron Bay is located on the French Atlantic coast and extends over about 170 km<sup>2</sup> between Oléron Island and the mainland (*figure 1*). It includes large intertidal mudflats that cover about 110 km<sup>2</sup>. The studied area is the largest eastern mudflat where sediment consists of silt and clay particles (95 % of sizes < 63  $\mu\text{m}$ ). General characteristics



**Figure 1.** Location of the sampling station on the study area in the Marennnes-Oléron Bay. Pale grey areas represented the intertidal zones.

for this sediment are shown in *table I*. Temperature variations in the surficial sediment are high and rapid. Sediment surface temperature ranges typically from 10 °C in winter to 30 °C in summer, but it can be as low as 3 °C or can peak at 34 °C. In the surface sediment, the maximum temperature daily change during the course of a single emersion period in June can reach 18 °C at a rate of 3 °C h<sup>-1</sup> (Guarini et al., 1997).

## 2.2. Sediment sampling and composition

Sediment samples were collected at low tide in the central part of the eastern intertidal mudflat of the Marennnes-Oléron Bay (*figure 1*). A first set of 3 cores sampled in May 1997 was used to adapt the <sup>15</sup>N isotope dilution technique to this silt-clay sediment and to measure the ammonification and NH<sub>4</sub><sup>+</sup> incorporation kinetics at *in situ* temperature (22 °C). A second set of 3 cores were sampled in June 1997 at the same site and were used to study the temperature effects on these processes. Sediments were quickly brought to the laboratory and stored at 4 °C before being processed (Sumi and Koike, 1990). Sediments

were sliced into two sections: 0–2 cm and 2–4 cm. Plant and shell particles were removed and each sediment layer was thoroughly homogenized.

Specific density was determined by weighing a known sediment volume and water content by drying to constant weight at 105 °C. Organic C and N contents were measured with a CHN analyzer (Carlo Erba Instruments 1500) after decarbonatation of the dried sediment using 1N HCl. Some sediment characteristics are shown in *table I*.

**Table I.** Main characteristics of the studied sediment.

Sediment characteristics	0–2 cm	2–4 cm
pH	7.02 ± 0.11	7.00 ± 0.06
Eh (mV)	236 ± 74	149 ± 12
Specific density	1.37 ± 0.01	1.41 ± 0.01
Water content (% ww)	50.4 ± 0.7	46.7 ± 0.6
Granulometry <63 µm (%)	94	96
Total N (mg.g <sup>-1</sup> dw)	1.6 ± 0.2	1.3 ± 0.0
Organic C (mg.g <sup>-1</sup> dw)	10.3 ± 0.9	8.6 ± 0.3
C/N	6.5 ± 0.1	6.6 ± 0.1
Organic matter (% dw)	1.85 ± 0.16	1.55 ± 0.06

### 2.3. $^{15}\text{N}$ labelling and extraction procedures

$^{15}\text{N}$  labelling and extraction procedures were carried out on sediment slurries as described by Laima (Laima, 1994). Slurrying was used to achieve a homogeneous tracer repartition and Blackburn and Henriksen (1983) showed that compared to intact cores, slurrying did not affect the  $\text{NH}_4^+$  production rates in muddy sediments. Nitrification was inhibited by adding a dicyandiamide solution ( $450 \text{ mg.l}^{-1}$ ) to the sediment to obtain a final concentration of  $15 \mu\text{g}$  inhibitor per gram of wet sediment (Guiraud et al., 1989; Laima, 1994). The initial  $\text{NH}_4^+$  pool (porewater plus exchangeable  $\text{NH}_4^+$ ) was extracted anaerobically with 2M KCl by shaking for 160 min at  $0^\circ\text{C}$  in the dark. Supernatants were centrifuged at  $3000 \times g$  for 10 min and were frozen at  $-20^\circ\text{C}$  until analysis.

An  $^{15}\text{N}$ -labelled  $\text{NH}_4\text{Cl}$  solution, containing  $49.4 \mu\text{mol NH}_4^+.\text{ml}^{-1}$  with 2 atom %  $^{15}\text{N}$ , was added to N-free artificial sea water (ASW) for homogeneous tracer dispersion. The mixture was then added to the homogenized sediment in a proportion of 4 ml ASW per gram of wet sediment, equivalent to the addition of about  $50 \mu\text{g NH}_4^+\text{-N}$  per gram of sediment dry weight.

Subsamples of the slurries were transferred into  $4 \times 3$  glass tubes under  $\text{N}_2$  and incubated in the dark. Sediments sampled in May 1997 were incubated at *in situ* sediment temperature ( $22^\circ\text{C}$ ) for 0, 24, 48 and 120 hours. The second set of samples (June 1997) was incubated during 24 h at 10, 20 and  $30^\circ\text{C}$  to reflect the range of sediment surface temperatures generally recorded during the year in the study area.

After each incubation period,  $(^{14}+^{15})\text{NH}_4^+$  was extracted by 2M KCl (1:1, v:w) and analyzed for  $(^{14}+^{15})\text{NH}_4^+$  concentration. An aliquot of this extract was stored at  $-20^\circ\text{C}$  for later isotopic analysis.

The microdiffusion technique was used to transfer the  $(^{14}+^{15})\text{NH}_4^+$  from the KCl extracts to an acidified  $\text{Al}_2\text{O}_3$  matrix (Laima, 1993). The maximum  $\text{NH}_4^+$  transfer rate onto the  $\text{Al}_2\text{O}_3$  matrix (95%) was obtained after 96 hours of diffusion time.

The  $^{15}\text{N}$ -labelled sediment residues were dried at  $60^\circ\text{C}$  for 2 days, ground into fine powder and stored in the dark.

### 2.4. $\text{NH}_4^+$ and $^{15}\text{N}$ analysis

The KCl-extractable  $\text{NH}_4^+$  in unlabelled and  $^{15}\text{N}$ -labelled samples was measured with a Skalar autoanalyzer. The %  $^{15}\text{N}$  excess of  $\text{NH}_4^+$  fixed on the  $\text{Al}_2\text{O}_3$  matrix and in the N of the dried residues of sediment was measured using a mass spectrometer coupled to a CHN analyzer (Isochrom, Fisons).

KCl-extractable  $\text{NH}_4^+$  is defined as the inorganic N pool, whereas the organic N pool is given by the fraction measured in the dried residues of sediment. The  $\text{NH}_4^+\text{-N}$  and the N contents ( $^{15}\text{N}$ -labelled and unlabelled) in the inorganic N and organic N pools of sediment are expressed relative to sediment dry weight, as  $\mu\text{g NH}_4^+\text{-N.g}^{-1} \text{ dw}$  and  $\mu\text{g N.g}^{-1} \text{ dw}$ , respectively.

### 2.5. Rate calculations

The kinetics of  $\text{NH}_4^+$  at different incubation times was used to calculate the gross ammonification and  $\text{NH}_4^+$  incorporation rates, as shown by the model of Kirkham and Bartholomew (1954, 1955). A simplified form of this model is often used for marine sediments (Blackburn, 1979). However, this model does not take into account  $^{15}\text{N}$  losses from the system (volatilization). In this study, we used Guiraud's model (Guiraud, 1984), which is also simplified assuming that (i) the variation of the isotopic excess of the inorganic nitrogen fraction during incubation is linear for short intervals as few days and (ii) the incorporated  $^{15}\text{N}$  is not remineralized during the experiment.

We first calculated leaks (f), which represented the decrease of  $^{15}\text{N}$  in the inorganic N pool during one incubation, by using the following equation:

$$f = \frac{P_0 E_0 - P E}{t \left( \frac{E_0 + E}{2} \right)}$$

where  $P_0$  and  $P$  are the pool size of  $\text{NH}_4^+$  ( $\mu\text{g NH}_4^+\text{-N.g}^{-1} \text{ dw}$ ) at the beginning and end of incubation,  $E_0$  and  $E$  are the corresponding atom %  $^{15}\text{N}$  excesses in the  $\text{NH}_4^+$  pool and  $t$  is the incubation period (days).

The gross ammonification rate (m) is calculated as:

**Table II.** Parameters used for the calculation of gross ammonification and  $\text{NH}_4^+$  incorporation at incubation times at 22 °C (May 1997), in the 0–2 cm and 2–4 cm layers. The variations with incubation time of the  $\text{NH}_4^+$  pool (P) and the atom % excess in  $\text{NH}_4^+$  pool (E) are presented before ammonification rates (m). Values of  $P_{\text{org}}$  represent the organic N pool,  $E_{\text{org}}$  the atom % excess in the organic N pool and i the  $\text{NH}_4^+$  incorporation rate. The values are the means of three replicates. Standard deviations are given only for the rates ( $\pm$  SD).

Incubation time (h)	Ammonification			$\text{NH}_4^+$ incorporation		
	P ( $\mu\text{g NH}_4^+\text{-N.g}^{-1}$ dw)	E (%)	m ( $\mu\text{g NH}_4^+\text{-N.g}^{-1}$ dw.d $^{-1}$ )	$P_{\text{org}}$ ( $\mu\text{g N.g}^{-1}$ dw)	$E_{\text{org}}$ (%)	i ( $\mu\text{g N.g}^{-1}$ dw.d $^{-1}$ )
Layer (0–2 cm)						
0	58	1.466		1483	0.0035	
24	60	1.243	$9.7 \pm 3.1$	1483	0.0078	$4.7 \pm 1.2$
48	58	1.124	$5.7 \pm 3.8$	1483	0.0116	$4.8 \pm 1.3$
120	73	0.828	$6.6 \pm 1.5$	1483	0.0150	$1.7 \pm 0.7$
Layer (2–4 cm)						
0	64	1.404		1409	0.0033	
24	59	1.288	$5.3 \pm 3.0$	1409	0.0061	$3.0 \pm 1.1$
48	53	1.196	$4.1 \pm 4.1$	1409	0.0109	$5.4 \pm 1.4$
120	58	1.069	$2.1 \pm 1.1$	1409	0.0140	$1.3 \pm 0.8$

$$m = f + \frac{P - P_0}{t} \quad (\mu\text{g NH}_4^+\text{-N.g}^{-1} \text{ dw.d}^{-1})$$

The  $\text{NH}_4^+$  incorporation rate (i) in the organic pool is calculated as:

$$i = \frac{P_{\text{org}} E_{\text{org}} - P_{\text{org}}^0 E_{\text{org}}^0}{t \left( \frac{E_0 + E}{2} \right)} \quad (\mu\text{g N.g}^{-1} \text{ dw.d}^{-1})$$

where  $P_{\text{org}}^0$  and  $P_{\text{org}}$  are the pool sizes of organic N ( $\mu\text{g N.g}^{-1}$  dw) at the beginning and end of incubation and  $E_{\text{org}}^0$  and  $E_{\text{org}}$  are the corresponding atom %  $^{15}\text{N}$  excesses in the organic N pool.

### 3. RESULTS AND DISCUSSION

#### 3.1. Kinetics of gross ammonification and $\text{NH}_4^+$ incorporation

Variations with incubation time of the inorganic and organic pools and isotopic excesses are shown in *table II*. In the inorganic pool of both layers, the  $\text{NH}_4^+\text{-N}$  contents (P) exhibited only small changes, whereas the  $^{15}\text{N}$  isotopic excess (E) decreased continuously

with incubation time. This  $^{15}\text{N}$  decrease, indicating  $^{14}\text{NH}_4^+$  production owing to mineralization of organic matter, was used to estimate the ammonification rates (m) which ranged between 2.1 and 9.7  $\mu\text{g NH}_4^+\text{-N.g}^{-1} \text{ dw.d}^{-1}$ . These rates decreased with incubation time in both layers and were higher in the 0–2 cm layer. The organic N content ( $P_{\text{org}}$ ) of both layers was constant during the incubation. The isotopic excesses ( $E_{\text{org}}$ ) increased with time in both layers, contrasting with the decrease in the inorganic pool (E). This accumulation of  $^{15}\text{N}$  in the organic pool indicated  $\text{NH}_4^+$  incorporation in living cells.  $\text{NH}_4^+$  incorporation rates (i) ranged between 1.3 and 5.4  $\mu\text{g N.g}^{-1} \text{ dw.d}^{-1}$ . The influence of incubation time depended on the analysed stratum: in the 0–2 cm layer, rates remained constant up to 48 h of incubation and then decreased afterwards, whereas no clear trend was observed in the 2–4 cm layer.

The experimental procedure involved incubation under anoxic atmosphere although surface sediment was oxidized. But the penetration of  $\text{O}_2$  was small, about 2 mm depth (Laima et al., 1999), and influenced only slightly the rates.

The general decrease or instability of ammonification and  $\text{NH}_4^+$  incorporation rates after 24 h of incuba-

tion in a closed environment (*table II*), is probably due to the decreasing intensity of biological processes. This implies a depletion of available substrate with time because of its use by microbial communities. This suggests that the rates measured with incubations exceeding 24 h no longer reflect the *in situ* dynamics. Therefore, incubation time must be short to minimize the differences between laboratory and *in situ* conditions (Bowden, 1984), and furthermore to avoid  $^{15}\text{N}$  remineralization (Blackburn, 1993).

Comparing the *m* and *i* rates obtained in this study with other studies, our results lie in the same range as Blackburn (1979) and Blackburn and Henriksen (1983) but are somehow lower than in other reports (*table III*). Most of these sites are subtidal, thus differing in parameters such as temperature, sediment type and biotic communities. In the Mokbaai Bay, which is an intertidal sandy site with similar temperatures (Goeyens et al., 1987), the *m* values were much higher than those calculated in the Marennes-Oléron Bay.

In Marennes-Oléron intertidal sediments, ammonification and  $\text{NH}_4^+$  incorporation rates are higher in the 0–2 cm layer than in the 2–4 cm layer, as often reported by others (Blackburn, 1979; Iizumi et al., 1982; Sumi and Koike, 1990; Hansen and Blackburn, 1991). This is attributed to higher availability of fresh organic matter in surface sediments (Bowden, 1984).

Labile organic N, which is the first mineralized organic matter fraction, can be estimated from measurements of the labile protein N. Garet (1996) found on the Marennes-Oléron mudflat that protein N represented, on average, 32 % of the particulate N content in the surficial sediment. Furthermore, it was found in an intertidal mudflat in the USA that only 25 % of protein N was degradable by bacterial enzymatic activity at the sediment–water interface (Mayer and Rice, 1992). With a total N content of 1442 mmol N per  $\text{m}^{-2}$  of sediment wet weight (mmol  $\text{N}\cdot\text{m}^{-2}$  ww) in the surficial sediment, we estimated the labile protein N content to be about 115 mmol  $\text{N}\cdot\text{m}^{-2}$  ww in May 1997. In our experiments, ammonification rates (0–24 h incubations) reached 9.5 mmol  $\text{NH}_4^+$   $\cdot\text{m}^{-2}$   $\text{ww}\cdot\text{d}^{-1}$  in the 0–2 cm layer at 22 °C. Assuming that all the labile protein N has been ammonified, we estimated that the time necessary to mineralize the labile protein N fraction in the surficial sediment of the Marennes-Oléron Bay is 12 days at 22 °C.

### 3.2. Temperature effects on the gross ammonification and $\text{NH}_4^+$ incorporation rates

Taking into account the results of the first experiment, incubations were limited to 24 h in the second experiment.

The rates of ammonification and  $\text{NH}_4^+$  incorporation obtained at 10, 20 and 30 °C are shown in *figure 2*. There was a major effect of temperature on both rates, which strongly increased from 10 to 30 °C. Although lower rates were obtained in the 2–4 cm layer compared to the 0–2 cm layer, rates increased in both layers. A one-way ANOVA was performed to test whether the mean ammonification and  $\text{NH}_4^+$  incorporation rates differed between temperatures and a multiple comparisons Tukey test showed that the rates at 30 °C were significantly different from those obtained at 10 and 20 °C ( $P < 0.05$ ) (*figure 2*). However, in the 2–4 cm layer, the  $\text{NH}_4^+$  incorporation rate at 30 °C was significantly different only from the rate obtained at 10 °C ( $P < 0.05$ ).

A higher increase of the ammonification rates was observed between 20 and 30 °C than between 10 and 20 °C. The corresponding  $Q_{10}$  values between these temperature ranges (10–20 °C and 20–30 °C) were 1.7 and 3.0 in the 0–2 cm layer and 2.3 and 4.0 in the 2–4 cm layer. The  $Q_{10}$  values for  $\text{NH}_4^+$  incorporation were constant (around 2.5) in the 0–2 cm layer from 10 to 30 °C and increased in the 2–4 cm layer (1.7 between 10 and 20 °C; 2.7 between 20 and 30 °C).

$Q_{10}$  values for the ammonification lie in the range values reported by Bowden (1984) in surficial coastal sediments ( $Q_{10} = 2.6$ ) and by Sumi and Koike (1990) ( $Q_{10} = 2.2$ ). Processes were stimulated between 20–30 °C compared to 10–20 °C, thus reflecting a more intense activity of microbial communities at higher temperatures. An exception was noticed for  $\text{NH}_4^+$  incorporation process in the 0–2 cm layer, where microbial communities were similarly active between 10–20 °C as between 20–30 °C. This could be due to the microalgae activity in the surface sediment, which is already high between 10 and 20 °C (Blanchard et al., 1997). As shown by their high  $Q_{10}$ , ammonification rates seem to be more sensitive to temperature than  $\text{NH}_4^+$  incorporation rates at high temperatures. This could be explained by a different adaptation to temperature of microbial communities involved in each process.

**Table III.** Comparison with literature of *m* and *i* values obtained in the present paper, using the  $^{15}\text{N}$  isotope method.

Location	Sediment type	Sections (cm)	Ammonification rates (m)	$\text{NH}_4^+$ incorporation rates (i)	Temperature in situ ( $^{\circ}\text{C}$ )	References
Denmark	Coastal marine	0–2 and 2–4	103–367 <sup>a</sup>	22–120 <sup>a</sup>	17	Blackburn, 1979
Alaska (USA)	Eelgrass beds	0–3	46–150 <sup>b</sup>	38–77 <sup>b</sup>	11–17	Iizumi et al., 1982
Japan		0–1	24–35 <sup>b</sup>	11–21 <sup>b</sup>	20–22	
Denmark	Coastal	0–2 and 2–4	24–152 <sup>a</sup>	18–84 <sup>a</sup>	3.5–10	Blackburn and Henriksen, 1983
The Netherlands	Intertidal marine	0–2	1170 <sup>a</sup>	–	20	Goeyens et al. 1987
Japan	Coastal and Estuarine	0–1	5.8–220 <sup>b</sup>	2.5–110 <sup>b</sup>	6–24	Sumi and Koike, 1990
Jamaica	Seagrass beds	0–4 and 4–8	204 <sup>a</sup>	13 <sup>a</sup>	29–31	Blackburn et al., 1994*
France	Intertidal Mudflat	0–2 and 2–4	111–475 <sup>a</sup> 3–14 <sup>b</sup>	69–288 <sup>a</sup> 2–8 <sup>b</sup>	22	Present paper**

<sup>a</sup>  $\text{nmol}\cdot\text{cm}^{-3}$  wet sediment. $\text{d}^{-1}$ .

<sup>b</sup>  $\text{nmol}\cdot\text{g}^{-1}$  wet sediment. $\text{h}^{-1}$ .

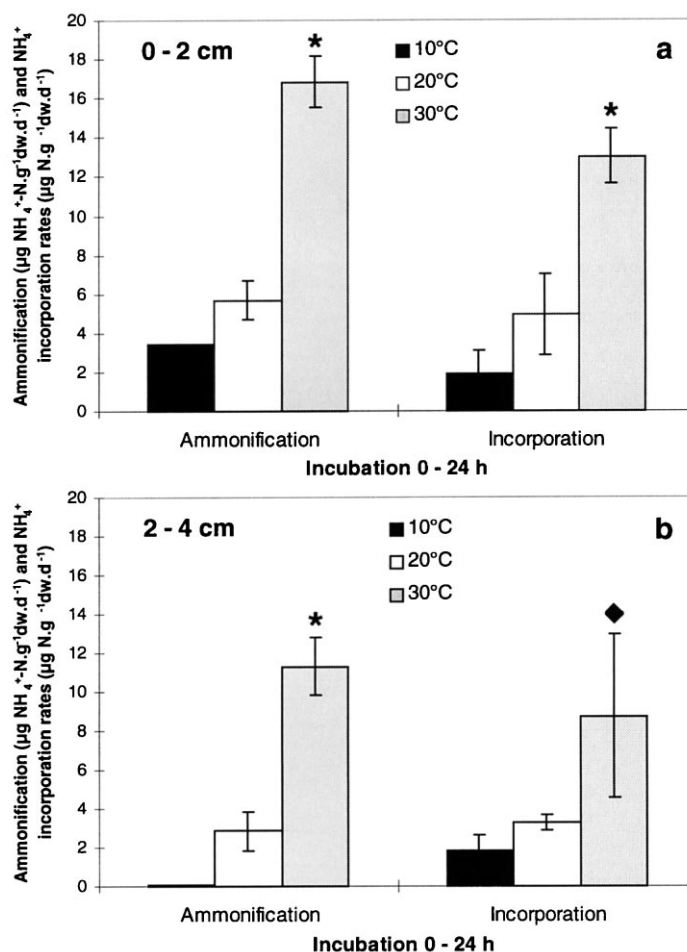
\* Recalculated rates.

\*\* Rates are expressed in the 2 different units for comparison purposes.

Another way to assess the temperature effect on both rates is to calculate the *i:m* ratio, which indicates the fraction of produced  $\text{NH}_4^+$  that is assimilated by the sediment microorganisms (table IV). In the 0–2 cm layer, the highest ratio (0.87) indicates that almost all regenerated  $\text{NH}_4^+$  has been incorporated by microorganisms. This incorporation is higher than that reported by Sumi and Koike (1990) for surficial coastal sediments, where about 50 % of the regenerated  $\text{NH}_4^+$  was assimilated by benthic microorganisms. In our experiments, the highest *i:m* ratio was obtained at 20  $^{\circ}\text{C}$ . This variation of the *i:m* ratio could be explained by differences in the activities of the microbial communities in the sediment. For example, benthic microalgae activity was maximum at around 25  $^{\circ}\text{C}$  on this mudflat and decreased above this temperature due to thermo-inhibition effects (Blanchard et al., 1997). Bacterial populations involved in the  $\text{NH}_4^+$  turnover could also have such maximal activities at different temperatures, leading to variations in the *i:m* ratio. In the 2–4 cm layer, the *i:m* ratios showed that  $\text{NH}_4^+$  incorporation was much higher than  $\text{NH}_4^+$  production at 10 and 20  $^{\circ}\text{C}$ . This was due to a weakness of the ammonification process at these temperatures, as indicated by the low rates (figure 2). This was also observed in deeper layers in Danish sediments (Blackburn, 1980).

In surficial sediments where the microorganism activity is intense, figure 2 shows that produced  $\text{NH}_4^+$  from ammonification is always available in excess compared to microorganism consumption between 10 and 30  $^{\circ}\text{C}$ . This clearly demonstrates that there is no limitation for benthic microorganisms concerning  $\text{NH}_4^+$ . Since this form of inorganic nitrogen is preferentially assimilated by microalgae (Admiraal et al., 1987; Dortch, 1990), this thus suggests that benthic microalgae are not N-limited in our study site, in the sediment temperature range.

The excess of produced  $\text{NH}_4^+$  can be estimated by calculation of net ammonification rates defined as the difference between gross  $\text{NH}_4^+$  production and  $\text{NH}_4^+$  incorporation rates (*m* – *i*). This net ammonification represents the part of  $\text{NH}_4^+$  which can diffuse to overlying water by flushing during immersion period (Rocha, 1998) and can become available for phytoplankton (Blackburn and Henriksen, 1983). The net ammonification rates vary from 1.5 to 3.8  $\mu\text{g NH}_4^+\text{-N}\cdot\text{g}^{-1}$  dw. $\text{d}^{-1}$  for 10 and 30  $^{\circ}\text{C}$ , respectively, and show the lowest value 0.8  $\mu\text{g NH}_4^+\text{-N}\cdot\text{g}^{-1}$  dw. $\text{d}^{-1}$  at 20  $^{\circ}\text{C}$ . As previously for *i:m* ratio, this low value at 20  $^{\circ}\text{C}$  could be due to high  $\text{NH}_4^+$  incorporation by benthic microalgae which have the maximal activity around 25  $^{\circ}\text{C}$  (Blanchard et al., 1997). This net ammonification represents 44, 14 and 23 % of produced



**Figure 2.** Comparison of gross ammonification and  $\text{NH}_4^+$  incorporation rates obtained at 10, 20 and 30 °C during a 0–24 h incubation period. Experimentations were made in June 1997 in the 0–2 cm (a) and 2–4 cm (b) layers. Values are means of three replicates  $\pm$  standard deviation, except for rates at 10 °C for ammonification, where standard deviation could not be calculated. Results of ANOVA show that rates at 10 and 20 °C are never significantly different. The symbol \* indicates that the rates at 30 °C are significantly different from those at 10 and 20 °C ( $P < 0.05$ ), while the symbol ◆ shows that rate at 30 °C is significantly different only from that at 10 °C ( $P < 0.05$ ).

$\text{NH}_4^+$  at 10, 20 and 30 °C, respectively. We can conclude that, in addition to benthic microalgae, phytoplankton potentially benefits of an important part, up to 44 %, of produced  $\text{NH}_4^+$  from mineralization of organic matter in surface sediments of this bay.

The turnover time of  $\text{NH}_4^+$ , defined as the ratio of average  $\text{NH}_4^+$  pool to gross  $\text{NH}_4^+$  production rates, was estimated in surficial sediments. It is inversely related to temperature: 15 days at 10 °C, 9 days at 20 °C and 3 days at 30 °C. These values are similar to Bowden (Bowden, 1984), who calculated an  $\text{NH}_4^+$  residence time of about 1–2 weeks at 13 °C in surface marsh sediments. These results showed that the sedi-

ment temperature strongly influences the  $\text{NH}_4^+$  turnover, which becomes shorter at higher temperatures. This fast return of  $\text{NH}_4^+$  back to the environment is essential during spring and summer, because  $\text{NH}_4^+$  rapidly becomes available to primary producers.

**Table IV.** Variations with sediment temperature of the ratio between  $\text{NH}_4^+$  incorporation rates and gross ammonification rates (*i:m*).

( <i>i:m</i> ) ratio	10 °C	20 °C	30 °C
0–2 cm	0.57	0.87	0.77
2–4 cm	18.77	1.15	0.77



#### 4. CONCLUSION

Our results show that  $\text{NH}_4^+$  production and incorporation rates greatly depend on sediment temperature. Hence, the emersion period should be considered for the  $\text{NH}_4^+$  turnover studies in intertidal sediments where the variations of sediment temperature are high. However, a more detailed relationship between rates and temperature could be gained by shortening the temperature interval between measurements and increasing the temperature range.

This paper highlights that benthic regeneration of  $\text{NH}_4^+$  is significant in intertidal sediments of Marennes-Oléron bay as source of nutrient for primary production. In the range of field sediment temperature, this regeneration provides all the  $\text{NH}_4^+$  necessary to benthic microalgae in surface sediment and supports also a part of the phytoplankton production in overlying water.

Since the quality of organic matter inputs is highly variable on the Marennes-Oléron intertidal mudflat (Galois et al., 1996), a seasonal study should show the influence of the nature of the organic matter on the  $\text{NH}_4^+$  turnover. Future work should also include the development of a benthic nutrient model taking into account these short-term temperature fluctuations in the ecosystem functioning.

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