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Effects of algal sedimentation and *Monoporeia affinis* on nutrient fluxes, pore water profiles and denitrification in sediment microcosms

Liisa Autio*, Kalervo Mäkelä, Kari K. Lehtonen, Jorma Kuparinen and Hannu Haahti

Finnish Institute of Marine Research, P.O. Box 33, FIN-00931 Helsinki, Finland

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A flow-through experiment was conducted to study the effects of a simulated algal sedimentation (8.0 g C m^{-2}) and the amphipod *Monoporeia affinis* (5000 ind. m^{-2}) using well-oxidised sediment from the Gulf of Bothnia. Pore water nutrient profiles, nutrient fluxes, sediment denitrification and O_2 consumption as well as NH_4^+ excretion of *M. affinis* were followed for 7 days. After the algal enrichment, the NO_3^- concentration fell to near zero in the pore water and a decrease in the denitrification rate was observed. This occurred since the concentration of NO_3^- in the flow-through water was low and coupled nitrification-denitrification formed the main denitrification pathway. The share of denitrification compared to other loss processes of N from the sediment reduced from 37% in the control cores to 0.7% in the algae treated cores. The efflux of NH_4^+ from the sediment formed 99% of the total loss of N in the algae treated cores. A massive efflux of NH_4^+ and PO_4^{3-} was observed after the algal enrichment. The amphipods did not increase denitrification.

Introduction

A typical feature of the northern Baltic Sea is the distinct sedimentation maximum in late spring after the spring phytoplankton bloom (e.g. Leppänen 1988, Kankaanpää *et al.* 1997, Lehtonen and Andersin 1998). Other typical factors in the Baltic Sea affecting the O_2 status of the sediment are the irregular inflows of saline O_2 rich water from the North Sea. These inflows aerate the deep sediments, whereas stagnation periods lead to sediment anoxia (Wulff *et al.* 1990) and subsequent disappearance of benthic animals (Andersin *et al.* 1978, Laine *et al.* 1997). This affects the capacity of denitrification by sediment bacteria, a process which is beneficial for the Baltic Sea as it removes N from the aquatic ecosystem. The heavy spring sedimentation of algae

* Current address: City of Helsinki, Environment Centre, P.O. Box 500, FIN-00099 Helsinki, Finland

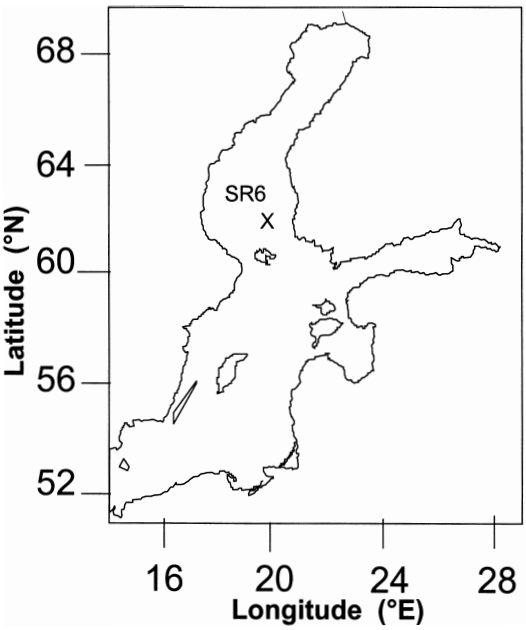

Fig. 1. Map showing the sampling station SR6 in the Gulf of Bothnia, the Baltic Sea.

and the subsequent increased consumption of O_2 by the sediment would, as such, be beneficial for benthic denitrification (the reduction of NO_3^- to N_2). However, nitrification (the oxidation of NH_4^+ to NO_3^-) may be suppressed by limiting O_2 conditions, leading to restrained denitrification due to the shortage of NO_3^-. In addition, the activity of bottom-dwelling animals has been found to be crucial for intensive denitrification (e.g. Gran and Pitkänen 1999).

Sediment microcosms have widely been used to study the effects of algal additions and of benthic animals on nutrient fluxes and biogeochemical processes in surface sediments (Kristensen 1988, Nowicki and Oviatt 1990, Andersen and Jensen 1992, van Raaphorst *et al*. 1992, Caffrey *et al*. 1993, Enoksson 1993, Jönsson *et al*. 1993, Hansen *et al*. 1998, Törnblom and Rydin 1998, Svensson *et al*. 2001). In the Baltic Sea (excluding Kattegat), the only microcosm experiments treating nutrient turnover at the sediment surface have been conducted using sediment from near the Landsort Deep at the depth of 82–85 m (Conley and Johnstone 1995, Stockenberg 1998) and from the Gulf of Finland at the depth of 75 m (Tuominen *et al*. 1999). The biogeochemical response in nutrient cycling triggered by the intensity and quality of sedimentation and shifts in the abundance and activity of benthic fauna strongly depends on the characteristics of the sediment (e.g. van Raaphorst *et al*. 1992). Therefore, more studies using different types of sediments, e.g. sediments with different oxidation status, concentration of organic matter and nutrients, and grain size are needed.

In the present mesocosm study, the effects of algal additions and of *Monoporeia affinis* on denitrification, pore water nutrient profiles, and nutrient fluxes between sediment and water were examined. We used sediment from the Gulf of Bothnia (Fig. 1), a mesotrophic area in the northern Baltic Sea characterised by a deep (2–3 cm) oxidised layer in the sediment. In this area, the sediment surface is constantly well oxidised. Near-bottom salinity has fluctuated between 6.2–6.6 in the past ten years (data from Finnish Institute of Marine Research). Details of the chemical and the biological characteristics of the area are found in a report of The Gulf of Bothnia Year 1991 (Anon. 1996). The dominant macrobenthic species in the area is the deposit-feeding, night-active amphipod *Monoporeia affinis* Lindström (Andersin *et al*. 1978, Elmgren 1978, Andersin *et al*. 1984, Laine *et al*. 1997).

The results of the present experiment are compared with results obtained from the sediment from the Gulf of Finland (Tuominen *et al*. 1999), where irregular fluctuations between oxic and anoxic periods occur and conditions are more eutrophic. In the Gulf of Bothnia, the O_2 concentration in the near-bottom water is much higher and the organic matter loading to the sediment much lower than in the Gulf of Finland (Kankaanpää *et al*. 1997, Laine *et al*. 1997). Consequently, in the Gulf of Bothnia sediment the oxidised layer is deeper and the pore water concentrations of NH_4^+ and PO_4^{3-} are ten times lower than in the Gulf of Finland (K. Mäkelä and L. Tuominen unpubl.). We hypothesized that these differences affect the responses observed after the addition of algae and *M. affinis*.

Material and methods

Sampling and experimental set-up

The sediment used in the experiment was collected aboard r/v *Aranda* in April 1998 at station SR6 in the Gulf of Bothnia, the Baltic Sea (61°03´N, 20°16´E; depth 100 m) (Fig. 1), with a van Veen grab. The brown-colored top layer of the sediment (2–3 cm) was scraped into one tub and the deeper, grey-colored clay layer into another tub. Sediments from several dozens of samples from the same site were combined. The surface sediment was passed through a 0.5-mm sieve to remove macrobenthic animals. The bottoms of the experimental cores (plastic, ∅ 10 cm, height 25 cm) were sealed with red earthenware clay (purchased in an artists' shop), and the cores were then filled with the deeper clay and the surface sediment collected (Fig. 2, details in Tuominen *et al*. 1999). The cores were stored filled with water uncapped in the dark at 4 °C. The water used in the experiments was collected from the same area and passed through a 0.2-μm filter to remove particles.

Specimens of the amphipod *Monoporeia affinis* were collected from the nearby station SR7 (depth 78 m) using a van Veen grab, and the amphipods were sieved gently upon a 0.5-mm mesh. The amphipods were rapidly transferred to the ship's cold room (4 °C) where they were kept in the dark in plastic aquaria containing natural surface sediment and water from the sampling site. The algal material was collected during the same cruise east of the Öland Island and at the Bornholm Deep (central-southern Baltic Sea) by towing with a 20-μm plankton net, mixed, and stored frozen at –20 °C. The sample was dominated by cyanobacteria *Nodularia spumigena* and *Aphanizomenon flos-aquae* as well as dinophyta *Dinophysis acuminata* and *Peridiniella catenata*.

The experiment was performed in May 1998, about two weeks after the collection of the sediment, amphipods and algae. The experimental set-up consisted of a flow-through system with 62 cores held at 4 °C in the dark. The flow-through water was divided into four tubs, one for each treatment. Water flow was regulated by multi-channel peristaltic pumps set at 5 ml min^{-1}, corresponding to a 2-h time for the complete exchange of water in the cores (tested with $KMnO_4$ dyed water, Tuominen *et al*. 1999). Water from the cores flowed passively through a hole (∅ 0.5 cm) in the lid into a plastic basin placed under the cores that belonged to the same treatment; from each basin the water returned back into the corresponding flow-through water tub. The flow was kept running for seven days before the experiment started. This time was adequate for the build-up of pore water nutrient profiles resembling those occurring naturally in the area (Fig. 3). The pore water nutrient profiles were not statistically analysed since the procedure allowed only one core squeezed per treatment. However, since the profiles were formed from several data points (profile depths) individual points possibly differing from the general trend can be discerned by eye.

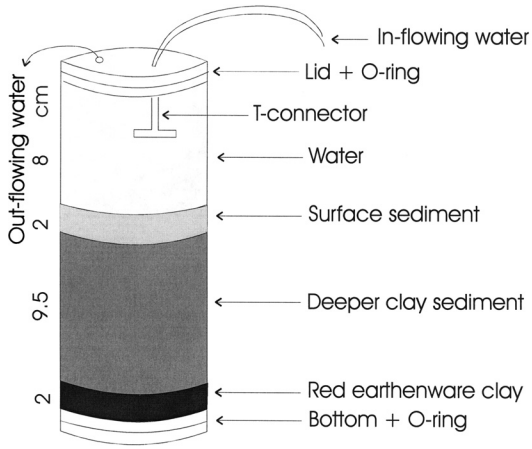

Fig. 2. The experimental core showing different layers of sediment. From the bottom: red earthenware clay to seal the bottom of the core, then deeper and surface sediment collected from the sampling station.

The cores were manipulated by additions of algae (A), *M. affinis* (B) and algae + *M. affinis* (AB). Unmanipulated cores served as controls (C). The algae (80 ml thawed algal suspension corresponding to 48 g dry mass m^{-2}, 29 mg chl *a* m^{-2}, 8.0 g C m^{-2}, and 1.7 g N m^{-2}) were added after removing an equal volume of water, and allowed to settle for 4–5 hours before the cores were closed

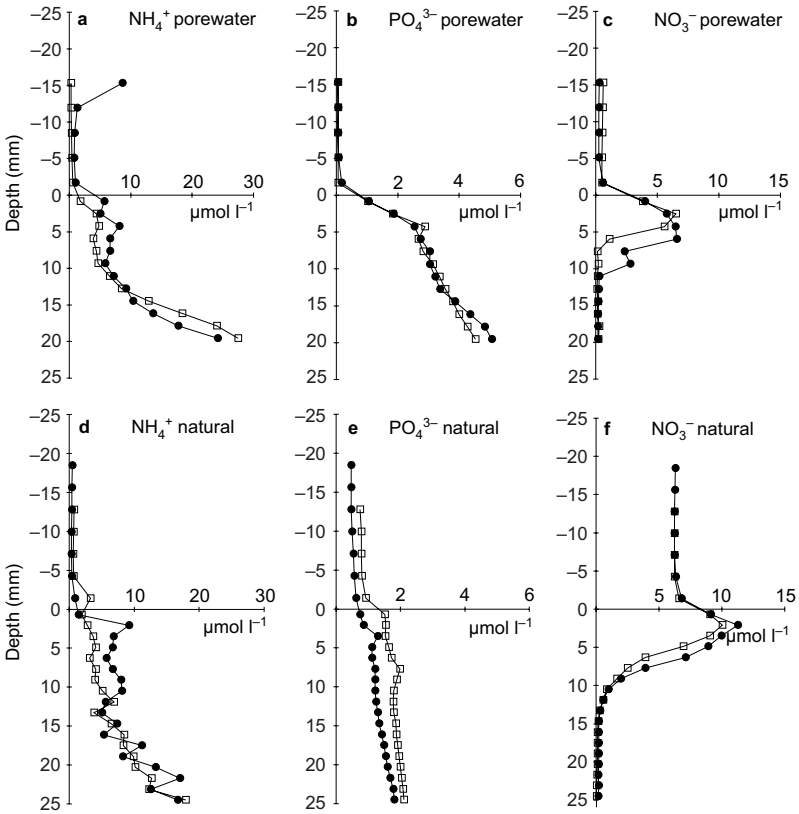

Fig. 3. Two replicate pore water profiles of (**a**) NH_4^+, (**b**) PO_4^{3-} and (**c**) NO_3^- on day 0 before algae and/or amphipods were added in comparison with natural pore water profiles measured from a nearby station SR5 (depth 125 m) in April 1997 and 1998 (**d**–**f**).

and connected to the flow-through system again. The addition of *M. affinis* (length 7–10 mm) was 39 specimens per core, corresponding to 5000 ind. m^{-2} which is within the natural density range in the Gulf of Bothnia area (Lehtonen 1995).

The experiment lasted for seven days. Of the 62 cores, 6 were used for pore water nutrient profiles, 8 for nutrient fluxes and O_2 consumption, and 48 for denitrification (Table 1). Day 0 measurements were made before algae and/or amphipods were added. Nutrient fluxes were always analysed from the same set of cores during the experiment, but for the rest of the analyses the incubations were terminated on each sampling day.

Table 1. Number of replicate cores and timetable of the different analyses.

Treatments	No. of replicate cores analysed per day																							
	Pore water profiles								Nutrient flux & O_2 consumption								Denitrification							
Day ->	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
A								1	2		2					2	3	3	3					3
B								1	2		2					2	3	3	3					3
AB								1	2		2					2	3	3	3					3
C	2							1	2		2					2	3	3	3					3
Total no. of replicate cores per analysis	6								8[a]								48[b]							

[a] Analyses were made from the same cores on the different days.
[b] Two incubation cores subsamples from each of these cores.

Analyses

Pore water nutrient profiles

High resolution pore water nutrient profiles were obtained by a whole-core squeezer (Bender *et al*. 1987) used in a modified form (Tuominen *et al*. 1999). The sediment core was fixed into a rack with a solid plastic clamp. A required volume of water (ca. 4 cm) was suctioned off the core to give space for the upper piston. The upper piston (∅ 10 cm) was provided with a supporting screen (Nitex 500 μm), a filter (pore size 0.45 μm, Versapor, Gelman Sciences) covering the whole filtering area, and a tube for the water samples. A hydraulic jack (ca. 26 kg cm^{-2}) was used to squeeze the core from underneath against the upper filtering piston. The pressure used was so low that negligible lysing of cellular material was expected from the algae (cf. the lowest tested pressure ca. 50 kg cm^{-2} by Bolliger *et al*. 1992). Each nutrient profile contained 4–5 water subsamples above and usually 12 pore water subsamples under the sediment-water interface. Every sample corresponded to 1.7 mm on the vertical scale.

The pore water samples were stored in the dark at 4 °C and analysed within 3 h of squeezing. NH_4^+ was analysed by the manual method of Koroleff (1983). Other nutrients (NO_3^-, NO_2^-, PO_4^{3-}) were analysed by a Skalar 5100 autoanalyser according to the methods described by Grasshoff *et al*. (1983).

Nutrient flux and O_2 consumption measurements

For the nutrient flux and O_2 consumption measurements the cores were withdrawn from the flow-through system and sealed with air-tight acrylic lids equipped with a magnetic stirrer. Nutrient fluxes were calculated as differences in concentration in the water overlying the sediment after 5-h incubations. Nutrients were analysed as described for pore water. The sediment O_2 uptake was measured as the decrease of O_2 in the water overlying the sediment during a 3-h incubation using a Clark-type oxygen electrode (Radiometer E 5046) mounted in a temperature regulated cuvette (Radiometer D616) and connected to a blood gas analyser (Radiometer PHM 72 Mk 2).

Denitrification

Sediment denitrification was assayed using the isotope pairing method (Nielsen 1992) following the procedure of Tuominen *et al*. (1998). Briefly, two replicate incubation cores (plastic cylinders with a height of 100 mm and ∅ 26 mm) were subsampled from each of the 3 replicate experimental cores. $K^{15}NO_3$ solution (99 atom%, Europa Scientific Ltd) was added to the water phase of the incubation cores to give a final concentration of 100 mM (found to be optimal, Tuominen *et al*. 1999), and the cores were closed with caps equipped with magnetic stirring bars. After a 3-h incubation denitrification was stopped with 1 ml of $ZnCl_2$ (1 g ml^{-1}), the cores were gently mixed using a glass stick, and 10 ml gas-tight exetainers were filled with the slurry. The mass ratios of N_2 formed were analysed from the exetainers using a mass spectrometer at the National Environmental Research Institute in Silkeborg, Denmark. Denitrification was calculated from the ratios of formed $^{29}N_2$ and $^{30}N_2$ as described by Nielsen (1992).

NH_4^+ excretion rate of the amphipods

After the denitrification subsamples were taken on days 2 and 7, all living amphipods were carefully pipetted from the cores and placed in plastic aquaria containing filtered sea water (Whatman GF/C) and a 1.5-cm layer of sand boiled and acid-washed (< 0.4 mm grain size) for 4–6 h. They were then placed individually into acid-washed scintillation vials filled with filtered sea water, incubated for 17 h at 4 °C, and NH_4^+ was analysed from the water. Each measurement series contained 4 control vials with filtered seawater only. The analysis was done also on day 0 for 11 animals left over from the cores. The excretion rate was calculated using an allometric model (Lehtonen 1995, Tuominen *et al*. 1999). O_2 consumption of the amphipods was calculated from the dry weights using the empirical

weight-rate regressions determined for animals from the same area (Lehtonen 1996).

Chlorophyll *a* and C and N concentrations

Chlorophyll *a* and C and N concentrations were analysed from additional surface sediment material before the start of the experiment (duplicate samples), as well as from the algae suspension used in the treatments. At the end of the experiment, the brown surface sediment layer in the nutrient flux cores (two replicates) was discerned by eye, removed from the cores, and analysed. Chl *a* was analysed fluorometrically after ethanol extraction using a method described by HELCOM (visit http://www.helcom.fi/Monas/CombineManual2/PartC/CFrame.htm and choose Annex C-4) and total C and N with a Leco CHN-900 analyser (carbonates not removed).

Statistical analyses

All data sets except the nutrient flux data were tested to have homogenous variances (Cochran's test). Data of the denitrification and nutrient flux measurements were analysed as a 2^3 factorial design using algae, *M. affinis* and time as factors. Data on denitrification were analysed using nested ANOVA with two incubation cores nested with each experimental core. Nutrient flux and O_2 consumption data (two replicates) were analysed using repeated measures ANOVA since the fluxes and O_2 consumption were measured from the same cores on each experiment day. Since no

Table 2. O_2 and nutrient concentrations in the flow-through water (SD). Treatments: C = control, A = algae, B = *M. affinis*, AB = algae and *M. affinis*. na = not analysed.

Day	Treatment	O_2 (μM)	NH_4^+ (μM)	NO_3^- (μM)	NO_2^- (μM)	PO_4^{3-} (μM)
0		328 (12)	0.4 (0.1)	0.07 (0.10)	0.03 (0.05)	0.03 (0.03)
2	C	348 (40)	0.7 (0.1)	0.3 (0.1)	0.1 (0.01)	0.1 (0.00)
2	A	245 (30)	42.4 (1.3)	1.1 (0.1)	0.5 (0.01)	27.5 (0.05)
2	B	350 (4)	0.7 (0.2)	0.7 (0.3)	0.1 (0.00)	0.5 (0.16)
2	AB	223 (9)	299.9 (49.0)	1.4 (0.01)	0.8 (0.01)	21.7 (0.4)
7	C	367 (6)	2.5 (0.4)	1.7 (0.2)	0.3 (0.01)	0.1 (0.01)
7	A	na	427.6 (20.4)	1.8 (0.03)	1.0 (0.03)	29.2 (0.5)
7	B	na	1.7 (0.1)	1.4 (0.05)	0.2 (0.05)	0.04 (0.01)
7	AB	311 (2)	428.6 (1.5)	1.7 (0.04)	1.0 (0.00)	31.3 (0.7)

Table 3. C, N and chl *a* concentration in the surface sediment layer of the cores at the start and the end of the experiment (g m^{-2} and mass-%), C, N and chl *a* addition introduced in the algal enrichment, the percentage of addition compared with the initial concentration of C, N and chl *a* in the surface sediment, and the percentage of the addition found in the surface sediment after 7 days (= increase in the surface sediment concentration in 7 days compared to the addition). Treatments: C = control, A = algae, B = *M. affinis*, AB = algae and *M. affinis*.

Day	Treatment	C		N		Chl *a*	
		g m^{-2}	%	g m^{-2}	%	mg m^{-2}	%
0		129	2.8	16	0.35	143	
7	C	129	2.8	16	0.35	129	
7	A	134	2.9	17	0.37	161	
7	B	129	2.8	16	0.35	129	
7	AB	134	2.9	17.5	0.38	166	
Addition		8	6.2	1.7	10.6	29	20
Found	A		63		59		
Found	AB		63		88		

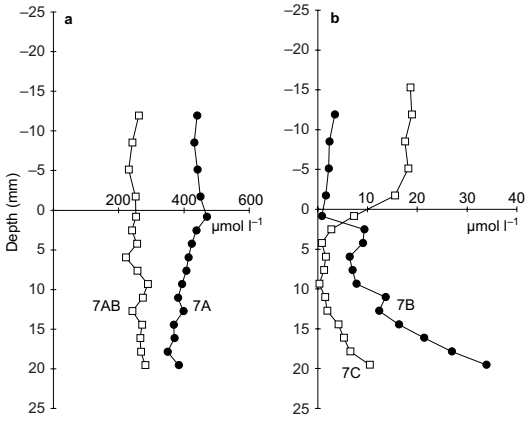

Fig. 4. Pore water profiles of NH_4^+ on day 7 (**a**) in algae enriched cores (A) and algae + *M. affinis* enriched cores (AB), and (**b**) in control cores (C) and *M. affinis* enriched cores (B). Please note different scales.

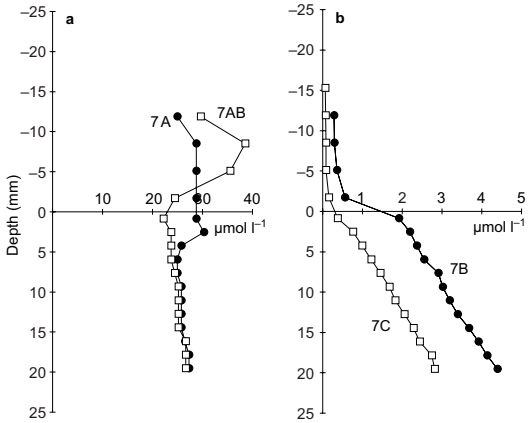

Fig. 5. Pore water profiles of PO_4^{3-} on day 7 (**a**) in algae enriched cores (A) and algae + *M. affinis* enriched cores (AB), and (**b**) in control cores (C) and *M. affinis* enriched cores (B). Please note different scales.

nonparametrical repeated measures ANOVA was available, a parametrical analysis was performed in spite of the unhomogeneity of variances. The error caused by deviations from the assumptions is usually small (Underwood 1997). The data of NH_4^+ excretion by *M. affinis* was analysed using *t*-test. The software product used was SAS version 6.12.

Results

General observations

The water phase remained well-oxidised in all treatments although the O_2 concentration was significantly lower in the A and AB cores compared to C and B (Table 2; ANOVA, $p < 0.01$). In one control core measured on day 7 the water pH was 7.4 and in the treatment AB 8.0 (other treatments not measured). The addition of algae increased the NH_4^+ and PO_4^{3-} concentrations in the flow-through water more than a hundred times (Table 2). The increase in the NH_4^+ concentration corresponded to 40%–70% of the total N content of the added algae. Since the same water was circulating during the experiment (however discrete water for each treatment), on later days the algae enriched cores received water which contained high concentrations of nutrients. The algal addition increased the surface sediment C content by 6.2%, the N content by 10.6%, and the chl *a* content by 20% (Table 3). Large numbers of dead animals (about half of the added number of individuals) were observed in the AB cores contrary to none in the B cores.

Pore water nutrient profiles and fluxes

The replicate nutrient profiles measured on day 0 differed very little (Fig. 3a–c) and resembled the natural profiles previously measured in the area. Due to the high concentrations of NH_4^+ and PO_4^{3-} in the flow-through water in the A and AB cores, the concentrations of these nutrients in the pore water were much higher than in the treatments C and B after 7 days, and they showed almost constant concentrations in the near bottom and the pore water (Figs. 4 and 5). The NO_3^- concentration approached zero in the pore water of the A and AB cores while in the B and C cores a NO_3^- peak was observed at the depth of a few millimeters (Fig. 6).

In the treatment B on day 7, the profiles of NH_4^+, PO_4^{3-} and NO_3^- resembled considerably the profiles at the start of the experiment (Figs. 4b, 5b and 6b). In the control the NH_4^+ and PO_4^{3-} concentrations in the pore water decreased and the peak of NO_3^- grew during the course of the experiment compared to the situation on day 0 (Figs. 4b, 5b and 6b). The NO_2^- concentration

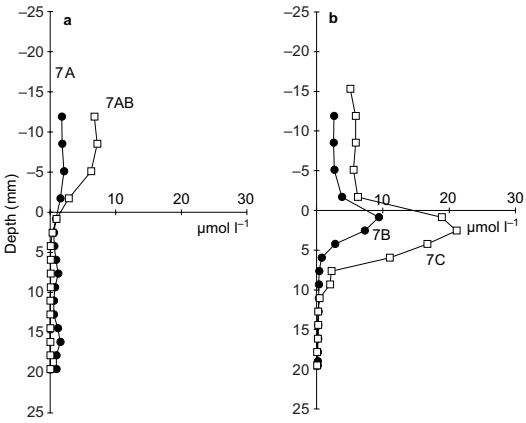

Fig. 6. Pore water profiles of NO_3^- on day 7 (**a**) in algae enriched cores (A) and algae + *M. affinis* enriched cores (AB), and (**b**) in control cores (C) and *M. affinis* enriched cores (B).

was very low in all cores with a maximum of 0.4 μM.

On day 2 (after the algal addition), the NH_4^+ fluxes differed markedly between the two algal treatments: in the A cores an extremely large efflux was observed while in the AB cores the flux was directed into the sediment (Fig. 7a; $p = 0.0355$ for the effect of A × B on day 2; Appendix). By day 7, both the A and AB cores showed a moderate efflux of NH_4^+.

In treatment A contrary to the other cores, the flux of NO_3^- was directed into the sediment (Fig. 7b; $p = 0.0007$ for the effect of A on day 7; Appendix). Treatment B had increased an efflux of NO_3^- by day 7 ($p = 0.0006$; Appendix). By day 2, a very high efflux of NO_2^- was observed in the treatment AB (Fig. 7c; $p = 0.0001$ for A × B; Appendix), while by day 7 an increased influx of NO_2^- was observed in treatments A and AB (Fig. 7c; $p = 0.0013$ for A; Appendix). The efflux of PO_4^{3-} was very high in the A cores on day 2 (Fig. 7d; $p = 0.009\ 4$ for A; Appendix), but on day 7 the fluxes in both the A and the AB cores were directed into the sediment ($p = 0.0001$ for A; Appendix).

NH_4^+ excretion of *M. affinis*

By day 2 the NH_4^+ excretion rate of the amphipods in the algae-treated cores (AB: 0.080 ± 0.052 [SD] μmol NH_4^+ d^{-1} per "standard" 2-mg individual) was significantly elevated in comparison with the initial values on day 0 (0.059 ± 0.048 [SD] μmol NH_4^+ d^{-1}; *t*-test $p < 0.01$, $n = 23$) and with cores without the algal addition on day 2 (B: 0.026 ± 0.020 [SD] μmol NH_4^+ d^{-1}; *t*-test $p < 0.01$, $n = 18$). Using these excretion rate measurements and the number of individuals in each core, the amphipods were calculated to release 128 and 397 μmol NH_4^+ m^{-2} d^{-1} on day 2 in treatments B and AB, respectively.

Denitrification

The total denitrification rate decreased in the algae enriched cores (Fig. 8). The effect was statistically significant for denitrification based on $^{14}NO_3^-$ produced in the sediment by nitrification (Dn; $p = 0.04$ for the effect of A; Appendix). On the contrary, the proportion of denitrification based on the water column NO_3^- increased in the A, B and AB cores (dw; Fig. 8, Appendix). In the B cores the proportion of dw was yet minimal all the time. The total denitrification rate in the C and the B cores did not differ from each other or from the start of the experiment (Fig. 8).

O_2 consumption

Sediment O_2 consumption increased in the algae enriched cores on both days 2 and 7 (only the results of C and AB cores available on day 7 due to technical reasons) (Fig. 9; $p = 0.0374$ and $p = 0.0180$, respectively). The O_2 consumption by *M. affinis* was calculated to be 5.4 mmol O_2 m^{-2} d^{-1} in the treatment B (42% of total consumption on day 2), and 7.0 and 3.6 mmol O_2 m^{-2} d^{-1} (12%–13% of total consumption) in the treatment AB on days 2 and 7, respectively (half of the animals were assumed to be alive in the AB cores on day 7).

Discussion

To get homogenous microcosm samples sieving and homogenising the sediment is needed.

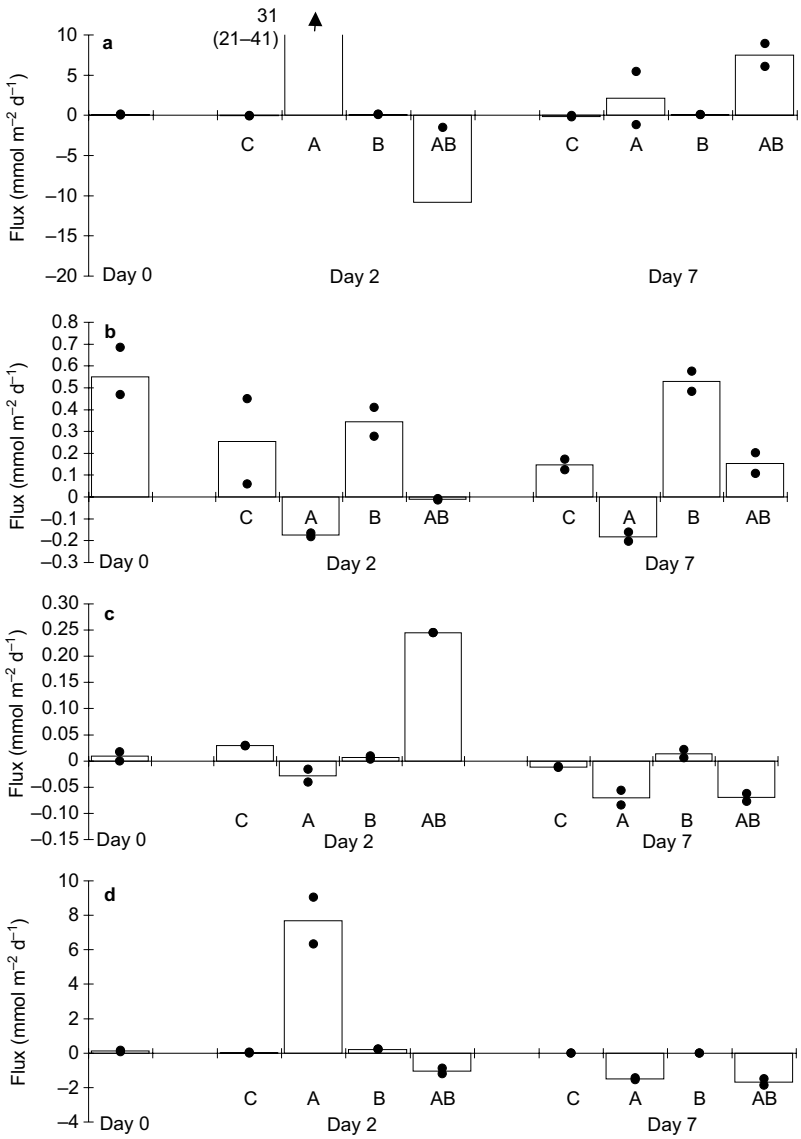

Fig. 7. Fluxes of (**a**) NH_4^+, (**b**) NO_3^-, (**c**) NO_2^-, and (**d**) PO_4^{3-} between sediment and water in control (C), algae enriched (A), *M. affinis* enriched (B), and algae + *M. affinis* enriched cores (AB). Positive fluxes are out of sediment. The dots denote values of two replicates, except on day 0 where they indicate the largest and the smallest of 6 replicates. Where only one dot is visible the replicates are near each other.

We found that after seven days pre-incubation the pore water nutrient profiles resembled the profiles found in the study area *in situ*. Svensson *et al*. (2001) found, as well, that denitrification and nitrification were not affected by sieving and homogenisation. Therefore we believe that the results from the mesocosm experiment can be extrapolated to nature. However, it must be kept in mind that the present experiment mainly studied the immediate mechanisms affecting the nutrient cycling after a disturbance. The observed effects may smoothen with time and the long-term effects may be different.

Effects of *M. affinis*

The pore water profiles of NH_4^+, NO_3^- and PO_4^{3-} in the *M. affinis* treated cores (without the algal addition) resembled the profiles measured at the start of the experiment. On the contrary, in the control cores the NH_4^+ and PO_4^{3-} concentrations in the pore water decreased and the NO_3^- concentration increased during the experiment. However, no statistical analysis could be performed for the pore water profiles as only one profile was possible to obtain per day and treatment. Similarly, the NH_4^+ and NO_3^- fluxes in the *M.*

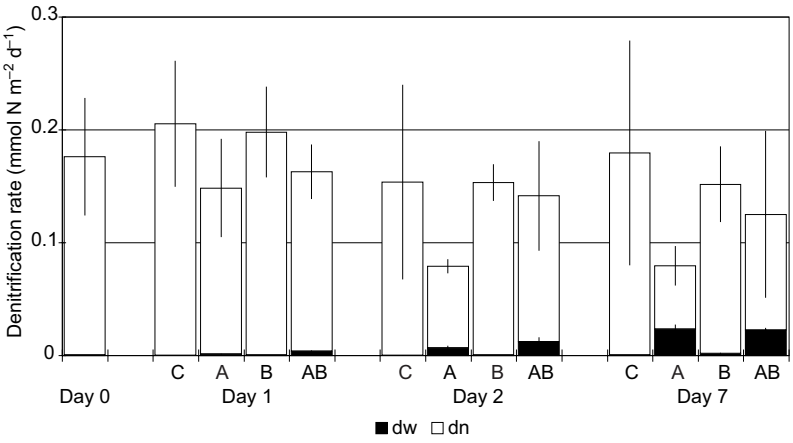

Fig. 8. Denitrification rate in the sediment cores (mean ± SD, n = 6). Dw = denitrification based on $^{14}NO_3^-$ from the water; Dn = denitrification based on $^{14}NO_3^-$ produced in the sediment by nitrification. Total denitrification = Dw + Dn. Treatments: C = control, A = algae, B = *M. affinis*, AB = algae + *M. affinis*.

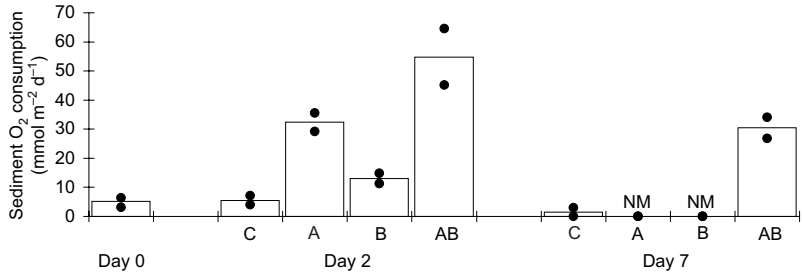

Fig. 9. Sediment O_2 consumption in control (C), algae enriched (A), *M. affinis* enriched (B), and algae + *M. affinis* enriched cores (AB). The dots denote values of two replicates, except on day 0 where they indicate the largest and the smallest of 6 replicates. NM = not measured.

affinis treated cores resembled more the fluxes at the start than the control cores. It appeared that the amphipod treated cores resembled most the natural situation since the sediment in the Gulf of Bothnia is naturally dominated by a dense amphipod population (Lehtonen 1995).

The bioturbating activity of the amphipods in the algae + *M. affinis* treated cores decreased the high concentration of NH_4^+ in the pore water observed after the algal enrichment. Similar decreased pore water NH_4^+ concentrations or increased effluxes of NH_4^+ by benthic animal activity have been observed in many studies (Blackburn and Henriksen 1983, Pelegrí and Blackburn 1994, 1995, Svensson 1997, Hansen *et al*. 1998), although an increase in NH_4^+ concentration in pore water has also been presented (Jönsson *et al*. 1993).

The presence of the amphipods did not increase the total denitrification as compared with the control cores. This agrees with the results obtained in our previous experiment (Tuominen *et al*. 1999) but not with most earlier studies (Pelegrí and Blackburn 1994, Pelegrí *et al*. 1994, Pelegrí and Blackburn 1995, Rysgaard *et al*. 1995, Svensson 1997, Gilbert *et al*. 1998, Svensson 1998, Gran and Pitkänen 1999). It must be noted that in some of these studies (Svensson 1997, 1998) only the denitrification based on NO_3^- originating from the water was increased due to the increased flushing of NO_3^- rich water through the animal burrows. This could not happen in our study since the NO_3^- concentration in the water was naturally very low (Table 2). Theoretically, a stimulation in denitrification is achieved by increased oxygenation of the sediment and/or increased availability of NH_4^+ by animal excretion which both should enhance nitrification (production of NO_3^-), the usually rate-limiting step in denitrification. The excretion rates were, however, quite low in the present experiment as observed also

earlier for *M. affinis* (Lehtonen 1996). Pelegrí and Blackburn (1994) obtained total excretion of 749 μmol NH_4^+ m^{-2} d^{-1} for *Corophium volutator* in a density of 6000 ind. m^{-2}, 2–6 times higher than recorded by us for the *M. affinis* population of 5000 ind. m^{-2}.

About half of the animals in the algae + *M. affinis* enriched cores were found dead on day 7. The O_2 concentration and pH in the flow-through water should not have been too low or high, respectively, for the animals to survive. However, the very large NO_2^- efflux on day 2 showed that anoxic situations may have prevailed in the sediment during the experiment. The NH_4^+ concentration in the pore water was below the lethal values determined for the amphipod *Hyalella azteca* (96-h LC_{50} 1000–2000 μmol l^{-1}, depending on other environmental factors; Ankley *et al.* 1995). We presume that the mortality was due to synergistic effects of an all-round deterioration of the physicochemical environment caused by a large, sudden input of organic matter. It should also be noted that *Nodularia spumigena* introduced to the cores is a potentially toxic cyanobacterium and the toxins may have affected the amphipods. Also, the activity of the remaining population may have been somewhat different than in natural conditions.

The difference in the sediment O_2 consumption between the control cores and the cores containing *M. affinis* on day 2 equalled the calculated respiration by the amphipods. On the contrary, the difference between the algae and algae + *M. affinis* treated cores could only partially be explained by the respiration of the amphipods.

Effects of algal addition

The algae were collected from the Baltic Proper in order to get a sufficient amount of material. This, however, introduced a different species composition (in favour of cyanobacteria) compared to the normal dominant taxa in spring in the Gulf of Bothnia (dinoflagellates and diatoms). However, cyanobacteria are not uncommon in the Gulf of Bothnia and *Nodularia* and *Aphanizomenon* blooms are often observed in late summer (Kononen *et al.* 1993, Piippola and Kononen 1995). The algal addition (48 g dry mass m^{-2}, 8 g C m^{-2} and 1.7 g N m^{-2}) was proportionate to that measured during the spring bloom sedimentation in the area (8.1–13.9 g C m^{-2} and 0.8–1.3 g N m^{-2}, registered for the mid-April–mid-July period; Lehtonen and Andersin 1998). Although not being unrealistically high and being comparable or even lower than the enrichments used in some other experiments (Andersen and Jensen 1992, van Raaphorst *et al.* 1992, Caffrey *et al.* 1993, Enoksson 1993, Conley and Johnstone 1995, Hansen *et al.* 1998, Törnblom and Rydin 1998), the addition caused a 200–400-fold increase in the NH_4^+ and PO_4^- concentrations in the flow-through water (Table 2). Pore water profiles of NH_4^+ and PO_4^- were therefore strongly affected by diffusion from the water column and by day 7 almost linear profiles with very high concentrations in the near-bottom water and the surface sediment were observed.

In our earlier experiment (Tuominen *et al.* 1999), we found a dramatic decrease in the denitrification rate in the Gulf of Finland sediment (from 270 to 9 μmol m^{-2} d^{-1}) following the algal enrichment of only 6 g dry mass m^{-2} (0.34 g C m^{-2}). A decrease in the denitrification rate was observed also in the present experiment although it was not as pronounced (from 180 to 80 μmol m^{-2} d^{-1}; Fig. 8). In both experiments, the pore water NO_3^- concentration approached zero soon after the enrichment indicating that the sediment became too hypoxic for effective nitrification or nitrification was inhibited by the added carbon through competition for NH_4^+ by heterotrophic bacteria (Strauss and Lamberti 2000). Subsequently the coupled nitrification-denitrification decreased. The proportion of denitrification based on NO_3^- diffusing from the water increased slightly which agrees with the influx of NO_3^- observed in the algae enriched cores. A decrease in denitrification after algal enrichment has also been observed by van Raaphorst *et al.* (1992), but denitrification has been shown to increase after the addition of organic matter in conditions where the water column concentration of NO_3^- is high (Caffrey *et al.* 1993). It is interesting to note that the share of denitrification compared to other loss processes of N from the sediment was reduced from 37% in the control cores to 0.7% in the algae treated cores (Table 4) although the absolute value did

not decline as dramatically. In our previous experiment a similar decrease from 15% to 0.6% was observed in the Gulf of Finland sediments (Tuominen *et al*. 1999).

The fluxes of N in the algae-treated cores were dominated by NH_4^+. An especially high release of NH_4^+ as well as PO_4^{3-} was found on day 2 (Fig. 7), obviously caused by the release of NH_4^+ and PO_4^{3-} from the algae lying as a visible layer on the sediment surface. On the contrary, in the algae + *M. affinis* treated cores the amphipods had mixed the algal addition deeper into the sediment. On day 7, an efflux of NH_4^+ was observed in both treatments (Fig. 7) which is in concert with many previous studies using algal additions (Andersen and Jensen 1992, Enoksson 1993, Conley and Johnstone 1995, Hansen *et al*. 1998). Calculated as a share of all the N loss processes occurring in the sediment, the efflux of NH_4^+ formed 99% in the algae treated cores (Table 4).

From the added C, 63% was found in the sediment after 7 days, implying that 37% had been mineralised (Table 3). From the N enrichment, 41% had been mineralised in the cores enriched only with algae and 12% in the algae + *M. affinis* enriched cores. Therefore, the calculated "half-life" was 2.5 wk for C and 2.5–8 wk for N. The value for N agrees with 4 wk recorded by Enoksson (1993) and 2–8 wk by Garber (1984). The average O_2 consumption in the algae and algae + *M. affinis* enriched cores was 40 mmol O_2 m^{-2} d^{-1}. If an RQ of 0.8 is assumed for the whole sediment community, 32 mmol C m^{-2} d^{-1} = 380 mg C m^{-2} d^{-1} was mineralised during the experiment. This agrees with the observed disappearance of the added algal C (420 mg C m^{-2} d^{-1}, i.e. 37% of 8 g C m^{-2} during 7 days). An RQ of 0.8 indicates metabolism based on mixed substrates (carbohydrate, fat and protein). Since *M. affinis* contains large amounts of fat and very little carbohydrate, the RQ is more balanced towards 0.7 than 1.0. Because protein metabolism is also present (NH_4^+ excretion) it is reasonable to assume that 0.8 is close to reality.

Conclusions

Our earlier experiment (Tuominen *et al*. 1999) was conducted using sediment from the Gulf of Finland, while in the present experiment sediment from the Gulf of Bothnia was used. In natural conditions, these sediments differ in the depth of oxidised layer (light brown colour) which in the Gulf of Finland is ca. 0.1–1 cm depending on the hydrographic situation and in the Gulf of Bothnia ca. 2–3 cm. In addition, the sediment surface in the Gulf of Bothnia is constantly oxic while in the Gulf of Finland oxic and anoxic periods vary. The concentrations of chl *a*, C and N were lower in the Gulf of Bothnia sediment than in the Gulf of Finland (chl *a*: 31 and 84 μg g^{-1}; C: 2.8 and 4.0%; and N: 0.35 and 0.54%, in the Gulf of Bothnia and the Gulf of Finland, respectively). These two experiments revealed that while the sediment in the Gulf of Finland was at the border to dump into the "vicious circle" (Kemp *et al*. 1990) where denitrification can no longer remove N from the ecosystem, there seems to be still more margin left in the Gulf of Bothnia as can be seen from the modest decline in denitrification by the added organic matter. An extensive increase in sedimenting organic matter

Table 4. The average loss of N from the sediment porewater during the course of the experiment (μmol N m^{-2} d^{-1}). In parenthesis are the percentages calculated from positive fluxes. Treatments: C = control, A = algae, B = *M. affinis*, AB = algae and *M. affinis*.

	Treatment			
Loss process	C	A	B	AB
Denitrification (N_2)	165 (37)	100 (0.7)	161 (23)	142 (37)
Efflux of NH_4^+	–52	14587 (99)	83 (12)	–1893
Efflux of NO_3^-	270 (61)	–41	452 (64)	156 (41)
Efflux of NO_2^-	10 (2)	–37	10 (1)	83 (22)
Sum	393 (100)	14609 (100)	706 (100)	–1512 (100)

can, however, dramatically change the proportions of denitrification and efflux of NH_4^+ from the sediment in the Gulf of Bothnia. This would have pronounced effects on the N cycling and therefore on ecosystem functioning in the Gulf of Bothnia.

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Appendix

Significant effects found in the repeated measures ANOVA for nutrient fluxes and O_2 consumption and in the nested ANOVA for denitrification results. Dn = denitrification based on $^{14}NO_3^-$ produced in the sediment by nitrification; Dw = denitrification based on $^{14}NO_3^-$ from the water. Treatments: A = algae, B = *M. affinis*. A(core) means the effect of A nested with the sediment core.

		Source	Degrees of freedom	Sum of squares	Mean square	*F*-value	*p*
NH_4^+-flux	Between subjects effects	A	1	154 047 677	154 047 677	8.78	0.0414
		B	1	222 357 950	222 357 950	12.67	0.0236
		A × B	1	232 540 561	232 54 0561	13.25	0.0220
	Within subject effects	time × B	2	679 087 061	339 543 530	8.34	0.0110
		time × A × B	2	680 330 813	340 165 406	8.36	0.0110
	Effects on each exp. day	B on day 2	1	886 146 271	886 146 271	9.59	0.0363
		A × B on day 2	1	899 475 606	899 475 606	9.73	0.0355
NO_3^--flux	Between subjects effects	A	1	289 997	289 997	28.45	0.0059
		B	1	117 819	117 819	11.56	0.0273
	Within subject effects	time	2	938 981	469 490	59.18	0.0001
		time × A	2	278 975	139 487	17.58	0.0012
		time × B	2	179 374	89 687	11.31	0.0047
	Effects on each exp. day	A on day 7	1	248 314	248 314	91.24	0.0007
		B on day 7	1	257 192	257 192	94.50	0.0006
NO_2^--flux	Between subjects effects	B	1	13 245	13 245	89.48	0.0007
		A × B	1	10 590	10 590	71.54	0.0011
	Within subject effects	time	2	38 150	19 075	399.75	0.0001
		time × A	2	26 165	13 082	274.17	0.0001
		time × B	2	18 366	9 183	192.45	0.0001
		time × A × B	2	33 669	16 834	352.79	0.0001
	Effects on each exp. day	A on day 2	1	16 398	16 398	206.79	0.0001
		B on day 2	1	31 261	31 261	394.21	0.0001
		A × B on day 2	1	43 770	43 770	551.96	0.0001
		A on day 7	1	10 127	10 127	64.35	0.0013
PO_4^{3-}-flux	Between subjects effects	B	1	12 593 561	12 593 561	41.22	0.0030
		A × B	1	13 951 168	13 951 168	45.66	0.0025
	Within subject effects	time	2	25 922 880	12 961 440	40.25	0.0001
		time × A	2	23 786 263	11 893 131	36.93	0.0001
		time × B	2	23 768 971	11 884 485	36.90	0.0001
		time × A × B	2	25 850 336	12 925 168	40.13	0.0001
	Effects on each exp. day	A on day 2	1	20 479 467	20 479 467	22.01	0.0094
		B on day 2	1	36 347 629	36 347 629	39.06	0.0033
		A × B on day 2	1	39 782 633	39 782 633	42.75	0.0028
		A on day 7	1	5 066 194	5 066 194	284.00	0.0001
O_2 consumption	Effects on each exp. day	A on day 2	1	1 214 971	1 214 971	25.22	0.0374
		A on day 7	1	419 611	419 611	54.00	0.0180
Dn		model	31	218 333	7 043	1.77	0.0277
		error	62	246 126	3 969		
		A(core)	2	27 205	13 602	3.43	0.0388
		time	3	66 774	22 258	5.61	0.0018
Dw		model	31	5 609	180	54.72	0.0001
		error	62	205	3.3		
		A(core)	2	1 650	825	249.50	0.0001
		B(core)	2	36	18	5.45	0.0066
		time	3	1 943	647	195	0.0001
		A × time(core)	6	1 670	278	84.19	0.0001
		A × B × time(core)	6	47	7.9	2.41	0.0370