NOTES

Limnol. Oceanogr., 44(8), 1999, 1993–1999

An experimental study on effects of submersed macrophytes on nitrification and denitrification in ammonium-rich aquatic systems

Abstract—We have examined the role of microbial communities on the surface of submersed macrophytes and in the underlying sediment for nitrification and denitrification in light and dark in NH_4^+-enriched microcosm systems using isotope pairing and dilution techniques. *Potamogeton pectinatus* L. and intact sediment cores were collected in a shallow reservoir receiving treated municipal wastewater and containing dense submersed vegetation. Chambers containing *P. pectinatus* shoots, sediment, or both *P. pectinatus* shoots and sediment were exposed to 6 h of darkness, 6 h of light, and 6 h of darkness. $^{14}NH_4^+$ and $^{15}NO_3^-$ were added at ambient concentrations of 15 and 5 mg N liter^{-1}, respectively. NH_4^+ was primarily nitrified in the epiphytic microbial communities, and NO_3^- was denitrified in the underlying sediment. In chambers containing macrophytes, there was a net production of O_2 and NO_3^- in light and a net consumption in dark, and nitrification was higher in light than in dark. In chambers with only sediment, there was always a net consumption of NO_3^-, and nitrification was similar in light and dark. The results show that submersed macrophytes can be important for the N metabolism in NH_4^+-rich freshwaters (e.g., wastewater treatment systems) by stimulating nitrification through providing surfaces for attached nitrifying bacteria and possibly also through diurnal changes in the water chemistry.

Submersed macrophytes provide a large accessible surface area for attached microorganisms (Sculthorpe 1967). Epiphytic microbial communities can play a major role in the transformation of inorganic nutrients in shallow freshwater environments with submersed vegetation (Mickle and Wetzel 1978). Submersed macrophytes can sustain high denitrification rates in nutrient-rich freshwater environments (Eriksson and Weisner 1996, 1997) and may support high abundances of attached nitrifying bacteria (Eighmy and Bishop 1989). Several studies involving N budgets in freshwater systems support the idea that submersed macrophytes can enhance N removal by offering surfaces that can hold populations of both nitrifiers and denitrifiers (Reddy and De Busk 1985; Eighmy and Bishop 1989; Körner 1997).

In submersed vegetation, there is an exchange of photosynthetic gases, i.e., O_2 and CO_2, between leaf surfaces and surrounding water. Due to the often reduced water movements in dense stands of submersed macrophytes (e.g., Loose and Wetzel 1993), limiting gas exchange with the atmosphere and water exchange with areas of air-equilibrated water, the metabolic activity of submersed macrophytes and their epiphytes can produce conspicuous changes in the concentrations of O_2, dissolved inorganic carbon (DIC), and pH (Pokorny et al. 1984; Prahl et al. 1991). However, although changes in the water chemistry produced by submersed macrophytes have been recognized in several studies, they have not been put together with activity measurements of bacterial N transformations.

The gas exchange at submersed leaf surfaces may have a strong impact on bacterial N transformations in epiphytic microbial communities and, through water column–sediment interactions, on N transformations in the sediment. Macrophytes may affect sediment processes not only through the growth and metabolism of their roots, which has been shown earlier (e.g., Reddy et al. 1989), but also by influencing the characteristics of the overlying waters. The transition of NH_4^+ to NO_3^- in sediment and epiphytic populations of nitrifiers may, in daylight, be stimulated by the photosynthetic release of O_2 from submersed vegetation. In addition to producing O_2, the primary production of the epiphyte–macrophyte association may, because of the dependence of NH_4^+-oxidizing bacteria on an alkaline pH and of the generation of acid that necessarily accompanies nitrification, promote epiphytic nitrification by raising pH of the interstitial water within the epiphytic communities. Epiphytic nitrifying bacteria in eutrophic waters may also be supported by the presence of precipitated particulate $CaCO_3$ on the leaves, which may buffer pH within the epiphytic community during nighttime when primary production is absent. Thus, the metabolism of the macrophyte substrata may create a favorable environment for attached nitrifiers. As opposed to daytime, when there is a net production of O_2, the macrophyte–epiphyton complex at night may be of importance in lowering the O_2 concentration of the water by respiratory consumption (Sand-Jensen et al. 1985). Respiration in dense stands of submersed vegetation at night may cause a shift from aerobic to anaerobic bacterial respiration, i.e., denitrification in epiphytic communities, and may also stimulate sediment denitrification by lowering O_2 concentrations of the overlying water. Consequently, it appears that the metabolic activity of submersed vegetation in shallow aquatic environments and the associated macrophyte–water gas exchange may promote a coupling between nitrification and denitrification, i.e., the sequence of NH_4^+ oxidation to NO_3^- by nitrifying bacteria and subsequent denitrification of NO_3^- to N_2.

In the aquatic ecosystem from which we collected macrophytes and sediment (*see below*), Eriksson and Weisner (1997) measured denitrification in epiphytic microbial communities and in the sediment. However, denitrification was measured only in darkness and in systems containing sediment or macrophytes, but not both. Furthermore, Eriksson and Weisner (1997) did not measure nitrification and coupled

Table 1. Constitution of the simulated wastewater used in the experiment.

Macro-constituents	mM	Microconstituents	nM
$CaCl_2$	1.08	H_3BO_3	3,000
$MgSO_4 \cdot 7\ H_2O$	0.244	$CuSO_4 \cdot 5\ H_2O$	157
$NaHCO_3$	0.5	$ZnSO_4 \cdot 7\ H_2O$	336
KCl	0.6	$CoCl_2$	170
$Na_2HPO_4 \cdot 2\ H_2O$	0.035	$MnCl_2 \cdot 4\ H_2O$	3,300
$FeCl_2 \cdot 4\ H_2O$	0.03	$(NH_4)_6MO_7O_{24} \cdot H_2O$	63

nitrification–denitrification. The aim of the present study was to investigate the importance of the metabolic activity of submersed vegetation on nitrification, denitrification, and coupled nitrification–denitrification and to study interactions between the bacterial N transformations in the epiphytic microbial communities and in the sediment. The aim was also to investigate the role of these habitats for nitrification and denitrification in light and dark. In a laboratory experiment, $^{14}NH_4^+$ and $^{15}NO_3^-$ were added to chambers with intact sediment, submersed macrophytes with epiphytic communities, or both sediment and macrophytes. Rates of nitrification and denitrification were determined using isotope pairing and dilution techniques (Koike and Hattori 1978; Nielsen 1992).

Sediment and macrophytes were collected on 18 September 1996 in a shallow reservoir receiving treated municipal wastewater and containing dense stands of *P. pectinatus* L. Influent water contained 10–25 mg NH_4^+ N liter^{-1} and 2–8 mg NO_3^- N liter^{-1}. The residence time for water in the reservoir was 1–2 d. The system has previously been described in more detail (Eriksson and Weisner 1997). The water depth at the sampling site was 0.4–0.5 m. Sediment samples were taken by inserting Plexiglas cylinders (50-cm length, 7-cm inner diameter, volume = 1.9 liter) into the sediment and enclosing sediment and overlying water in the cylinders. Sediment-column depths in the chambers were 10–12 cm (volume = ca. 0.4 liter). Submersed *P. pectinatus* shoots, 30–40-cm stem length, were cut off into separate chambers, which were sealed and brought to the surface. Roots and rhizomes were not included. All of the chambers were sealed with butyl rubber stoppers. The plant material and intact sediment cores were transported to the laboratory. The chambers were stored for 24 h in darkness prior to incubation. During storage and subsequent incubation, a temperature of 20°C was maintained. Prior to incubation, the overlying water in 10 chambers containing sediment was carefully siphoned out of the chambers. They were then completely filled by carefully siphoning simulated wastewater (Table 1) into the systems without disturbing the sediment. To avoid unnatural illumination of the sediment during incubation, the lower part of the sediment chambers was wrapped in aluminum foil. Water was also siphoned out of five chambers containing macrophyte shoots, and simulated wastewater was added. The shoots in five additional macrophyte chambers were transferred to five of the sediment chambers to which simulated wastewater had been added. The macrophytes were floating in the water and were not in contact with the underlying sediment.

The experimental design consisted of five chambers containing shoots of *P. pectinatus*, sediment, or both shoots and sediment, respectively. There was no gas phase in the incubation chambers, which were sealed with closely fitting Plexiglas caps with jointing rubber rings. The water column in each chamber was gently stirred by a small 3-cm Teflon-coated rotating magnet, placed 10 cm below the cap and driven by an external magnet (47 rpm). Prior to incubation, $^{15}NO_3^-$ N (99.9 atom % enrichment) and $^{14}NH_4^+$-N were added to obtain a final concentration of 5 and 15 mg liter^{-1}, respectively, in the water phase of the chambers. These concentrations are similar to those in the reservoir (Eriksson and Weisner 1997) and are also commonly found among treatment wetlands or reservoirs (Hammer and Knight 1994). The chambers were incubated for 18 h, during which time they were exposed to 6 h of darkness, 6 h of light (200 μmol quanta m^{-2} s^{-1}, mercury lamp), and 6 h of darkness, in that order. Initially and after every 6 h, 60-ml water samples were taken from each chamber using a plastic syringe and replaced by simulated wastewater. Prior to each sampling, the cap of the chambers was removed, and the water phase was very gently mixed with a 30-cm-long stainless steel forceps, making sure not to stir the water surface or the sediment. Twenty milliliters of the samples was immediately frozen and later analyzed for NH_4^+ N and (NO_3^- + NO_2^-) N by using colorimetric methods (Chaney and Marbach 1962; Wood et al. 1967). The remaining water was transferred to three rubber-stoppered airtight 12-ml glass vials (Exetainer, Labco) and used for analysis of O_2, DIC, and the $^{14}NO_3^-$ and $^{15}NO_3^-$ content of the water (these vials were frozen), respectively. Immediately after sampling, the O_2 samples were fixed using the Winkler method, and DIC was analyzed with a Shimadzu TOC-5000 C analyzer. No more than 12 h after fixation, dissolved O_2 concentrations were determined by using an automatic potentiometric titrator (Mettler DL 21). The $^{14}NO_3^-$ and $^{15}NO_3^-$ content of the water was determined using a previously described assay (Risgaard-Petersen et al. 1993). Nitrification was determined using the isotope-dilution method of Koike and Hattori (1978). To examine the production of $^{29}N_2$ and $^{30}N_2$, water was transferred to two airtight 12-ml vials (*see above*) initially, and replaced by simulated wastewater, and at the end of the incubation after sediment and water was mixed. The N_2 samples were fixed by adding 200 μl of 7.3 M ZnCl. The frequencies of the N_2 isotopes in the samples were analyzed by mass spectrometry using the procedure of Davidsson et al. (1997). Rates of denitrification of $^{15}NO_3^-$ and $^{14}NO_3^-$ were calculated by the method of Nielsen (1992). The macrophytes were collected after the experiment, dried for 48 h at 85°C, and weighed. Macrophyte biomass in the chambers was on average (±SE, n = 10) 0.53 ± 0.022 g dry weight (DW), corresponding to 140 ± 6 g DW m^{-2}, which is similar to biomasses of *P. pectinatus* that are commonly observed in shallow aquatic environments (van Wijk 1988; Prahl et al. 1991).

We used repeated measures analysis of variance (ANOVA) (von Ende 1993) to determine differences in O_2, NO_3^-, NH_4^+, and nitrification, respectively, among treatments and time periods. One-factor ANOVA was used to determine differences in denitrification among the treatments. Contrast tests (von Ende 1993) were done to examine whether the

Table 2. O_2 saturation (mean ± 1 SE, n = 5; %) before and after 6-h periods of light or darkness and rates of change in O_2 concentration during the periods of light and darkness, in microcosms containing shoots of *P. pectinatus*, sediment, or both shoots and sediment, respectively. Average rates (±1 SE, n = 5; mg O_2 h^{-1} m^{-2} sediment surface area) are given. Average dark includes both periods of darkness (±1 SE, n = 10). Total represents the mean rate of change in O_2 concentration during the whole incubation period, i.e., the difference between final and initial concentrations divided by 18 h.

Time (h)	O_2 saturation (%) Macrophyte	Sediment	Macrophyte and sediment	Period of time (h)	Rates of change in O_2 concentration (mg O_2 h^{-1} m^{-2}) Macrophyte	Sediment	Macrophyte and sediment
0	78 ± 2	89 ± 1	71 ± 3	0–6 (dark)	−266 ± 24	8.3 ± 12	−270 ± 8.3
6	43 ± 5	90 ± 1	24 ± 3	6–12 (light)	690 ± 82	−84 ± 3.2	629 ± 42
12	134 ± 6	75 ± 1	133 ± 5	12–18 (dark)	−471 ± 58	15 ± 8.0	−469 ± 54
18	72 ± 2	78 ± 2	52 ± 5	Average dark	−368 ± 64	12 ± 9.7	−369 ± 59
				Total	−16 ± 6.7	−20 ± 4.0	−37 ± 6.7

macrophytes affected the microbial processes of nitrification and denitrification. Contrast tests were also conducted to compare nitrification in light and dark. Data were transformed to logarithms to meet the assumptions of ANOVA. However, when the data consisted of percentages, we used arcsine transformation (arcsine $\sqrt{p}$), where p is a proportion and $\sqrt{}$ is the square root. Differences were accepted as significant at the $P \leq 0.05$ level.

The submersed macrophytes produced conspicuous changes in the water chemistry of the aquatic microcosm systems. Low and high O_2 concentrations and high and low DIC at dark and light periods, respectively, were displayed in chambers with only macrophytes and with both macrophytes and sediment (Table 2). In the macrophyte chambers, the DIC concentration ranged between 8 mg C liter^{-1} in light and 16 mg C liter^{-1} in dark. In the chambers with only sediment, the DIC concentration ranged between 13 and 15 mg C liter^{-1}. The production and consumption rates of O_2 in the chambers with macrophytes (with and without sediment) were similar to those that have been recorded in shallow freshwaters with submersed vegetation (Table 2; Prahl et al. 1991). During the experiment as a whole, there was a net production of NO_3^- in chambers with only macrophytes and a net consumption of NO_3^- in chambers with sediment (with and without macrophytes; Table 3). In light, there was a net production of NO_3^- in chambers with macrophytes (with and without sediment) and a net consumption of NO_3^- in chambers that contained only sediment (Table 3). In darkness, there was a net consumption of NO_3^- in all chambers (Table 3). In all treatments, the rates of change in NO_3^- concentration were higher during the second than during the first dark period. Since the production of NO_3^-, i.e., nitrification, was similar during the dark periods, the differences in NO_3^- concentration between these periods were most likely due to changes in the NO_3^- consumption. The diffusional penetration of NO_3^- into the sediment and biofilms during the time course of the experiment probably resulted in a continuous widening of the denitrification zones and a steady increase in the NO_3^- consumption. At high NO_3^- concentrations, NO_3^- stimulates denitrification by extending the denitrification zone with depth and not by increasing the specific activity of the denitrifying bacteria (Sørensen and Revsbech 1990). In addition, the O_2 production in light resulted in extended penetration of O_2 into the sediment and biofilms, and since O_2 inhibits denitrification, NO_3^- could penetrate deeper during the light period without being used by denitrification (Sørensen and Revsbech 1990; Jensen et al. 1994). O_2 is rapidly consumed after a light/dark switch, and denitrification will predominate (Sørensen and Revsbech 1990). Because sediment and biofilms presumably contained more NO_3^- after the light period than before, there were higher consumption rates of NO_3^- by denitrification during the second than during the first dark period. Rates of change in NH_4^+ concentration were similar among the treatments and among the different time periods (repeated measures ANOVA: treatment, $F_{2,12} = 1.2$, $P = 0.34$; time, $F_{2,24} = 3.0$, $P = 0.070$; treatment × time, $F_{4,24} = 2.8$, $P = 0.05$). The NH_4^+ level decreased during the experiment at an average (±SE, n = 15) of 3.5 ± 1.3%, which corresponds to 0.55 ± 0.20 mg NH_4^+ N liter^{-1}.

Table 3. Rates of change in NO_3^- concentration during periods of light and darkness in chambers containing shoots of *P. pectinatus*, sediment, or both shoots and sediment, respectively. Average rates (±1 SE, n = 5; mg N h^{-1} m^{-2} sediment surface area) are given. Average dark includes both periods of darkness (±1 SE, n = 10). Total represents the mean rate of change in NO_3^- concentration during the whole incubation period, i.e., the difference between final and initial concentrations divided by 18 h. Rates of change in NO_3^- concentration differed significantly among the treatments and among the different time periods (repeated measures ANOVA: treatment, $F_{2,12} = 12$, $P = 0.0014$; time, $F_{2,24} = 70$, $P < 0.0001$; treatment × time, $F_{4,24} = 2.3$, $P = 0.082$).

Period of time (h)	Macrophyte	Sediment	Macrophyte and sediment
0–6 (dark)	5.0 ± 3.4	−6.0 ± 3.0	−4.8 ± 1.1
6–12 (light)	18 ± 6.6	−10 ± 2.0	2.8 ± 3.1
12–18 (dark)	−16 ± 4.3	−30 ± 3.3	−26 ± 2.6
Average dark	−5.7 ± 3.8	−18 ± 2.6	−16 ± 1.5
Total	2.3 ± 4.4	−16 ± 1.2	−9.4 ± 0.57

To accurately measure rates of bacterial N transformations and to determine the coupling between nitrification and denitrification, controlled short-term enclosure studies using isotope techniques are necessary (Mosier and Schimel 1993). An important limitation of chamber experiments is that enclosing a system more or less affects its functioning (Carpenter 1996). However, in the present study, the changes in

Table 4. Denitrification of $^{15}NO_3^-$ denitrification of $^{14}NO_3^-$, and $^{14}NO_3^-$ denitrification in percentage of the total denitrification rate during 18 h of incubation in aquatic systems containing shoots of *P. pectinatus*, sediment, or both shoots and sediment, respectively. Average rates ($\pm$1 SE, $n = 5$; mg N h^{-1} m^{-2} sediment surface area) are given. Significance of difference between treatments is according to one-factor ANOVA.

Process	Macrophyte	Sediment	Macrophyte and sediment	Significance
Denitrification of $^{15}NO_3^-$	0.19 $\pm$ 0.047	7.2 $\pm$ 0.59	4.9 $\pm$ 0.88	$P < 0.0001$ ($F = 35$)
Denitrification of $^{14}NO_3^-$	0.041 $\pm$ 0.008	0.22 $\pm$ 0.042	0.30 $\pm$ 0.086	$P = 0.01$ ($F = 6.9$)
$^{14}NO_3^-$ denitrification in % of total denitrification	21 $\pm$ 5.6	2.9 $\pm$ 0.55	5.5 $\pm$ 0.81	$P = 0.0007$ ($F = 14$)

O_2, total inorganic C, and NO_3^- are in line with what has been recorded in natural environments with abundant submersed vegetation (Pokorny et al. 1984; Prahl et al. 1991). Sediment nitrification and denitrification were also in accordance with earlier studies. Furthermore, the consumption of NO_3^- showed the same pattern in all of the chambers, i.e., higher NO_3^- consumption during the second than during the first dark period, indicating that the treatments were comparable.

Denitrification was several times higher in chambers that contained sediment (with and without macrophytes) than in chambers with only macrophytes, indicating that the epiphytic microbial communities were of minor importance for denitrification (Table 4). It has been shown in earlier studies that the level of denitrification can be relatively high in epiphytic microbial communities (Eriksson and Weisner 1996, 1997). However, the low rates of epiphytic denitrification measured in the present study are not contradictory to the studies mentioned, because epiphytic communities may vary widely in their biomass composition and functioning within the same system (Eriksson and Weisner 1996; Strand and Weisner 1996). In the present study, the majority of the added $^{15}NO_3^-$ was denitrified in the sediment. The sediment functioned almost as the sole source of $^{29}N_2$ and $^{30}N_2$ to the overlying water. The function of the sediment as the major site of denitrification is confirmed by the measurements of NO_3^- exchange. In the chambers with sediment, denitrification was of the same magnitude whether or not macrophytes were present. A conspicuous effect macrophytes have on the N metabolism of aquatic systems is the inhibition of denitrification in light by elevation of the O_2 concentrations of the water (Sørensen and Revsbech 1990; Weisner et al. 1994; Eriksson and Weisner 1996). However, the results indicate that even though the macrophytes increased the O_2 level of the overlying water several folds, the sediment bacteria consumed O_2 at such an efficiency that the sediment denitrification could remain at a high level throughout the incubation.

Nitrification was several times higher in chambers with macrophytes (with and without sediment) than in chambers that only contained sediment, indicating that the epiphytic communities were the major sites of nitrification. Nitrification is most commonly the limiting step in the N removal process in wetlands or reservoirs used for wastewater treatment (Hammer and Knight 1994). Submersed macrophytes may provide a positive benefit on the N removal in these environments by supplying a substratum for the establishment of nitrifying bacteria in the water column. The present study indicates that nitrifiers in microbial communities on the surfaces of submersed macrophytes can support higher rates of nitrification than sediment populations of nitrifiers. However, nitrification in epiphyton per unit shoot surface area was similar to that of the sediment per unit surface area. A macrophyte abundance of 140 g DW m^{-2} amounts to 22 m^2 shoot surface area m^{-2}, based on a value of 0.16 m^2 shoot surface g^{-1} DW (Eriksson and Weisner 1997), and a nitrification of 10 mg N h^{-1} m^{-2} therefore corresponds to 0.5 mg N h^{-1} m^{-2} shoot surface area. Nitrification was greater in the macrophyte chambers (with and without sediment) than in the chambers with only sediment, probably because the macrophytes provided more surface area for attached nitrifying bacteria than the sediment.

Nitrification was higher in light than in dark in the chambers that contained macrophytes (contrast test: $F = 7.5$, $P = 0.018$; Fig. 1). Nitrification was similar in light and dark in the chambers with only sediment (contrast test: $F = 2.8$, $P = 0.14$). The results indicate that the submersed vegetation, presumably by its photosynthetic O_2 production, stimulated biofilm nitrification in light. Sand-Jensen et al. (1985) showed that O_2 concentrations at macrophyte surfaces could change from zero in the dark to 2.5 times oversaturation in light, even if O_2 equilibration was maintained in the surrounding water. Moreover, respiratory activity causes a depletion of O_2 in the epiphyton within minutes after a light/dark switch (Sand-Jensen et al. 1985). The activities of the macrophyte–epiphyte assemblage can have large effects on the chemical conditions within the epiphytic communities. Nevertheless, in the present study, we found only a minor difference in nitrification between the periods of light and darkness. This indicates that although an elevation of the O_2 level in light promoted nitrification, the nitrifying bacteria could still maintain a high activity in darkness. Thus, the transport of O_2 from the surrounding water was sufficient to support nitrification in the epiphytic communities during the periods of darkness.

In the chambers with sediment (with and without macrophytes), $^{14}NO_3^-$ denitrification comprised a small part of the total denitrification (Table 4). Nitrification produced on average 0.0087 and 0.21 mg $^{14}NO_3^-$ N liter^{-1} in the sediment and sediment + macrophyte chambers during the 18 h of incubation, respectively. The concentrations of $^{14}NO_3^-$ were much lower than the concentration of $^{15}NO_3^-$, which was 5 mg N liter^{-1}. $^{14}NO_3^-$ denitrification could therefore, in this short-term experiment, never reach the same magnitude as

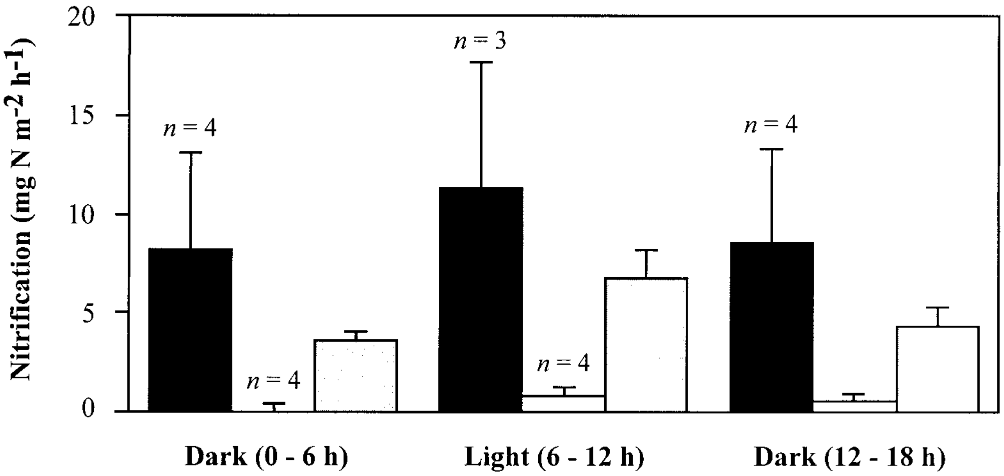

Fig. 1. Average rates of nitrification ($\pm$1 SE; mg N h^{-1} m^{-2} sediment surface area), during 6-h periods of light or darkness, in aquatic systems containing shoots of *P. pectinatus* (black bars), sediment (white bars), or both shoots and sediment (gray bars), respectively. The number of replicates differs because three samples were damaged when frozen, and the NO_3^- data of these samples could not be used in the calculations of nitrification. Statistics are based on five replicates, if the number of replicates is not given on top of the error bars. Nitrification differed significantly among the treatments and among the different time periods (repeated measures ANOVA: treatment, $F_{2,9}$ = 9.4, P = 0.0063; time, $F_{2,18}$ = 5.5, P = 0.014; treatment $\times$ time, $F_{4,18}$ = 1.5, P = 0.25).

$^{15}NO_3^-$ denitrification. However, the $^{14}NO_3^-$ denitrification comprised a much larger part of total denitrification in the chambers with only macrophytes than in the chambers with both macrophytes and sediment (Table 4). Because nitrification was similar in these chambers, the ratio between $^{14}NO_3^-$ and $^{15}NO_3^-$ denitrification also ought to have been similar. However, since it was not, $^{14}NO_3^-$, which was denitrified in the epiphytic communities, may not have been thoroughly mixed with $^{15}NO_3^-$ in the overlying waters before it was used by the epiphytic denitrifiers. These results indicate that the denitrification in the epiphytic communities occurred in close connection with the sites of nitrification.

There were about 40 times higher rates of nitrification than denitrification in the epiphytic communities. A major portion of the produced $^{14}NO_3^-$ diffused out of the epiphytic communities without being used by the epiphytic denitrifiers. As a result, there was much more $^{14}NO_3^-$ in the overlying water of the chambers with macrophytes and sediment than in those with only sediment. However, there was no significant difference in $^{14}NO_3^-$ denitrification between these treatments (contrast test: F = 1.2, P = 0.30; Table 4), indicating that little of the $^{14}NO_3^-$ in the overlying water of the chambers was transferred to the sediment. To avoid sediment resuspension or disruption of the epiphytic assemblages, the overlying water of the chambers was gently stirred by a spinning magnet, creating relatively small water movements. The water movements within the chambers were probably within the lower range of what is found in natural stands of submersed vegetation (Loose and Wetzel 1993), which are exposed to wind-induced water turbulence, currents, and the action of large biota such as fish and waterfowl. Sediment–water column exchange of nutrients increases with increasing water movements (Miller-Way and Twilley 1996). There is probably a higher transfer of NO_3^- produced within nitrifying biofilms on submersed macrophytes to the sediment and therefore also a better coupling between nitrification and denitrification in many natural environments with submersed vegetation than in the chambers. In conclusion, the rate of coupling between nitrification in epiphytic communities and denitrification in the sediment probably depends to a large extent on the hydrodynamic conditions of the aquatic system.

Nitrification and denitrification require extremely different redox conditions. They are obligatory oxic and anoxic processes, respectively, and are therefore always spatially or temporally separated from each other. The present study indicates that submersed vegetation may be a link by which nitrification and denitrification can be efficiently connected. NO_3^- may be produced within the water column by microbial communities attached to submersed macrophytes and, by forces of mass flow or diffusion, enter the sediment in which it can be reduced to N_2 by denitrification. Furthermore, nitrification balanced denitrification when both macrophytes and sediment were present (i.e., the production rate of NO_3^- by nitrification in the epiphytic communities equaled the consumption of NO_3^- by sediment denitrification). Thus, the production of NO_3^- by epiphytic communities may support denitrification in the underlying sediment.

In conclusion, the present study has displayed a pattern by which NH_4^+ is nitrified in attached microbial communities at the surfaces of submersed macrophytes, and NO_3^- is mainly denitrified in the underlying sediment. In the presence of both macrophytes and sediment, rates of nitrification and denitrification were similar (Fig. 2). The transformation of NH_4^+ to N_2 by the sequential action of nitrification and denitrification may be greatly enhanced by submersed vegetation. The results show that submersed macrophytes can be of importance for the N metabolism in NH_4^+-rich freshwater ecosystems (e.g., wastewater treatment systems) by stimu-

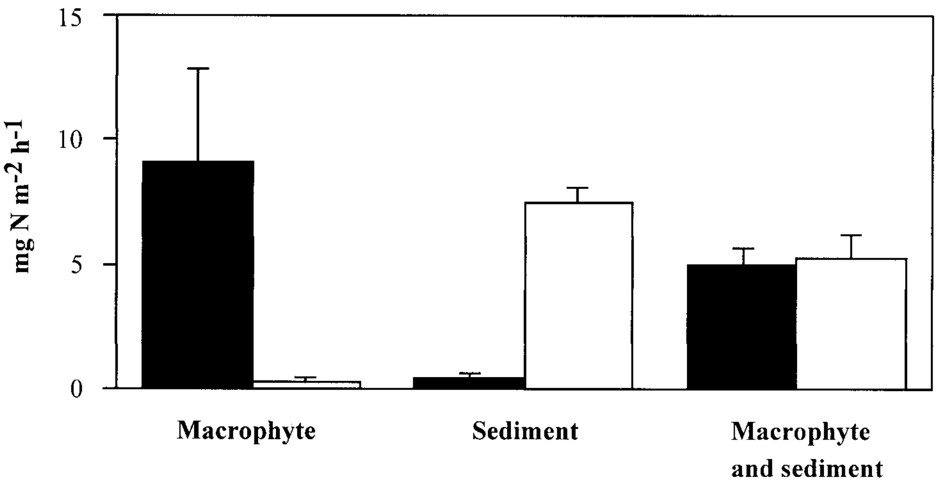

Fig. 2. Nitrification (black bars) and total denitrification (white bars), during 18 h of incubation, in aquatic systems containing shoots of *P. pectinatus,* sediment, or both shoots and sediment, respectively. Average rates (± 1 SE, $n = 5$; mg N h^{-1} m^{-2} sediment surface area) are given.

lating nitrification through providing surfaces for the attachment of nitrifying bacteria and possibly also through the diurnal changes in the water chemistry occurring in the submersed vegetation.

Peder G. Eriksson
Stefan E. B. Weisner

Limnology, Department of Ecology
Lund University
Ecology Building
S-223 62 Lund, Sweden

References

CARPENTER, S. R. 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology **77:** 677–680.

CHANEY, A., AND E. P. MARBACH. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. **8:** 130–132.

DAVIDSSON, E. T., R. STEPANAUSKAS, AND L. LEONARDSON. 1997. Vertical patterns of nitrogen transformations during infiltration in two wetland soils. Appl. Environ. Microbiol. **63:** 3648–3656.

EIGHMY, T. T., AND P. L. BISHOP. 1989. Distribution and role of bacterial nitrifying populations in nitrogen removal in aquatic treatment systems. Water Res. **25:** 947–955.

ERIKSSON, P. G., AND S. E. B. WEISNER. 1996. Functional differences in epiphytic microbial communities in nutrient-rich freshwater ecosystems: An assay of denitrifying capacity. Freshwater Biol. **36:** 555–562.

———, AND ———. 1997. Nitrogen removal in a wastewater reservoir: The importance of denitrification by epiphytic biofilms on submersed vegetation. J. Environ. Qual. **26:** 905–910.

Acknowledgments

We thank Lars Leonardson, John Strand, Viveka Vretare, and Göran Ewald for comments on earlier drafts of the manuscript. This study was supported by grants from the Swedish EPA. The purchase of the mass spectrometer was possible because of a grant provided by the Skandinaviska Enskilda Bankens foundation for economic and technical research.

HAMMER, D. A., AND R. L. KNIGHT. 1994. Designing constructed wetlands for nitrogen removal. Water Sci. Technol. **29:** 15–27.

JENSEN, K., N. P. SLOTH, N. RISGAARD-PETERSEN, S. RYSGAARD, AND N. P. REVSBECH. 1994. Estimation of nitrification and denitrification from microprofiles of oxygen and nitrate in model sediment systems. Appl. Environ. Microbiol. **60:** 2094–2100.

KOIKE, I., AND A. HATTORI. 1978. Simultaneous determinations of nitrification and nitrate reduction in coastal sediments by a ^{15}N dilution technique. Appl. Environ. Microbiol. **35:** 853–857.

KÖRNER, S. 1997. Nutrient and oxygen balance of a highly polluted treated sewage channel with special regard to the submerged macrophytes. Acta Hydrochim. Hydrobiol. **25:** 34–40.

LOOSE, R. F., AND R. G. WETZEL. 1993. Littoral flow rates within and around submersed macrophyte communities. Freshwater Biol. **19:** 7–17.

MICKLE, A. M., AND R. G. WETZEL. 1978. Effectiveness of submersed angiosperm–epiphyte complexes on exchange of nutrients and organic carbon in littoral systems. I. Inorganic nutrients. Aquat. Bot. **4:** 303–316.

MILLER-WAY, T., AND R. R. TWILLEY. 1996. Theory and operation of continuous flow systems for the study of benthic–pelagic coupling. Mar. Ecol. Prog. Ser. **140:** 257–269.

MOSIER, A. R., AND D. S. SCHIMEL. 1993. Nitrification and denitrification, p. 181–208. *In* R. Knowles and T. H. Blackburn [eds.], Nitrogen isotope techniques. Academic.

NIELSEN, L. P. 1992. Denitrification in sediment determined from isotope pairing. FEMS Microbiol. Ecol. **86:** 357–362.

POKORNY, J., J. KVET, J. P. ONDOK, Z. TOUL, AND I. OSTRY. 1984. Production–ecological analysis of a plant community dominated by *Elodea canadensis* Michx. Aquat. Bot. **19:** 263–292.

PRAHL, C., E. JEPPESEN, K. SAND-JENSEN, AND T. MOTH-IVERSEN. 1991. A continuous-flow system for measuring in vitro oxygen and nitrogen metabolism in separated stream communities. Freshwater Biol. **26:** 495–506.

REDDY, K. R., AND W. F. DE BUSK. 1985. Nutrient removal potential of selected aquatic macrophytes. J. Environ. Qual. **148:** 459–462.

———, W. H. PATRICK, JR., AND C. V. LINDAU. 1989. Nitrification–denitrification at the plant root–sediment interface in wetlands. Limnol. Oceanogr. **34:** 1004–1013.

RISGAARD-PETERSEN, N., S. RYSGAARD, AND N. P. REVSBECH. 1993. A sensitive assay for determination of $^{14}N/^{15}N$ isotope distribution in NO_3^-. J. Microbiol. Methods **17:** 155–164.

SAND-JENSEN, K., N. P. REVSBECH, AND B. B. JÖRGENSEN. 1985. Microprofiles of oxygen in epiphyte communities on submerged macrophytes. Mar. Biol. **89:** 55–62.

SCULTHORPE, C. D. 1967. The biology of aquatic vascular plants. Edward Arnold.

SØRENSEN, J., AND N. P. REVSBECH. 1990. Denitrification in stream biofilm and sediment: In situ variation and control factors, p. 277–289. *In* N. P. Revsbech and J. Sørensen [eds.], Denitrification in soil and sediment. Plenum.

STRAND, J. A., AND S. E. B. WEISNER. 1996. Wave exposure related growth of epiphyton: Implications for the distribution of submerged macrophytes in eutrophic lakes. Hydrobiologia **325:** 113–119.

VAN WIJK, R. J. 1988. Ecological studies on *Potamogeton pectinatus* L. I. General characteristics, biomass production and life cycles under field conditions. Aquat. Bot. **31:** 211–258.

VON ENDE, C. N. 1993. Repeated measures analysis: Growth and other time-dependent measures, p. 113–137. *In* S. M. Scheiner and J. Gurevitch [eds.], Design and analysis of ecological experiments. Chapman and Hall.

WEISNER, S. E. B., P. G. ERIKSSON, W. GRANÉLI, AND L. LEONARDSON. 1994. Influence of macrophytes on nitrate removal in wetlands. Ambio **23:** 363–366.

WOOD, E. D., F. A. J. ARMSTRONG, AND F. A. RICHARDS. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. **47:** 23–31.

Received: 16 June 1998
Accepted: 14 June 1999

Limnol. Oceanogr., 44(8), 1999, 1999–2004

Preferential recycling of nutrients—the ocean's way to increase new production and to pass nutrient limitation?

Abstract—Uptake of atmospheric CO_2 by the oceans and the export of carbon into deeper waters via the biological CO_2 pump is driven by the production of particulate organic matter (POM). The elemental ratios of carbon, nitrogen, and phosphorus within POM follow Redfield ratios, suggesting that the concentrations of dissolved inorganic carbon (DIC) and nutrients decrease during new production corresponding to these ratios. Subsequently, new production and the efficiency of the biological CO_2 pump are usually estimated using Redfield ratios. However, our observations in the Baltic Sea and observations elsewhere show significantly greater decreases in DIC during the productive season than that predicted from the decline in nutrients with reference to Redfield ratios. Using new DIC, nutrient, and oxygen data from the Baltic Sea, we discuss this discrepancy and provide evidence that preferential recycling of nutrients fuels the productive community with nutrients. Limiting nutrients are preferentially recycled and become available for new production. These findings derived from hydrochemical bulk data confirm the picture of the microbial loop but question the common description of new production and nutrient limitation. Finally, we argue for a carbon-based efficiency estimate of the biological CO_2 pump probably exceeding significantly nutrient-based estimates.

Uptake of atmospheric CO_2 by the oceans via the biological CO_2 pump is driven by the new production of particulate organic matter (POM) and its export into deeper waters (e.g., Eppley and Peterson 1979). Phytoplankton produces POM during photosynthesis consuming dissolved inorganic carbon (DIC) and mainly nitrate (NO_3) and phosphate (PO_4). The elemental ratios of carbon (C), nitrogen (N), and phosphorus (P) within freshly produced POM that are found to be similar all over the world's oceans (e.g., Copin-Monteguet and Copin-Monteguet 1983; Toggweiler 1993) are expressed by Redfield ratios, suggesting that the concentration changes of DIC and nutrients during production and remineralization of POM are in the same ratios (Redfield et al. 1963). With reference to Redfield's idea of a coupling between DIC and nutrient concentrations and elemental ratios of POM, Dugdale and Goering (1967) deduced the widely accepted and applied description of new production and its limitation by nutrients: New production is defined as phytoplankton production based on nitrate uptake that can be converted to carbon units using the elemental ratios of POM. New production is limited by nutrient availability in the euphotic zone. Consequently, the export of CO_2 into the deeper waters via the biological CO_2 pump is estimated using the increases of nutrients.

However, several investigations on the relationship between the elemental composition of POM and changes of nutrients over depth show only weak consistency (Shaffer 1996, and review therein). There are even strong indications that the observed DIC decrease during production of POM is decoupled from the associated NO_3 decrease, because the ratio of DIC to NO_3 decreases, $\Delta DIC/\Delta NO_3$ is found to be significantly higher than predicted by the C/N ratio of POM. Here, we discuss some of these hydrochemical indications and refer to new DIC and NO_3 data from the Baltic Sea to provide evidence that the discrepancy between observed and predicted DIC decrease is caused by preferential recycling of nutrients within the euphotic zone. This mechanism enhances the efficiency of the biological CO_2 pump by allowing further net-CO_2 fixation, i.e., net community production, using recycled nutrients. We suggest that Redfield's idea of fixed elemental ratios of POM is consistent with this mechanism. However, in contradiction to this mechanism are (1) the assumption that the concentration changes of DIC and nutrients would reflect the Redfield ratios, (2) the application of the definitions of new production and nutrient limitation according to Dugdale and Goering (1967) to assess net community production, and (3) the description of the biological CO_2 pump with reference to nutrient concentrations and Redfield ratios.