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Interaction effects of flow velocity and oxygen metabolism on nitrification and denitrification in biofilms on submersed macrophytes

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Abstract. Effects of water flow velocity on nitrification, denitrification, and the metabolism of dissolved oxygen and inorganic carbon in macrophyte-epiphyton complexes were investigated in the present study. The metabolic rates were measured in microcosms containing shoots of *Potamogeton pectinatus* L. with epiphytic biofilms in the light and dark with no flow or with the flow velocities of 0.03 and 9 cm s^{-1}. Photosynthesis and respiration increased with increasing water flow velocity. Rates of oxygen respiration were positively correlated to the oxygen concentration of the water. Nitrification was not significantly affected by flow velocity, but nitrification was higher in light than in dark at 0.03 cm s^{-1}, but not at 9 cm s^{-1}. Denitrification was higher in stagnant water and at 9 cm s^{-1} than at 0.03 cm s^{-1} in the absence of oxygen, possibly due to complex effects of water flow velocity on the supply of organic matter to the denitrifying bacteria. Denitrification was always inhibited in light, and negatively correlated to the oxygen concentration in dark. Epiphytic denitrification occurred only at low oxygen concentrations in flowing water, whereas in stagnant water, denitrification was present in almost oxygen saturated water. Therefore, because there are little of water movements and high oxygen consumption in dense stands of submersed macrophytes, significant rates of epiphytic denitrification can probably be found within submersed vegetation despite high oxygen concentrations in the surrounding water. In conclusion, this study shows that the water flow and oxygen metabolism within submersed vegetation have minor effects on nitrification, but significantly affect denitrification in biofilms on submersed macrophytes.

Introduction

In aquatic environments, bacteria and other microorganisms attach to solid surfaces producing complex microbial communities, which are referred to as biofilms or periphyton (Fletcher & Marshall 1982). Zobell (1943) discovered that surfaces have a positive influence on bacterial activity, and it has later been shown that attached bacteria are more active and often present at higher densities than free-living bacteria (Fletcher & Marshall 1982). Macrophytes

are conspicuous components of shallow-water environments such as wetlands and the littoral zone of lakes, and provide habitats for attached microorganisms. Because of the large surface area provided in particular by submersed macrophytes, epiphytic biofilms have been shown to be important for the transformation and cycling of nutrients in shallow freshwater ecosystems, (Wetzel 1979; Eriksson & Weisner 1997). Epiphytic bacteria can in particular have a large impact on the amount and species of dissolved inorganic nitrogen (Eriksson & Weisner 1997, 1999). High densities of nitrifying and denitrifying bacteria have been reported on submersed macrophytes (Körner 1999). In nutrient-rich aquatic systems, nitrification can be ten times higher with than without submersed macrophytes and epiphytic denitrification can be of similar importance as sediment denitrification (Eriksson & Weisner 1997, 1999).

Submersed macrophytes are often present within a large part of the water column of shallow lakes and reservoirs, and are therefore exposed to the action of water currents and wind-induced waves. Flow velocities in streams and in the open water of lakes commonly lie between 0.01 and 1 m s^{-1} (Silvester & Sleigh 1985). Water flows are however modified by the physical obstruction of submersed macrophyte beds, and rates of flow velocities within submersed vegetation often differ from those in the open water (Madsen & Warnacke 1983; Loose & Wetzel 1993; Sand-Jensen & Mebus 1996). Sand-Jensen and Mebus (1996) found flow velocities of 1.6–6.2 cm s^{-1} among submersed macrophytes, whereas in the open water the flow velocity was 18–34 cm s^{-1}. Madsen and Warnacke (1983) measured flow velocities of 0–1.7 cm s^{-1} and Loose and Wetzel (1993) determined flow velocities ranging between 0 and 0.46 cm s^{-1}, with a mean flow velocity of 0.07 cm s^{-1}, within beds of submersed macrophytes. Thus, in natural aquatic environments, submersed vegetation is exposed to a wide range of water flow velocities. However, no studies have to my knowledge been done on effects of water flow velocity on bacterial nitrogen transformations in epiphytic microbial communities on submersed macrophytes.

Mass flow is the dominant mode of transport within the water column of aquatic systems, but in boundary-layers at solid-water interfaces, dissolved substances are mainly transferred by molecular diffusion, which is a relatively slow process (Silvester & Sleigh 1985; Stevens & Hurd 1997). The flow velocity of the overlying water controls the depth of the diffusive boundary layers, which can be fractions of a mm up to several mm thick (Silvester & Sleigh 1985; Kühl et al. 1996). As the flow velocity increases the boundary layer decreases resulting in shorter diffusive distances (Silvester & Sleigh 1985). Consequently, flowing water enhances the exchange of nutrients and gases

such as oxygen between the water and biofilm communities affecting their metabolic activity (Silvester & Sleigh 1985; Stevens & Hurd 1997).

The metabolism of oxygen by macrophyte-epiphyton complexes is related to the flow velocity of the surrounding water (Dawson et al. 1981; Stevens & Hurd 1997). Macrophyte-epiphyton respiration increases in relation to water flow up to about 20 cm s^{-1} (Dawson et al. 1981). Photosynthesis also increases with increasing flow velocity, but it may decline at high flow velocities possibly due to mechanical stress (Madsen et al. 1993). The oxygen concentration varies more in biofilms attached to aquatic macrophytes than in biofilms in general because, in addition to biofilm organisms, the macrophyte substrata produces oxygen when illuminated, and reduces oxygen concentrations in dark by its respiration (Sand-Jensen et al. 1985; Eriksson & Weisner 1999). Thus, the water flow velocity controls the oxygen concentrations in epiphyton by affecting the transfer of oxygen to the macrophyte surfaces from the surrounding water, as well as affecting the metabolic rates within the macrophyte-epiphyton communities. The water flow velocity may have significant effects on rates of nitrification and denitrification in epiphytic biofilms because the presence of oxygen promotes nitrification but inhibits denitrification (Tiedje 1988).

In conclusion, earlier studies have shown that the rate of water flow velocity controls the flux of matter to and from epiphytic biofilms on submersed vegetation and may affect the oxygen metabolism of the vegetation. As a result, the water flow velocity probably has a large impact on the metabolic processes of nitrification and denitrification in epiphytic biofilms. However, effects of flow velocity on epiphytic nitrification and denitrification have so far not been investigated. Therefore, the aim of the present study was to investigate the influence of water flow velocity on epiphytic nitrification and denitrification in submersed vegetation. The aim was also to study the effects of the photosynthetic and respiratory activities of the submersed vegetation on the nitrification and denitrification processes at different flow velocities. Three experiments were conducted using microcosms with shoots of *Potamogeton pectinatus* L. with no flow or recirculating water at 0.03 or 9 cm s^{-1}. Measurements were done both in the light and dark. The objective of Experiment I was to investigate whether or not the photosynthetic and respiratory activities of *P. pectinatus* shoots at different flow velocities could significantly affect the water chemistry and therefore also could affect epiphytic nitrification and denitrification. The objective of Experiment II and III was to investigate the influence of the water flow velocity and the oxygen metabolism of the vegetation on nitrification and denitrification in the epiphytic biofilms.

Material and methods

P. pectinatus shoots were collected in a series of shallow macrophyte-dominated ponds (55 °42′N, 13 °32′E), which received nutrient-rich water containing both ammonium and nitrate. The water depth in the ponds varied between 0.4 and 1 m. During the growth season water flow velocities were between 0.5 and 4 cm s^{-1} outside the vegetation. Velocities within the vegetation were probably lower because of the physical obstruction of flow by the plant surfaces. The submersed macrophyte community was dominated by *P. pectinatus*, which by mid-July reached the water surface forming dense canopies. The plants were free of filamentous macro-algae.

Submersed macrophytes growing in the outer sections of macrophyte stands were cut and transferred to buckets under water and then transported to the laboratory. At the laboratory, the submersed macrophytes were carefully washed in tap water to remove loosely attached epiphyton and animals, and inserted into transparent plastic gas-tight cylindrical microcosms (400 mm length, 26 mm inner diameter, volume 212 ml) containing nutrient solution (Table 1) with pH 7.8. The nutrient solution was recirculated through fresh gas-tight neoprene tubes. In once-flow through systems changes in the concentration of nutrients and gasses due to the activity of attached organisms are difficult to detect because of the fast renewal time of water. Therefore, recirculating-water systems similar to the ones used in present study are commonly used for estimating activities of attached organisms in flowing waters (e.g. Dawson et al. 1981; Sørensen et al. 1988). To obtain a water flow velocity of 0.03 and 9 cm s^{-1}, microcosms were connected to a peristaltic pump (Ole Dich Instrument Makers, type 110 SC.G9.CH8A) or equipped with magnetically driven centrifugal pumps (RENA C20). The pumps did not alter the temperature of the microcosm water, which was 20 °C. pH was measured initially and at the end of the experiments. After the experiments, the epiphytic material was removed from the macrophyte shoots as previously described by Eriksson and Weisner (1996). Macrophyte shoots and epiphyton were dried for 48 hours at 85 °C, weighed, combusted (450 °C, 4 h) and re-weighed.

In Experiment I, effects of water flow velocity on photosynthesis and respiration were investigated in eight microcosm systems with shoots of *P. pectinatus*, which were incubated at 0.03 and 9 cm s^{-1}, respectively, during five to six hours in light and dark. The macrophyte shoots were collected on August 5, 1996. A preliminary study showed that none or very little nitrification was present on the macrophyte shoots. Nitrifying bacteria could otherwise have confounded the measurements of photosynthesis and respiration, since oxygen and DIC are also used in the metabolic activities of nitrifying bacteria. Initially and at the end of the light and dark incubation

Table 1. Constitution of the nutrient solution used in the experiments

Macroconstituents (mg L^{-1})		Microconstituents (μg L^{-1})	
$^{15}NH_4Cl$	35	H_3BO_3	190
$CaCl_2$	120	$CuSO_4$ (5 H_2O)	40
$MgSO_4$ (7 H_2O)	60	$ZnSO_4$ (7 H_2O)	100
$NaHCO_3$	42	$CoCl_2$	20
KCl	45	$MnCl_2$ (4 H_2O)	600
Na_2HPO_4 (2 H_2O)	6	$(NH_4)_6MO_7O_{24}$ (H_2O)	80
$FeCl_2$ (H_2O)	6		

periods, water was collected, replaced by nutrient solution, and later analysed for dissolved oxygen and DIC. To check for nitrification activity, (NO_3^- + NO_2^-)-N and the isotope composition of dissolved nitrogen gas ($^{28}N_2$, $^{29}N_2$ and $^{30}N_2$) were also analysed. Oxygen was analysed, no more than 12 h after fixation, using the Winkler method and an automatic potentiometric titrator (Mettler^{TM} DL 21). DIC was analysed immediately after sampling using a Shimadzu TOC-5000 carbon analyser. (NO_3^- + NO_2^-)-N was analysed according to Wood et al. (1967). The frequencies of the nitrogen gas-isotopes in water, which were collected in airtight glass vials, were analysed by mass-spectrometry using the procedure of Davidsson et al. (1997) and coupled nitrification-denitrification was calculated according to Nielsen (1992).

In Experiment II, effects of water flow velocity on epiphytic nitrification were determined using four microcosm systems with shoots of *P. pectinatus*, which were incubated at 0.03 and 9 cm s^{-1}, respectively, during twelve hours in light followed by twelve hours in dark. The macrophyte shoots were collected on September 12, 1995. Initially and after 12 and 24 hours, water was collected, replaced by nutrient solution, and analysed for oxygen, (NO_3^- + NO_2^-)-N and the isotope composition of nitrogen gas (see above). Rates of nitrification were determined by adding net nitrate production to rates of coupled nitrification-denitrification. Assimilative nitrate uptake by microorganisms and aquatic vascular plants is inhibited in the presence of high ammonium concentrations (Nichols & Keeney 1976; Reddy & DeBusk 1987; Kirchman 1994), and did therefore probably not affect the nitrate concentrations in this experiment.

In Experiment III, effects of water flow velocity on epiphytic denitrification were determined using twenty-four microcosm systems with shoots of *P. pectinatus*. The macrophyte shoots were collected on August 15, 1996. A preliminary study showed that there was no nitrification activity on these

shoots. $^{15}NO_3^-$ was added to the microcosms obtaining a concentration of 10 mg $^{15}NO_3^- - N\ L^{-1}$. To determine effects of flow velocity on epiphytic denitrification in the absence of oxygen, the microcosm systems were pre-incubated during 12 hours in dark without flow to remove oxygen before start of denitrification measurements. The pre-incubation was followed by a 6-hour denitrification incubation in dark, in which eight microcosms were incubated at zero, 0.03 or 9 cm s^{-1}, respectively. At the start and at the end of the 6-hour incubation water was collected for analysis of dissolved oxygen and nitrogen gas. Thereafter, to determine the relationship between epiphytic denitrification and oxygen metabolism at the different flow velocities, the microcosms were incubated for 5 hours in light followed by an additional 6-hour period in dark. To determine oxygen respiration and denitrification during the dark period, water was collected after 0, 1, 2, 4 and 6 hours for analysis of oxygen and after 0, 2 and 4 hours for analysis of nitrogen gas. At the end of the experiment, water was collected for analysis of $(NO_3^- + NO_2^-)$-N, dissolved organic carbon (DOC) and bacterial abundance. DOC was analysed on a Shimadzu TOC-5000 carbon analyser. Microscopic enumeration of bacteria was done by epiflourescent direct counts on black polycarbonate filters. The specific fluorescing DNA stain, 4′6-damidino-2-phenylindole (DAPI) was used as outlined by Porter and Feig (1980).

Data was analysed for statistically significant differences at the $p = 0.05$ level. Two-sample comparisons were made with the Students *t*-test or the paired sample *t*-test when appropriate. Arithmetic means are presented with standard errors. Comparisons of more than two treatments were conducted using the method of analysis of variance. In Experiment III, regression analyses were done on the initial oxygen concentration versus denitrification during the first 6-hour incubation. Regression analyses were also done on oxygen concentrations versus denitrification or oxygen respiration during the second 6-hour incubation period i.e. the period after switching from light to dark.

Results

In Experiment I, the oxygen outflux, i.e. net photosynthesis, from the macrophyte-epiphyton complexes was similar (*t*-test, $p = 0.51$; Figure 1), but the net uptake of DIC was about twice as large at 9 as at 0.03 cm s^{-1} (*t*-test, $p < 0.001$; Figure 1). Consumption of oxygen and production of DIC in the dark was larger at 9 than at 0.03 cm s^{-1} (*t*-test, $p < 0.01$; Figure 1). Gross photosynthetic production of oxygen and consumption of DIC, which were estimated as the sum of the net change in oxygen or DIC concentration in light

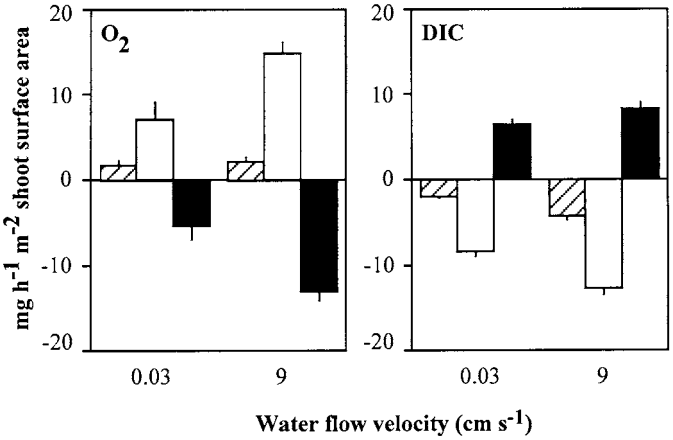

Figure 1. Net (striped bars) and gross (white bars) production of oxygen and consumption of dissolved inorganic carbon (DIC) in light, and oxygen consumption and DIC production in dark (black bars). Measurements were done in eight microcosms containing *P. pectinatus* and nutrient solution with a water flow velocity of 0.03 or 9 cm s^{-1} in Experiment I. Gross photosynthetic production of oxygen or consumption of DIC in light was estimated as the sum of the net flux of oxygen or DIC in light and dark. Error bars represent standard error. Initial concentrations were 6 ± 0.6 mg O_2 L^{-1} and 13 ± 0.3 mg DIC L^{-1} (means ± standard error, n = 16).

and in dark, was also larger at 9 than at 0.03 cm s^{-1} (t-tests, $p < 0.01$; Figure 1). There was no nitrification on the macrophyte shoots in Experiment I.

In Experiment II, there was no clear relationship between the epiphytic nitrification and the water flow velocity. In the light, rates of nitrification were similar at 0.03 and 9 cm s^{-1} (t-test, $p = 0.54$; Figure 2). In the dark, the results indicate that there were higher rates of nitrification at 9 than at 0.03 cm s^{-1} (Figure 2), but this difference was not statistically confirmed (t-test, $p = 0.23$). However, nitrification was almost twice as high in the light than in the dark at 0.03 cm s^{-1} (paired t-test, $p < 0.05$; Figure 2), but there were similar rates of nitrification in light and dark at 9 cm s^{-1} (paired t-test, $p = 0.24$; Figure 2). Initially, after 12 hours in light and after 12 hours in dark the mean oxygen concentration was 7.4 ± 0.11, 9.9 ± 0.15 and 5 ± 0.4 mg O_2 L^{-1} (n = 8). The change in oxygen concentration was similar at 0.03 and 9 cm s^{-1}, and the net production and consumption of oxygen was 4.6 ± 0.32 and 9.3 ± 0.6 mg O_2 m^{-2} h^{-1} in light and in dark, respectively.

In Experiment III, water flow velocity had significant, but complex effects on epiphytic denitrification. Denitrification was about six times higher in stagnant water and three times higher at 9 cm s^{-1}than at 0.03 cm s^{-1} during the first 6-hour incubation in dark (Figure 3). There were 1 ± 0.1 mg O_2 L^{-1} (n = 23) at the start and no oxygen at the end of this incubation period.

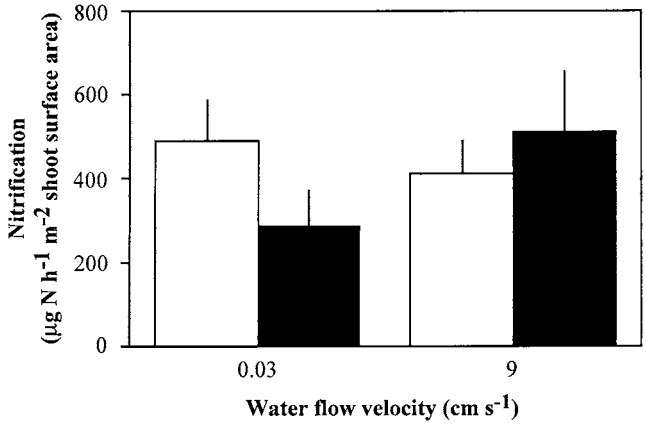

Figure 2. Rates of nitrification in four microcosms containing *P. pectinatus* and nutrient solution with a water flow velocity of 0.03 or 9 cm s^{-1} in light (white bars) and dark (black bars) in Experiment II. Error bars represent standard error.

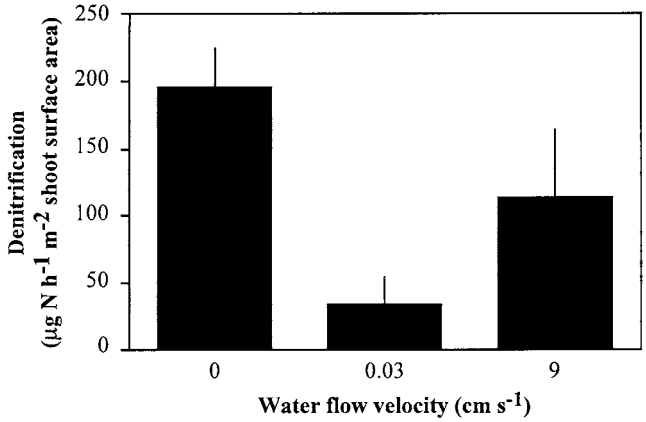

Figure 3. Rates of denitrification in eight microcosms containing *P. pectinatus* and nutrient solution with no flow (n = 7), or a water flow velocity of 0.03 or 9 cm s^{-1}, in dark and with oxygen-poor water in Experiment III. Error bars represent standard error.

No correlations were found between the initial very low oxygen concentrations and rates of denitrification during the first 6-hour incubation period (no flow: r^2 = 0.04, 0.03 cm s^{-1}: r^2 = 0.07, 9 cm s^{-1}: r^2 = 0.07, all data: r^2 = 0.01). No denitrification was detected during the light period or during the following 2 hours in dark. However, denitrification was present 2–4 hours after switching from light to dark, during which the oxygen concentration decreased to 1–3 mg O_2 L^{-1}in flowing water and remained high in stagnant water (Figure 4); hence, the oxygen consumption rates were higher in flowing than in stagnant water (Figure 5). Epiphytic denitrification was only

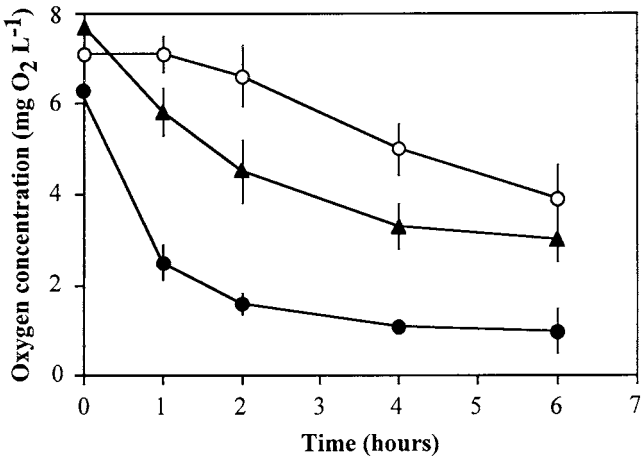

Figure 4. The change in oxygen concentration in eight microcosms containing *P. pectinatus* and nutrient solution with no flow (n = 7, white circles), or a water flow velocity of 0.03 cm s^{-1} (triangles) or 9 cm s^{-1} (black circles), after switching from light to dark in Experiment III. Error bars represent standard error.

present at low oxygen concentrations in flowing water, whereas in stagnant water denitrification could occur at high oxygen concentrations (Figure 6). Denitrification was inversely related to the oxygen concentration of the water (Figure 6). Contrasting to denitrification, oxygen respiration was positively correlated with oxygen concentration (Figure 5). The correlation was not significant with no flow, while it was clearly positively correlated with the oxygen concentration at 0.03 and 9 cm s^{-1} (Figure 5). Although oxygen respiration was positively and denitrification negatively correlated to the oxygen concentration of the water, significant rates of oxygen respiration and denitrification could occur simultaneously in the macrophyte-epiphyton complexes in stagnant water (Figures 5 and 6). In flowing water, denitrification only occurred when oxygen was absent or the concentration was low. Thus, epiphytic denitrification was significantly impeded in flowing water by oxygen, but it could occur in stagnant water at high oxygen concentrations and simultaneously with oxygen respiration.

At the end of Experiment III, the DOC concentration of the water was similar at 0.03 and 9 cm s^{-1}, but lower in stagnant water (Table 2). The bacterial abundance was similar in stagnant water and at 0.03 cm s^{-1}, but slightly higher at 9 cm s^{-1} (Table 2). In all of the experiments, pH was similar among the treatments and at the start and end of the experiments. The macrophyte DW and specific DW or AFDW of epiphyton on the macrophytes of the experiments are shown in Table 3. There were no significant differences in the specific DW or AFDW of epiphyton on macrophytes incubated at

Table 2. The concentration of dissolved organic carbon (DOC, $n = 8$) and bacterial abundance ($n = 3$) in the water of microcosm systems with no flow, or a flow velocity of 0.03 or 9 cm s^{-1} in Experiment III. Significance of difference between the treatments according to one-factor ANOVA is shown. Means ± standard error

	No flow	0.03 cm s^{-1}	9 cm s^{-1}	Significance
DOC (mg C L^{-1})	7.4 ± 1.7	16 ± 1.8	15 ± 0.6	$p < 0.001$
Bacteria (cells mL^{-1})	0.4 ± 0.1 × 10^6	0.4 ± 0.1 × 10^6	0.6 ± 0.2 x 10^6	$p = 0.64$

different water flow velocities (ANOVA analyses, p values between 0.30 and 0.57).

Discussion

Water flow had a positive effect on photosynthesis and oxygen respiration in the macrophyte-epiphyton complexes. The oxygen and DIC metabolism was probably controlled by the influx of oxygen and DIC from the surrounding water, since this has been shown to increase with increasing water flow velocity (Dawson et al. 1981; Madsen & Sand-Jensen 1991). Madsen and Sand-Jensen (1991) suggested that boundary layer resistance is the major determinant of the photosynthetic rate of submersed macrophytes. The linear relationship between dark respiration and oxygen concentration in Experiment III (Figure 5) was similar to that found by Dawson et al. (1981) and Strand et al. (1985). Conclusively, the results of the present study indicate that flow velocity can have a significant impact on the oxygen and DIC metabolism in submersed vegetation.

The effect of water flow velocity on nitrification was not as conspicuous as that on oxygen respiration and denitrification. Nitrification was higher in light than in dark at 0.03 cm s^{-1}, but not at 9 cm s^{-1}. Sand-Jensen et al. (1985) showed that oxygen concentrations in epiphyton could change from zero in the dark to 2.5 times over-saturation in light in systems with low water flow. Moreover, Kühl et al. (1996) showed that the oxygen concentration within biofilms decrease with increasing flow velocity in light, and suggested that produced oxygen is washed out of the biofilm communities at high flow velocities. These earlier studies indicate that photosynthetically produced oxygen could be concentrated at macrophyte surfaces at a flow of 0.03 cm s^{-1}, possibly promoting epiphytic nitrification in light, but to a large extent transported out of biofilm communities at a high flow velocity such as 9 cm s^{-1}. Thus, diurnal changes in oxygen concentrations within epiphytic

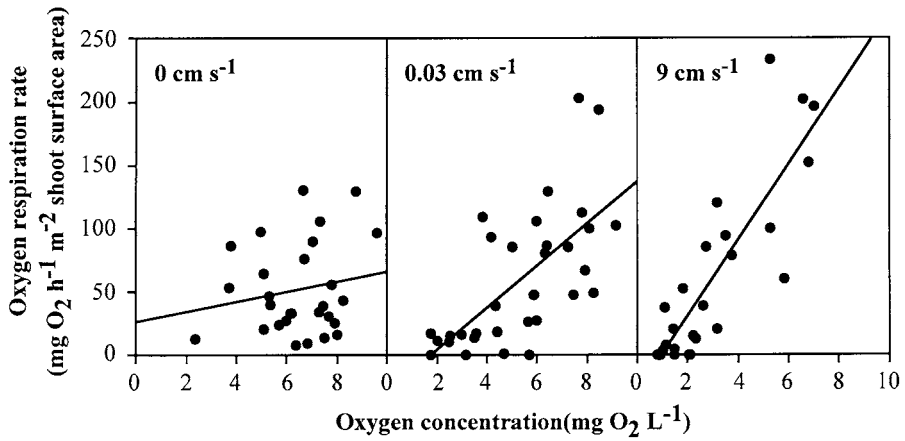

Figure 5. The oxygen respiration rate versus the oxygen concentration in eight microcosms containing *P. pectinatus* and nutrient solution with no flow ($r^2 = 0.18$, $p = 0.37$), 0.03 cm s^{-1} ($r^2 = 0.44$, $p < 0.0001$) or 9 cm s^{-1} ($r^2 = 0.74$, $p < 0.0001$) during the second incubation period in the dark, i.e. after switching from light to dark, in Experiment III.

communities due to the oxygen metabolism are probably more pronounced at low than at high flow velocities, which might explain why nitrification differed between light and dark at 0.03 cm s^{-1}, but not at 9 cm s^{-1}. However, although nitrification was affected by the light conditions at 0.03 cm s^{-1}, there were significant rates of nitrification in the light as well as in the dark. Conclusively, the results of the present study indicate that there can be a considerable nitrification in submersed vegetation in light and in dark and at very different water flow velocities. Therefore, if ammonium is available, epiphytic nitrification is probably present diurnally both in the outer sections of submersed vegetation, where there are large water movements and much of oxygen, and in the inner sections, where there can be low flow and poor oxygen conditions.

Denitrification has been found in various attached microbial communities both on different marine macro-algae and on submersed vascular freshwater plants (Law et al. 1993; Eriksson & Weisner 1996, 1997, 1999). In a nutrient-rich lowland stream, Sørensen et al. (1988) collected epilithic biofilms, which were incubated in recirculating water at a flow velocity of 15 cm s^{-1}, and denitrification ranged between 0 and 800 μg N m^{-2} h^{-1}. In epilithic biofilms of the San Francisco creek, Duff et al. (1984) recorded denitrification rates of 750 μg N m^{-2} h^{-1} in incubation systems with recirculating water. In these studies denitrification was measured in recirculating water systems, but only at a single flow velocity.

Table 3. The dry weight (DW) of the *P. pectinatus* shoots that were used in the experiments and the specific DW and ash free dry weight (AFDW) of the epiphyton on the macrophyte shoots. Means ± standard error

	n	Plant DW (mg)	Epiphyton DW (mg g^{-1} plant DW)	Epiphyton AFDW (mg g^{-1} plant DW)
Experiment I	8	290 ± 23	58 ± 10	41 ± 7
Experiment II	16	103 ± 6	144 ± 16	64 ± 8
Experiment III	23	115 ± 8	334 ± 39	274 ± 33

In the present study, the water flow velocity significantly affected epiphytic denitrification. Denitrification was higher in stagnant water and at 9 than at 0.03 cm s^{-1}in the first incubation period of Experiment III during which oxygen was mainly absent. The macrophyte-epiphyton complexes completely consumed the small amounts of oxygen initially present. At these low oxygen concentrations, the oxygen consumption rate was similar among the water flow velocities (Figure 5), and no correlations between the initial oxygen concentrations and denitrification were found ($r^2 \leq 0.07$ in all cases). Therefore, the differences in denitrification between the water flow treatments were most likely not due to differences in the inhibitory effect of dissolved oxygen on denitrification. Furthermore, since the microcosms contained excessive amounts of nitrate, differences in the supply of nitrate are also unlikely to have caused the differences in denitrification among the treatments. Earlier studies have shown that denitrification in anaerobic biofilm layers is limited by the supply of organic carbon in systems with high nitrate concentrations (e.g. Nielsen et al. 1990). Biofilm bacteria can be supported with organic carbon from the microbial exo-polysaccharide mucus, which constitutes a major part of biofilm communities (Freeman & Lock 1995). The organic products of enzymatic polysaccharide hydrolysis are retained in proximity to the biofilm bacteria in stagnant water, but can be washed away in flowing water. Attached bacteria in flowing water may be compensated for the losses of organic carbon by uptake from the water. Organic substances are efficiently transported from the surrounding water into biofilm communities at high flow velocities (Gantzer et al. 1988). Furthermore, Zobell (1943) and Freeman et al. (1995) among others have suggested that a biofilm acts as an exchange resin from which some organic compounds are released while others are adsorbed, and that the outcome is affected by environmental conditions such as water flow velocity. Lock et al. (1984) suggested that labile compounds such as amino acids more easily penetrate into biofilms at higher flow velocities. Thus, if the net in-flux of easily degradable organic

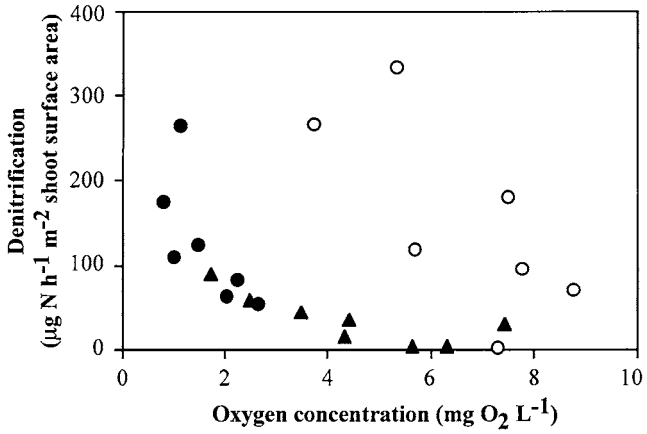

Figure 6. The denitrification rate versus the oxygen concentration in eight microcosms containing *P. pectinatus* and nutrient solution with no flow ($r^2 = 0.49$, $p = 0.07$, white circles), 0.03 cm s^{-1} ($r^2 = 0.62$, $p < 0.05$, triangles) or 9 cm s^{-1} ($r^2 = 0.53$, $p < 0.05$, black circles), during the second incubation period in the dark, i.e. after switching from light to dark, in Experiment III.

compounds compared to recalcitrant ones was higher at 9 cm s^{-1} than at 0.03 cm s^{-1}, then heterotrophic biofilm denitrification would be higher at 9 cm s^{-1}. In stagnant water, denitrification was probably supported by internal biofilm sources of organic compounds.

In the systems with flowing water, the response of denitrification rate to oxygen concentration was similar to that which has been recognised earlier by Tiedje (1988) and Law et al. (1993). Law et al. (1993) found in measurements of denitrification on marine macroalgae in a flow-cell with recirculating water that epiphytic denitrification was absent until the oxygen concentration was about 1.6 mg O_2 L^{-1}, and below this value epiphytic denitrification increased rapidly with decreasing oxygen concentration. Tiedje (1988) found that denitrification is dramatically lowered with a slight increase in the oxygen concentration from zero, and suggested the presence of an oxygen threshold value at which facultative bacteria distinctly shift from using nitrate to using primarily oxygen as electron acceptor. In the present study, the rapid increase in epiphytic denitrification between 1–2 mg O_2 L^{-1} and the fact that oxygen respiration was halted at this concentration support the idea of a conspicuous shift from aerobic to anaerobic respiration over a narrow range of oxygen concentrations.

The more distinct response of both denitrification and oxygen respiration on changes in oxygen concentration in flowing than in stagnant water, was probably due to steeper gradients in the oxygen concentration across the macrophyte-water interfaces, and a more efficient transfer of oxygen from

the water to the macrophyte surfaces in flowing water (Kühl et al. 1996). Due to thick boundary layers and a poor transfer of oxygen from the overlying water to the biofilms (Stevens & Hurd 1997), there were probably anaerobic microzones within the epiphytic biofilms allowing denitrification to occur simultaneously with oxygen respiration in the microcosms with stagnant water. Microsensor studies of biofilm communities in systems with absent or low flow have shown that oxygen respiration can occur in the outer layers of biofilms while denitrification is present in deeper anaerobic layers (Nielsen et al. 1990). Strand et al. (1985) found that oxygen respiration could occur simultaneously with denitrification in wastewater reactor biofilms in flowing water and at high oxygen concentrations, if the biofilms contained bacterial densities greater than 2×10^9 cells cm^{-2}. Significant rates of denitrification have also been determined in thick natural epilithic biofilms, which were collected from a highly nutrient-rich stream, and incubated at a flow of 15 cm $s^{-1}$and at an oxygen concentration above 50% of air saturation (Sørensen et al. 1988). However, in the present study, although there were high biofilm densities, epiphytic denitrification was significantly impeded in flowing water, except when the oxygen concentration of the surrounding water was very low.

Dense submersed vegetation has high oxygen consumption rates (Prahl et al. 1991; Eriksson & Weisner 1997, 1999), and flow velocities are significantly impeded by its presence (Madsen & Warnacke 1983; Loose & Wetzel 1993; Sand-Jensen & Mebus 1996). Submersed macrophytes and their epiphytes can therefore significantly lower oxygen concentrations in the water (Prahl et al. 1991; Eriksson & Weisner 1997, 1999). In a shallow macrophyte-dominated reservoir, Eriksson and Weisner (1997) found that the oxygen concentrations were lowered down to 1–3 mg O_2 L^{-1} during the night in the submersed vegetation. Prahl et al. (1991) found that submersed vegetation in a nutrient-rich stream could reduce oxygen concentrations from about 10 to 3 mg O_2 L^{-1} during the night. Thus, although high flow velocities are present in the open water of streams and lakes, the water flow velocities within submersed vegetation are much lower, and the metabolic activity of the macrophyte-epiphyton can induce low oxygen concentrations in dark promoting epiphytic denitrification.

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