TITLE: Periphyton contribution to nitrogen dynamics in the discharge from a wastewater treatment plant

SHORT TITLE:

Contribution of periphyton to N dynamics

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ABSTRACT

To evaluate the importance of periphyton to nitrogen dynamics in the discharge from wastewater treatment plants (WWTPs), we examined changes in total and inorganic nitrogen content downstream from a WWTP on the Kurose River in Hiroshima Prefecture, Japan. At 0.7 km downstream of the WWTP (point A), NH_4^+-N was the dominant form of inorganic nitrogen, but concentrations decreased rapidly to 5 km downstream (point B). In contrast, no significant change in the [NO_2^- + NO_3^-]-N concentration was observed between the two points. Total nitrogen (TN) load decreased significantly between the two points, suggesting that sorption and/or denitrification occurred in the river channel. Potential rates of nitrogen sorption and transformation by periphyton were determined in a laboratory experiment in which changes in the nitrogen content of river water were examined in an acrylic chamber with periphyton. Nitrification and nitrogen removal occurred mainly in the periphyton. The contributions of periphyton activity to TN and NH_4^+-N decrease in the field, as estimated from the results of the laboratory experiments, were 6%–18% and 23%–72%, respectively. These results suggest that periphyton plays an important role in decreasing NH_4^+-N concentration in the discharge from wastewater treatment plants.

KEY WORDS: nitrogen; periphyton; wastewater treatment plant; nitrification; ammonium nitrogen; TN; river

INTRODUCTION

Discharge from wastewater treatment plants (WWTPs) is one of the most important sources of nitrogen, especially ammonium nitrogen (NH_4^+-N), in rivers in urbanized areas (Brion and Billen, 2000; Féray and Montuelle, 2003; Cébron *et al*., 2004; Drolc *et al*., 2007). This nitrogen, especially as un-ionized ammonia (NH_3), has a substantial influence on water quality and the health of fish and other aquatic organisms in the receiving waters (e.g. Arthur *et al*., 1987; van Katwijk *et al*., 1997; Schuytema and Nebeker, 1999). Nitrogen input also stimulates algal growth in seas and lakes, ultimately leading to eutrophication (Ryther and Dunstan, 1971). In addition, oxidation of the NH_4^+-N (nitrification) results in an increase in biochemical oxygen demand (BOD) downstream of WWTPs (Deai *et al*., 1991; Brion and Billen, 2000; Ogura *et al*., 2006).

Nitrogen compounds in river water are transformed or removed by biological processes. Several authors have pointed out that periphyton is especially abundant in river channels downstream of WWTPs (Montuelle *et al*., 1996; Teissier *et al*., 2002; Ange *et al*., 2006). Periphyton is a complex matrix of algae and heterotrophic microbes attached to submerged substrata and is therefore likely to affect nitrogen dynamics in several ways. Abe *et al*. (2003) reported that algal biofilm removed inorganic nitrogen from wastewater in an experimental study. However, little is known about the role of periphyton in the nitrogen dynamics in the discharge from WWTPs in the field.

The aim of this study was to evaluate the importance of periphyton to the nitrogen dynamics in the discharge from WWTPs. First, we examined changes in total and inorganic nitrogen content downstream of a WWTP. Second, we determined the rates of nitrogen sorption and transformation by periphyton in a laboratory experiment. Using these data, we estimated the contribution of periphyton to the changes in total and inorganic nitrogen content observed in the field.

STUDY SITE AND METHODS

Study site

The study site was situated in the middle reach of the Kurose River in Hiroshima Prefecture, Japan (34˚23'N, 132˚43'E). The Kurose River (length, 50.6 km) has a catchment area of 238.8 km^2 and flows southward into the Seto Inland Sea. Higashi-Hiroshima WWTP receives wastewater from about 53 000 inhabitants in Higashi-Hiroshima City, and in 2005 it treated about 24.3×10^3 m^3 of sewage daily by a

classic activated sludge process (Higashi-Hiroshima City, 2007); the effluent was discharged into the Kurose River. The wastewater discharge of the Higashi-Hiroshima WWTP has been increasing because the population in Higashi-Hiroshima City has increased steadily during the last decade (Ogura and Nakatsubo, 2004).

Nitrogen dynamics in the field

To examine the changes in total and inorganic nitrogen content in the river channel, we collected water samples at two points, A and B, which were located 0.7 and 5.7 km, respectively, downstream of the WWTP. The average depth and width of the channel were 0.47 and 27.5 m at point A, and 0.58 and 13.8 m at point B. There were a few stream inflows between points A and B, but the effect of these inflows was assumed to be negligible.

Samples were collected at points A and B on 27 June, 19 July, and 31 July 2007. The surface water samples were collected with polyethylene bottles (100 mL) and brought back to the laboratory on each sampling date. Total nitrogen (TN) in un-filtered samples was determined by the ultraviolet absorption spectroscopic method coupled with oxidation by potassium peroxodisulphate (Nydahl, 1978). The samples were filtered through glass fiber filters (GC-50, Advantec, Tokyo, Japan) with a nominal particle retention size of 0.45 μm, and stored at 5 ˚C until measurement of inorganic nitrogen (within 6 days). NH_4^+-N was analyzed by the indophenol blue method (Ivančič and Degobbis, 1984). Nitrite nitrogen (NO_2^--N) was analyzed by the diazotization method (Bendschneider and Robinson, 1952). Nitrate nitrogen (NO_3^--N) was analyzed with an ion chromatograph (PIA-1000, Shimadzu, Kyoto, Japan) with a Shim-pack IC-A1 column (Shimadzu GLC Ltd, Tokyo, Japan). In all samples, the concentration of NO_2^--N was much lower than that of NO_3^--N. In the following text, therefore, we present the sum of NO_2^--N and NO_3^--N ([NO_2^- + NO_3^-]-N). Nitrogen loads were calculated from the concentrations and the river flow. River flow rate was measured by an electric velocity meter (Kamiyama-seisakusho Co., Ltd, Tokyo, Japan).

Laboratory experiments

Nitrogen sorption and transformation potentials by periphyton were determined by a simple experimental system composed of an acrylic incubation chamber (200 × 200 × 150 mm) and a pump (Fig. 1).

Stones (approximately 100–150 mm in diameter) with attached periphyton were collected on each sampling date at point A. Enough stones were placed in the experimental chamber to cover the bottom (usually 4 stones per chamber). The chamber

and connecting tubes were filled with river water (about 5–6 L) collected on each sampling date at point A. The water was circulated with the pump at a flow rate similar to that at the point of collection (approximately 12 L min^{-1}). Water temperature in the chamber was controlled at the average water temperature in the field (15 ˚C from April to May and 25 ˚C from June to July). Unless otherwise noted, light was provided by a 1000 W mercury lamp. Photon flux density (PFD) just above the chamber was about 1000 μmol m^{-2} s^{-1}. At 0, 1, 2, 3, 4, 5, and 6 h after the beginning of the experiment, an aliquot of the water (100 mL) was collected from the chamber and replaced with the same amount of deionized water. Total and inorganic nitrogen concentrations in the water samples were measured by the same procedures described above. A similar experiment without stones was carried out as a control. The experiments were conducted on 27 April, 9 July, and 19 July 2007.

To study the effect of light on the activity of periphyton, another set of experiments was conducted under light and dark conditions. These experiments were conducted on 16 May, 27 June, and 31 July 2007.

RESULTS AND DISCUSSION

Nitrogen dynamics in the field

At point A (0.7 km from WWTP), NH_4^+-N was the dominant form of nitrogen with an average concentration of 7.6 mg N L^{-1}, which is about 4 times larger than the [NO_2^- + NO_3^-]-N concentration. This agrees with previous studies of discharges from WWTPs by the classic activated sludge process (Brion and Billen, 2000; Ogura *et al*., 2006). The NH_4^+-N concentration dropped to 2.0 mg N L^{-1} at point B (5.7 km from WWTP). In contrast, there was no significant change in the [NO_2^- + NO_3^-]-N concentration between points A and B (*t*-test; Fig. 2a). Total nitrogen (TN) concentrations also decreased from points A to B (Fig. 2a) suggesting that sorption, denitrification, or both, occurred in the river channel.

Changes in nitrogen load (concentration × river flow) showed patterns similar to those of the nitrogen concentrations: the TN and NH_4^+-N load dropped significantly between points A and B, whereas no significant change in the [NO_2^- + NO_3^-]-N load was observed (Fig. 2b). However, the river flow at point B was about 25% less than that at point A (Fig. 2b). Therefore, the decreases in the TN and NH_4^+-N load were partly due to the decline in the river flow. Saito *et al*. (2005) reported similar nitrogen missing in a coastal alluvial fan catchment, in which they attributed the missing nitrogen to runoff into groundwater.

Laboratory experiments

In the laboratory experiment conducted on 27 April 2007, we found that, in the chambers with stones, the NH_4^+-N concentration decreased whereas [NO_2^- + NO_3^-]-N concentration increased significantly with time (one-way ANOVA, $p < 0.05$), suggesting that nitrification took place during the 6-h experimental period (Fig. 3a). TN concentrations also decreased in the chamber with stones (Fig. 3a). This indicates that nitrogen removal due to nitrogen sorption and/or denitrification occurred in this chamber. In contrast, there were no significant changes in any form of nitrogen in the control chamber (without stones) (Fig. 3b). These results suggest that nitrification took place mainly in the surface of stones (periphyton). The other two experiments conducted on 9 July and 19 July 2007 showed almost the same trends (data not shown).

Light conditions had little effect on the dynamics of inorganic nitrogen: NH_4^+-N concentration decreased with time (one-way ANOVA, $p < 0.05$) and the [NO_2^- + NO_3^-]-N concentrations increased significantly both in light and dark conditions (Fig. 4). However, TN concentration decreased with time only under light conditions (one-way ANOVA, $p < 0.05$). This light dependence of nitrogen removal may be explained partly by the activity of microalgae in periphyton. Microscopic observation revealed that microalgae were among the most numerous components of the periphyton at our study site. Pizarro *et al*. (2002) reported that algal turf removed TN from dairy wastewater at a rate of 0.72 g N m^{-2} day^{-1}. The rate of nitrogen removal in our laboratory experiment (1.19–2.58 g N m^{-2} day^{-1}), calculated from Table 1 was similar to the value reported by Pizarro *et al*. (2002).

Contribution of periphyton to the nitrogen dynamics in river water

The results of the laboratory experiments showed that periphyton on the stone surfaces played an important role in the nitrogen dynamics in river water. From these results, we estimated the contribution of periphyton activity to nitrogen removal from wastewater flowing through point A to point B. It takes about 6 h (3.9–8.8 h) for the river water to flow from point A to B (Nobuso *et al*., 1978). The amount of nitrogen removed or transformed during this period, Q_f (mg N), was calculated as:

$$Q_f = F_f T(C_A - C_B) \quad (1)$$

where F_f is river flow rate (L h^{-1}), T is the time to flow from point A to B (6 h), C_A is the nitrogen concentration at point A (mg N L^{-1}), and C_B is the nitrogen concentration at point B (mg N L^{-1}).

The amount of nitrogen removed by periphyton activity in laboratory experiments, Q_e

(mg N), was calculated as:

$$Q_e = F_e(C_i - C_a) \quad (2)$$

where F_e is the volume of water in the chamber (L), C_i is the initial nitrogen concentration in the chamber (mg N L^{-1}), and C_a is the nitrogen concentration in the chamber after 6 h (mg N L^{-1}). Therefore, the contribution of periphyton activity to the nitrogen decrease R (%) was calculated as:

$$R = Q_e / Q_f \times A_f / A_e \times 100 \quad (3)$$

where A_f is the area of the river bottom between points A and B (120×10^3 m^2), and A_e is the bottom area of the acrylic chamber (0.04 m^2). The values used for these calculations are summarized in Table 1. For these calculations, the river flow at point B was used as the value for F_f to avoid the effect of the river flow decrease between points A and B.

The contribution of periphyton (substratum) activity to the TN removal from river water flowing from point A to B was estimated to be 6%–18% (Table 1). This result shows that periphyton activity only partly explains the TN decrease observed in the field. Another factor that might contribute to the TN decrease is dilution by river–groundwater exchange. Saito *et al*. (2005), who discussed the nitrate transport process in a coastal alluvial fan catchment, suggested that the NO_3^--N load in the stream water declined as a result of incorporation into ground water. It is also likely that NH_4^+-N was adsorbed to cation-exchange sites on the surfaces of organic sediments and plants (Wittgren and Tobiason, 1995). Aquatic and emergent macrophytes were relatively rare at our study site; therefore, their contribution to the TN removal is likely to be small. Volatilization of NH_4^+-N may also contribute to the TN decrease, although Mahne *et al.* (1996) determined a maximum of 6% ammonia volatilization from highly nitrogenous (1600 mg NH_4^+-N L^{-1}) wastewater.

On the other hand, 23%–72% of the NH_4^+-N decrease can be attributed to periphyton activity (Table 1). Periphyton could affect the NH_4^+-N concentration in several ways: physical adsorption to cation-exchange sites, biological absorption, and nitrification. The results of the laboratory experiments indicate that nitrification is more important than physical adsorption and biological absorption in decreasing NH_4^+-N concentrations.

In conclusion, periphyton plays a major role in decreasing NH_4^+-N in the discharge from wastewater treatment plants, whereas the periphyton contribution to TN removal is limited. However, recent studies indicate that a significant amount of nitrogen is denitrified after it is incorporated into ground water (Spalding and Parrott, 1994; Dillon *et al*., 2007). Therefore, rapid nitrification by periphyton may promote removal of

nitrogen at the watershed level.

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Table 1. Values used for the calculation of the contribution of periphyton activity to nitrogen dynamics in the river channel. The values are from both field measurements and laboratory experiments on 27 June, 19 July, and 31 July 2007.

	NH_4^+-N			TN		
	27 Jun	19 Jul	31 Jul	27 Jun	19 Jul	31 Jul
Field measurements						
C_A: nitrogen concentration at point A (mg N L^{-1})	9.13	3.71	10.11	12.05	6.44	13.9
C_B: nitrogen concentration at point B (mg N L^{-1})	2.71	0.47	2.85	6.2	2.93	7.22
F_f: river flow rate (at point B; L h^{-1})	4.52×10^6	10.38×10^6	2.43×10^6	4.52×10^6	10.38×10^6	2.43×10^6
Q_f: nitrogen removed or transformed in 6 h in the field (mg N)	1.74×10^8	2.02×10^8	1.06×10^8	1.59×10^8	2.19×10^8	0.98×10^8
Laboratory experiments						
C_i: initial nitrogen concentrationin of chamber (mg N L^{-1})	8.71	3.62	8.23	12.06	6.46	11.55
C_a: nitrogen concentration of chamber after 6 hours (mg N L^{-1})	2.94	0.53	3.58	10.23	5.58	10.64
F_e: volume of chamber (L)	5.15	4.95	5.46	5.15	4.95	5.46
Q_e: nitrogen removed by periphyton activity in 6-h laboratory experiments (mg N)	29.72	15.3	25.39	9.42	4.36	4.97
R: contribution of periphyton activity to nitrogen decrease (%)	51	23	72	18	6	15

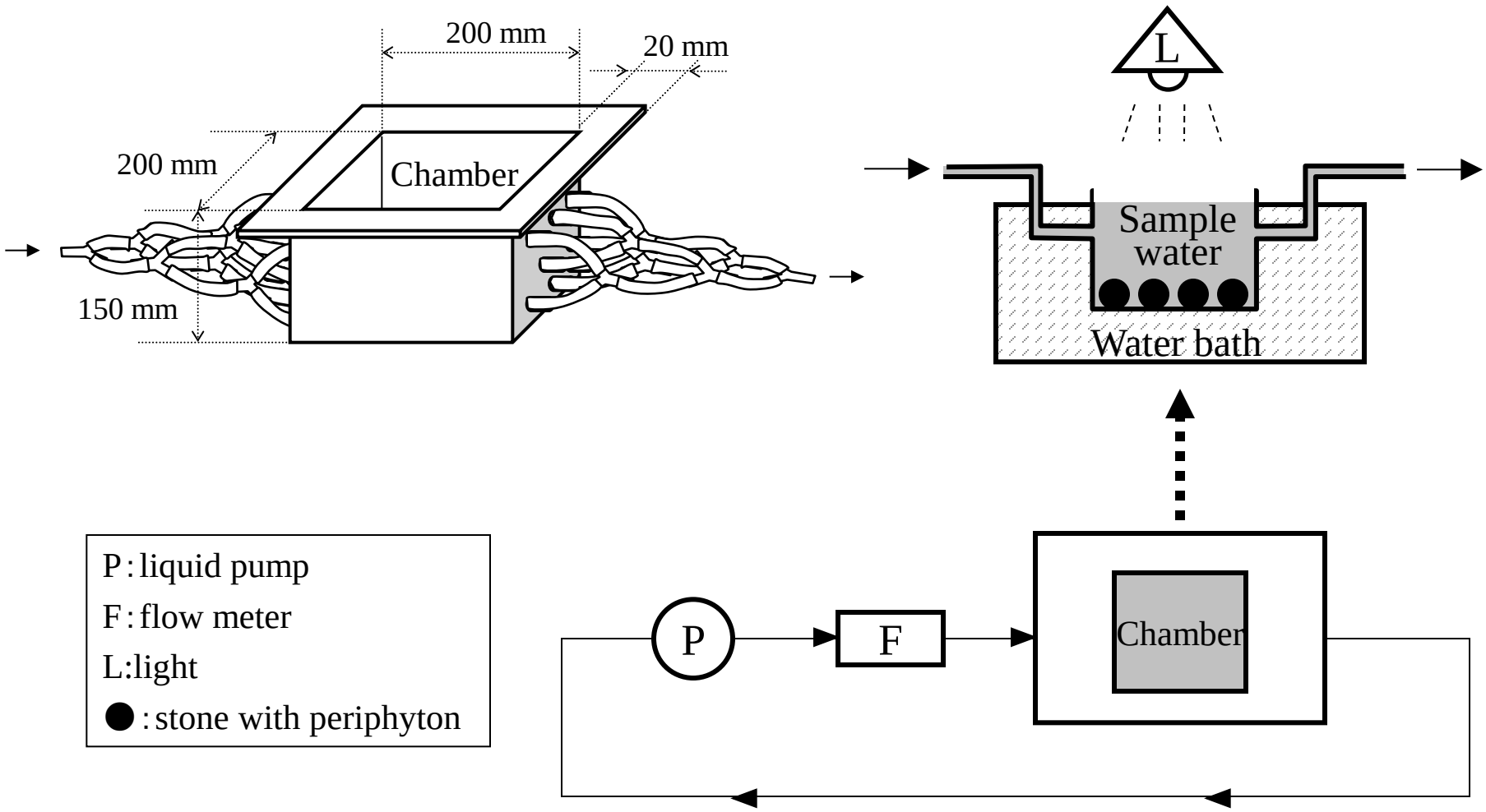

Figure.1 : Experimental system for the measurements of effect of periphyton activity on nitrogen dynamics in river. System for open-flow method: The sample water circulates through round the system at flow rate of 12 L min^{-1}.

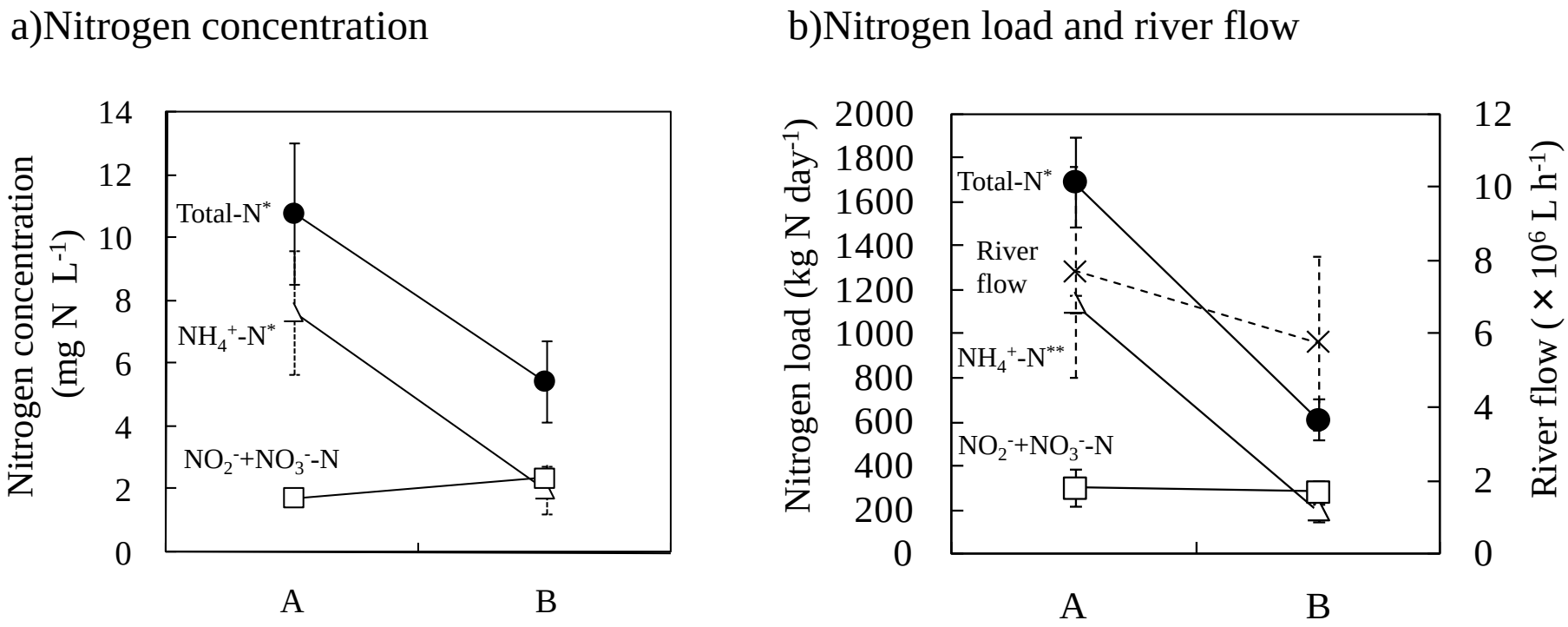

Figure.2: Difference in nitrogen concentration (a), nitrogen load and river flow rate (b) between point A and B (5 km intervals) in the Kurose River. Each value is the mean of 3 measurements (June27, July 19, and July 31, 2007). Vertical bars represent standard error (n=3). Significance levels (t-test): ** $P<0.01$, * $P<0.05$.

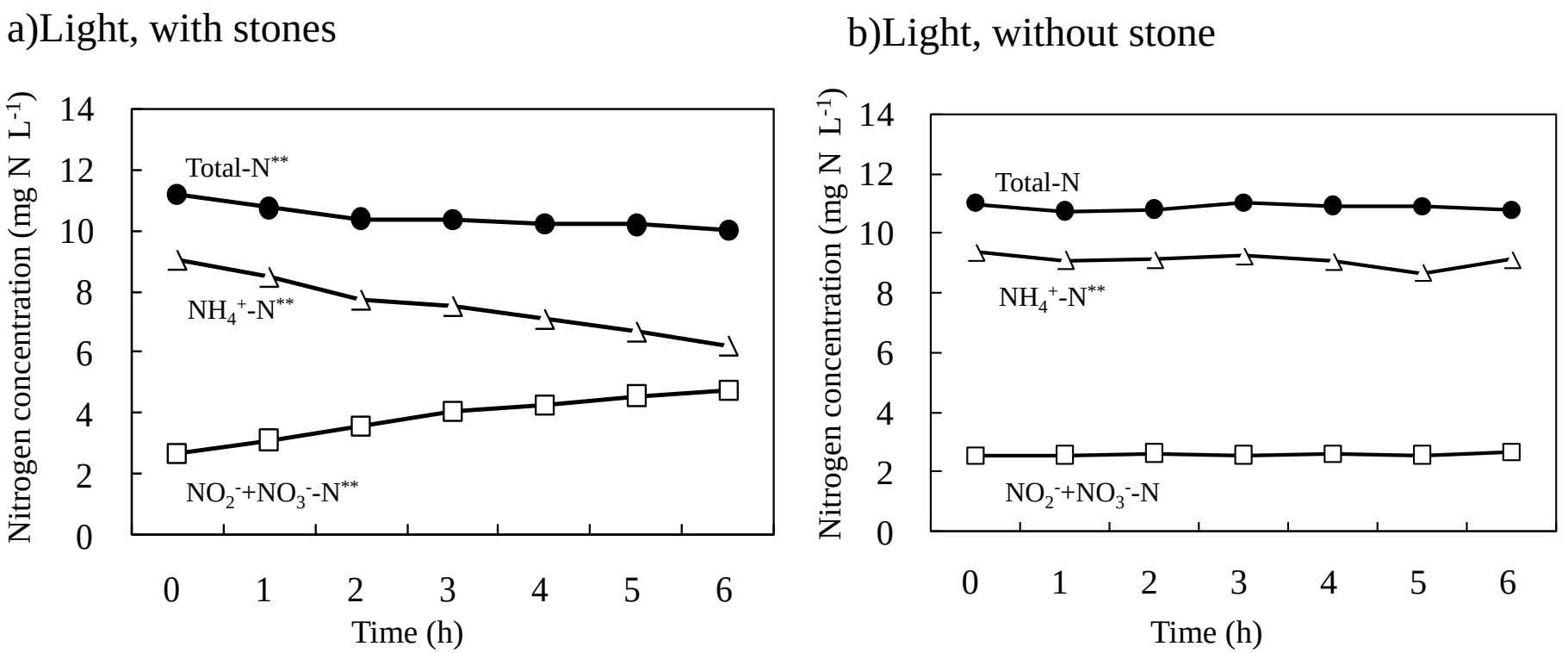

Figure.3: Variation in nitrogen concentration for 6 hours in acrylic chamber fill with river water and periphyton (stones) (a), and without stone (b) in light condition. The value measured in April 27, 2007. Vertical bars represent standard error (n=3). Significance levels (ANOVA): ** $P<0.01$, * $P<0.05$.

a)Light, with stones

b)Dark, with stones

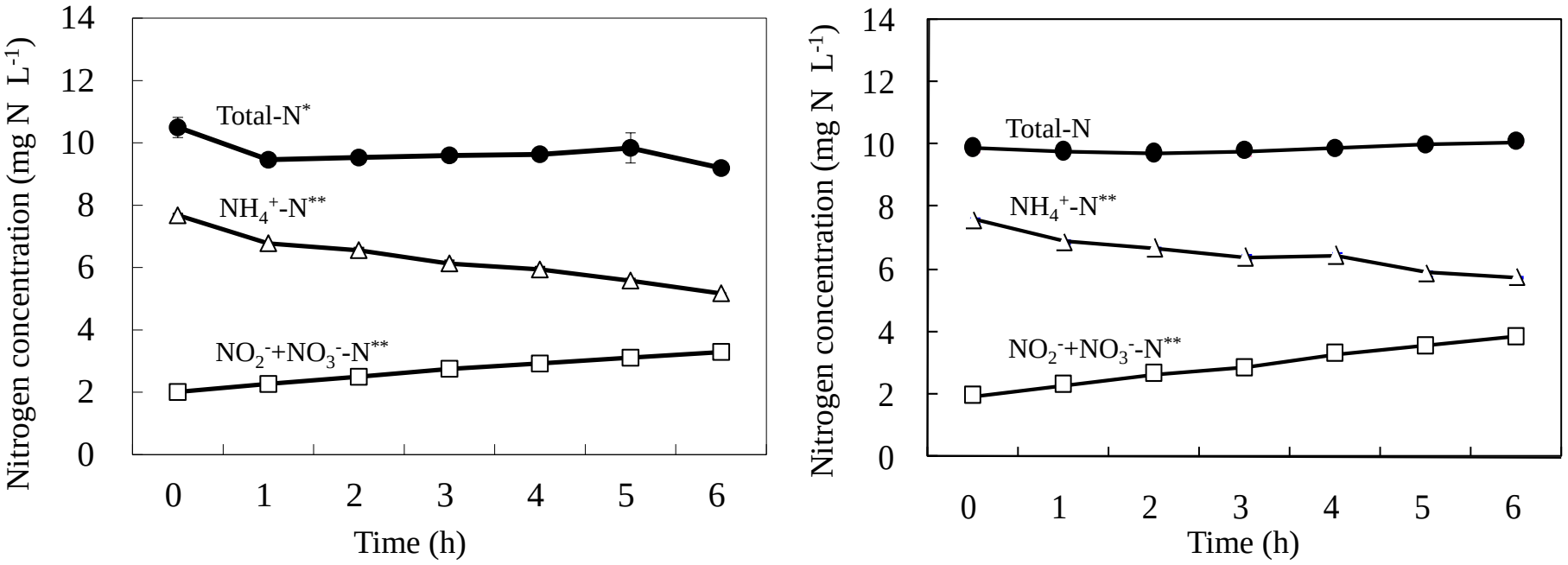

Figure.4: Variation in nitrogen concentration for 6 hours in acrylic chamber filled with river water and periphyton (stone), in light condition (a) and in dark condition (b) . The value measured in May 16, 2007. Vertical bars represent standard error (n=3). Significance levels (ANOVA): ** $P<0.01$, * $P<0.05$.

Table.1: Calculation value for the contribution of periphyton activity to nitrogen dynamics in river channel. The value is measured in June 27, July 19, and July 31, 2007, which performed both field measurements and laboratory experiments.

	NH_4^+-N			Total-N		
	27 June	19 July	31 July	27 June	19 July	31 July
Field measurements						
C_A: nitrogen concentration of point A (mg N L^{-1})	9.13	3.71	10.11	12.05	6.44	13.90
C_B: nitrogen concentration of point B (mg N L^{-1})	2.71	0.47	2.85	6.20	2.93	7.22
F_f: river flow rate (at point B) (L h^{-1})	4.52×10^6	10.38×10^6	2.43×10^6	4.52×10^6	10.38×10^6	2.43×10^6
Q_f: amount of nitrogen removed or transformed in 6 hours at fields (mg N)	1.74×10^8	2.02×10^8	1.06×10^8	1.59×10^8	2.19×10^8	0.98×10^8
Laboratory experiments						
C_i: initial nitrogen concentration of chamber (mg N L^{-1})	8.71	3.62	8.23	12.06	6.46	11.55
C_a: nitrogen concentration of chamber after 6 hours (mg N L^{-1})	2.94	0.53	3.58	10.23	5.58	10.64
F_e: volume of chamber (L)	5.15	4.95	5.46	5.15	4.95	5.46
Q_e: amount of nitrogen removed by periphyton activity in 6 hours at laboratory experiments (mg N)	29.72	15.30	25.39	9.42	4.36	4.97
R: contribution of nitrogen decrease by periphyton activity (%)	51	23	72	18	6	15