

Photochemically produced bioavailable nitrogen from biologically recalcitrant dissolved organic matter stimulates production of a nitrogen-limited microbial food web in the Baltic Sea

Anssi V. Vähätalo¹

Tvärminne Zoological Station, University of Helsinki, 10900 Hanko, Finland

Marko Järvinen²

Lammi Biological Station, University of Helsinki, 16900 Lammi, Finland

Abstract

Experiments were designed to assess whether the photochemical production of bioavailable nitrogen (N) from biologically recalcitrant dissolved organic nitrogen (DON) stimulates growth of N-limited plankton. Filtered Baltic Sea water was exposed to solar radiation for 12–19 days. Along with dark controls, samples were inoculated with an indigenous nanoplankton (<10 μm) community and incubated under photosynthetically active radiation and N-limiting conditions (molar dissolved inorganic nitrogen to PO_4^{3-} ratio of 0.2). The concentration of particulate nitrogen, chlorophyll *a* (Chl *a*), and the biomass of phytoplankton and protozoa increased more in the solar radiation-exposed waters than in dark controls, showing that the photochemical transformation of dissolved organic matter (DOM) supported both heterotrophic and autotrophic plankton. In order to calculate the apparent quantum yield (AQY) for the photochemical reactions, the experimentally determined photoproduced bioavailable N (the formed particulate N and mineralized DON) and the phytoplankton biomass stimulated by DOM photochemistry were related to the number of photons absorbed by chromophoric DOM. Model calculations based on AQY indicated that DOM photochemistry produces 22–26 μmol bioavailable N $\text{m}^{-2} \text{d}^{-1}$ and stimulates phytoplankton biomass by 12–14 μg Chl *a* $\text{m}^{-2} \text{d}^{-1}$ during summer in the northern Baltic Sea. Photoproduced bioavailable N potentially supports 1.2% and 3.6% of the new primary production and its N-demand, respectively, in this study region during summer. In N-limited surface waters, the photoproduction of bioavailable N from biologically recalcitrant, but photoreactive DOM can provide a new source of N and sustain the background productivity of a nanoplankton community, including phytoplankton.

The productivity of aquatic ecosystems is frequently limited by the amount of bioavailable nitrogen ([N] Rabalais 2002; Bergström et al. 2005). Bioavailable dissolved N consists of dissolved inorganic N ([DIN] = NH_4^+ , NO_2^- , and NO_3^-) as well as directly utilizable organic monomers (e.g., amino acids) and polymers such as peptides and nucleic acids, which can be hydrolyzed to utilizable monomers. Bacterio- and phytoplankton can also use a portion of N associated with biologically recalcitrant humic substances (Carlsson and Granéli 1993). Under N-limiting conditions, the concentration of DIN is low, but

the concentration of dissolved organic nitrogen (DON) can be high, because a large portion of it is biologically recalcitrant (Seitzinger et al. 2002; Stepanauskas et al. 2002).

Solar radiation can photochemically transform a portion (10–18%) of biologically recalcitrant DON into bioavailable forms (NH_4^+ or amino acids; Bushaw et al. 1996; Smith and Benner 2005; Vähätalo and Zepp 2005). Photochemical reactions can also incorporate bioavailable N into biologically recalcitrant forms (Kieber et al. 1997; Reitner et al. 2002). The direction of photochemical reactions (i.e., the production vs. the incorporation of bioavailable N) depends, in part, on the concentration of reactants. For example, photochemistry incorporates NH_4^+ into DON at high NH_4^+ concentrations (8–33 mmol m^{-3}), but produces NH_4^+ from DON at low NH_4^+ concentrations (<6 mmol m^{-3} ; Tarr et al. 2001; Koopmans and Bronk 2002; Vähätalo et al. 2003). Under N-limiting conditions and in the presence of biologically recalcitrant but photoreactive DON, photochemistry can be expected to produce predominantly bioavailable N.

Photoproduced bioavailable N is a potential nutrient source for N-limited plankton, but little is known about its fate in surface waters. The photoproduced N can support more bacterial biomass than photoproduced labile carbon substrates (Bushaw et al. 1996; Moran and Zepp 1997). Photoproduced substrates and nutrients can cascade

¹ Present address: Department of Biological and Environmental Sciences, P.O. Box 65, FIN-00014 University of Helsinki, Finland (anssi.vahatalo@helsinki.fi).

² Present address: Department of Ecological and Environmental Sciences, University of Helsinki, Niemenkatu 73, FIN-15140 Lahti, Finland.

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throughout the heterotrophic food web up to metazooplankton, indicating that the photochemical reactions of dissolved organic matter (DOM) can contribute to the overall productivity of surface waters (DeLange et al. 2003; Caesar et al. 2006). Although the need of N relative to phosphorus (P) is larger for phytoplankton (typical carbon (C):N:P ratio of 106:16:1) than for bacterioplankton (typical C:N:P ratio of 45:9:1; Goldman et al. 1987), the contribution of photoproduced N to the primary production or the transfer of photoproduced bioavailable N into the planktonic food web has not been investigated. This study examined the contribution of the photoproduced bioavailable N to the biomass of nanoplankton community and to the primary producers under the N-limiting conditions of northern Baltic Sea. Models were applied to translate experimentally determined photoproduction of bioavailable N and the response of phytoplankton to it in the environment at relevant rates.

Methods

Study region and sampling of water—This study was carried out in the coastal northern Baltic Sea in the western Gulf of Finland with salinities of 5–6. In the study region, the typical seasonal succession of plankton includes a vernal bloom dominated by diatoms and dinoflagellates (April–May), followed by a N-deplete period with pico- and nanophytoplankton in June to mid-July and diazotrophic filamentous cyanobacteria in mid-July to August (Kivi et al. 1993; Lignell et al. 2003). For the June and July experiments, water samples (0–5 m) were collected on 16 June 2004 and 12 July 2004, respectively, with a Limnos sampler from the Station (Sta.) Långskär (59°47'N 23°19'E) and passed through a 100- μm mesh net into acid-rinsed polyethylene containers. Thereafter, only acid-rinsed containers were used, and quartz- and glassware were additionally combusted in 450°C for 2 h before use. Within a few hours of the collection, the waters were filtered through a Pellicon GVPP-V cassette (0.22 μm). To guarantee N-limitation, KH_2PO_4 was amended into water to the final concentration of 1.7 mmol m^{-3} (July experiment) and 1.2 mmol m^{-3} (June experiment). The concentration in mmol m^{-3} equals $\mu\text{mol L}^{-1}$, is compatible with the theoretical model (Eqs. 1, 2), and can be converted to a depth-integrated value (mmol m^{-2}).

Experimental design—For the July experiment, the water was siphoned through a 0.2- μm filter (Sartobran 300, Sartorius) into quartz and glass bottles with glass stoppers (Fig. 1). For the exposure to solar radiation, the quartz bottles were placed into a matte black, outdoor pool flushed with tap water along with the glass bottles covered with aluminum foil (dark controls; Fig. 1). Earlier, we estimated that the daily summer solar radiation produces 31 $\mu\text{mol NH}_4^+ \text{m}^{-3} \text{d}^{-1}$ at the surface of Sta. LL12 25 km southwest from this study site (Vähätalo and Zepp 2005). Based on that rate, we estimated that about a 2-week exposure would produce enough bioavailable N to render a biological response. The solar radiation exposure of the July experiment lasted 12 days on the roof of Tvärminne

Zoological Station at 16–24.5°C corresponding roughly to in situ surface-water temperatures. Although filtration through a 0.2- μm filter removed most of the bacterioplankton initially, bacterial regrowth will take place within a few days under the conditions of this study (e.g., Vähätalo and Wetzel 2004; Vähätalo et al. unpubl. data). Therefore, the solar radiation exposure included a combined effect of solar radiation and bacteria.

At the beginning and end of the solar radiation exposure, absorption by chromophoric DOM (CDOM) was determined from 0.2- μm filtered (Minisart, Sartorius) waters against Milli-Q-water blank in a 10-cm quartz cuvette with a spectrophotometer (Shimadzu UV-2101PC). The absorbance spectrum was scanned three times with a new blank each time at 1-nm intervals and converted into an absorption coefficient ($a_{\text{CDOM},\lambda}$). The spectral slope of $a_{\text{CDOM},\lambda}$ (S) was calculated by the log-linear fitting method from 300 nm to ca. 475 nm, where $a_{\text{CDOM},\lambda}$ fell below 0.2 m^{-1} . For wavelengths longer than 475 nm, the absorption coefficient was calculated according to the determined S and $a_{\text{CDOM},300}$.

The number of photons absorbed by CDOM (the product of $Q_{s,z,\lambda}$ and $a_{\text{CDOM},\lambda}$ of Eq. 1) was estimated by a radiative transfer model (details in Vähätalo et al. 2000; Vähätalo and Wetzel 2004), which used the information about the solar radiation measurements as well as the optical characteristics of the cooling water and the water samples. The solar radiation incident to the pool was measured every 12 seconds with a Vantage Pro 6450 global radiation sensor and the mean of 50 measurements was logged every 600 s. The radiative transfer model separated solar radiation into direct and indirect parts according to the turbidity and the Rayleigh scatter. The specular reflection from the interface between the atmosphere and the cooling water in the pool was calculated according to the Fresnel's law of reflection. The attenuation of solar radiation caused by the cooling water was calculated according to the vertical attenuation coefficient of downward photon flux in that medium. The number of photons absorbed by the CDOM of exposed water was calculated as the difference of photons entering to the water sample and those passing the sample water after the mean depth of the sample (39.7 mm, the mean optical depth of sample = $0.5\pi r$, where r is the inner radius of bottle) (Vähätalo and Zepp 2005). The photochemical decomposition of CDOM during exposure was accounted for by calculating a mean $a_{\text{CDOM},\lambda}$ for the exposure period according to the first order kinetic decay. The reflectance from the matte black lining of the pool was considered negligible. The calculations of photon fluxes were done at 5-nm bands through 300–700 nm at 10-min intervals and summed across the time of exposure.

For the bioassay, the dark and the exposed waters were poured into transparent polycarbonate containers (25-liter Nalgene), and inoculated with an indigenous nanoplankton community from the surface of Sta. Storfjärden (10- μm reverse-phase filtrate into 10% of final volume, collected on 26 July 2004 from 59°49'N 23°17'E; Fig. 1). The waters were incubated under photosynthetically active radiation ([PAR] Philips, Natural daylight 35W/965) after an 18 : 6

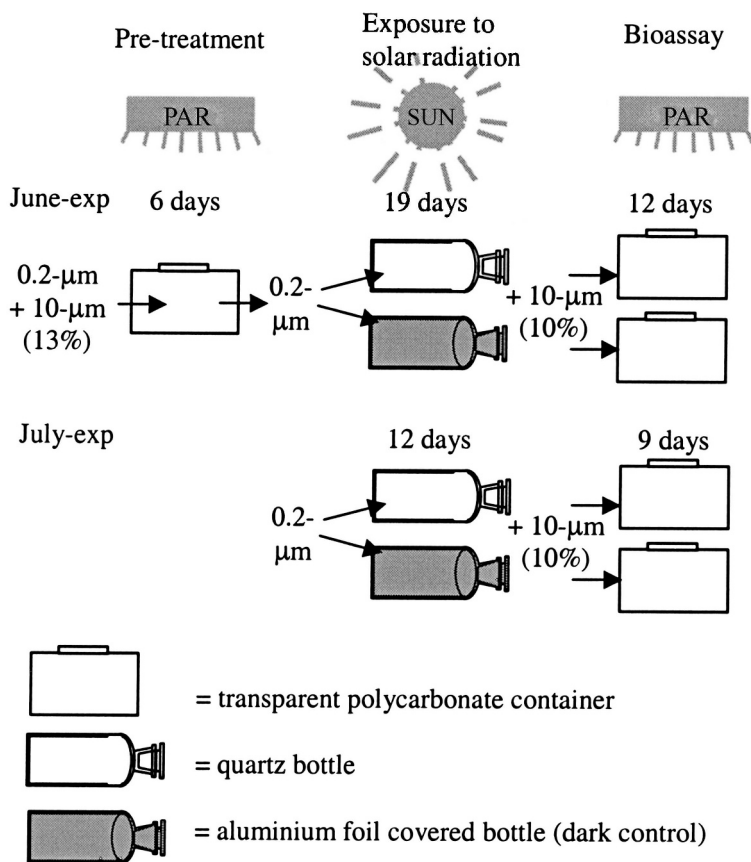


Fig. 1. Design of June and July experiments. The figure shows filtration steps (0.2 μm), the inoculations with indigenous nanoplankton communities (+ 10 μm with introduced vol/vol%), and the containers used in the 6–19-d phases of experiments consisting of the exposures to solar radiation and the incubations under photosynthetically active radiation lamps.

light : dark (LD) cycle with the scalar photon flux densities of 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 20–22°C (measured with a QSL2101, Biospherical Instruments; Fig. 1). The irradiance of PAR lamps was negligible at the wavelengths <430 nm (measured with a Macam SR 991 spectroradiometer). The ultraviolet (UV) range of the spectrum is mainly responsible for DOM photochemistry (Moran and Zepp 1997; Vähätalo and Zepp 2005), and therefore DOM photochemistry under the PAR lamps was minimal. The length of bioassays was designed long enough (9–12 days) to detect the effect of photoproduct N on the introduced nanoplankton community, which consisted of phytoplankton and a three-trophic level heterotrophic food web (bacteria–flagellates–ciliates). Although bacterioplankton can respond to photoproduct N quickly, the detection of a cascading primary response through the food web and the following secondary responses, such as the grazing-assisted release of nutrients, requires a bioassay across several days (Vähätalo et al. unpubl. data). During the bioassay, the water was mixed 1–3 times per day and sampled periodically for chlorophyll *a* (Chl *a*), nutrients, and plankton community composition.

The June experiment was similar to the July experiment, except it included a pretreatment for the removal of

bioavailable dissolved N (Fig. 1). For the pretreatment, the 0.2- μm filtrate (Pellicon GVPP-V) of collected water was inoculated with a 10- μm reverse-phase filtrate (13% of final volume) of the original water sample. This nanoplankton culture was incubated under the PAR light for 6 days as described above except at 11°C, corresponding to the in situ temperature at the time of sampling. At the end of the pretreatment, the waters were filtered (0.2 μm , Sartobran 300, Sartorius) to remove particulate N (PN). The pretreatment was excluded from the July experiment, because the N-limiting in situ conditions during summer acted as a natural pretreatment. The filtrate was siphoned into quartz and glass bottles and placed into the outdoor pool as described above for 19 days at 12.5–20.5°C. For the bioassay, the exposed and dark controls were inoculated with a 10- μm reverse-phase filtrate (10% of final volume) of the water collected from Sta. Långskär on 13 July 2004 and were incubated under the PAR light as during the pretreatment.

Theoretical model for photoreaction rates of DOM producing bioavailable N and stimulating phytoplankton— The photoproduction rate of bioavailable N in an optically thin water layer at the depth z (pN_z , mol N $\text{m}^{-3} \text{d}^{-1}$) can be

expressed as

$$pN_z = \int_{\lambda_{\min}}^{\lambda_{\max}} \phi_{N,\lambda} Q_{s,z,\lambda} a_{CDOM,\lambda} / d\lambda \quad (1)$$

where $\phi_{N,\lambda}$ is the apparent quantum yield for the photoproduction of bioavailable N at wavelength λ (mol bioavailable N produced mol absorbed photons⁻¹ nm⁻¹), $Q_{s,z,\lambda}$ is the scalar photon flux density at λ and depth z (mol photons m⁻² d⁻¹ nm⁻¹), and $a_{CDOM,\lambda}$ is the absorption coefficient of CDOM at λ (m⁻¹ nm⁻¹). The integration of Eq. 1 is over the wavelength range from λ_{\min} to λ_{\max} , the minimum and maximum wavelengths (nm), respectively, contributing to the photoproduction of bioavailable N.

Over the entire water column, the photoproduction rate of bioavailable N (pN , mol N m⁻² d⁻¹) is

$$pN = \int_{\lambda_{\min}}^{\lambda_{\max}} \phi_{N,\lambda} Q_{a,\lambda} a_{CDOM,\lambda} / a_{tot,\lambda} d\lambda \quad (2)$$

where $Q_{a,\lambda}$ represents mol of photons absorbed by the water column (mol photons m⁻² d⁻¹ nm⁻¹), and the $a_{CDOM,\lambda} / a_{tot,\lambda}$ ratio is the fractional contribution of CDOM to the total absorption coefficient, $a_{tot,\lambda}$.

The apparent quantum yield for the photoproduction of bioavailable N was assumed to increase exponentially with decreasing wavelength as has been found for many photochemical reactions with natural DOM (Vähätalo et al. 2000 and references therein),

$$\phi_{N,\lambda} = c e^{-d\lambda} \quad (3)$$

where c (mol N mol photons⁻¹) and d (nm⁻¹) are positive parameters, and λ is a wavelength in nanometers. The stimulus to the phytoplankton from the photochemical reactions of DOM can be calculated when $\phi_{Chla,\lambda}$ (g Chl a mol photons⁻¹ nm⁻¹) is used instead of $\phi_{N,\lambda}$ in Eqs. 1 and 2.

Determination of apparent quantum yields—The experiments were designed to reveal the rates of those photochemical reactions of DOM that produced bioavailable N and later stimulated the phytoplankton biomass under conditions representing those at the surface of the Baltic Sea. The experimentally determined bioavailable N consisted of N that accumulated into PN (i.e., into plankton biomass) or was mineralized during the experiments. The accumulated PN was the difference between PN at the end (at time = t) and the beginning (at time = 0) of the experiment (i.e., = $PN_t - PN_0$). Analogously, the mineralized N was the difference between the concentration of DIN at the end and the beginning of the experiment (i.e., $DIN_t - DIN_0$). The photoproduction of bioavailable N and DOM photochemistry-stimulus to phytoplankton was the difference in the concentration of bioavailable N and Chl a , respectively, between the solar radiation exposed and the dark controls.

The apparent quantum yield spectrum for the photo-reactions of DOM producing bioavailable N ($\phi_{N,\lambda}$) and those stimulating phytoplankton biomass ($\phi_{Chla,\lambda}$) were calculated from experimentally determined photoproduction of bioavailable N (pN_z applicable to Eq. 1) and DOM photochemistry-stimulus to Chl a ($pChla_z$ applicable to Eq. 1). Photoproduction of bioavailable N or DOM photochemistry-stimulated Chl a was related to the number of photons absorbed by CDOM at 5-nm bands in the spectral range of 300–700 nm, which includes those wavelengths of solar radiation contributing to DOM photochemistry (Moran and Zepp 1997; Vähätalo et al. 2000). The solar radiation below 300 nm was ignored, because of the large uncertainty in its determination, its low intensity, and the negligible contribution to DOM photochemistry in our study region (Vähätalo et al. 2000; Vähätalo and Zepp 2005). Each of the 81 spectral bands was assumed to contribute to the photoreactions with the spectral weighing described by Eq. 3, where weighing increases exponentially with decreasing wavelength in a manner specified by the parameters c and d . The 81 bands of absorbed photons were related to the measured photoproduction of bioavailable N or to the stimulus in the phytoplankton biomass by unconstrained nonlinear optimization (the `fminsearch`-function of Matlab6) so that the resulting c and d of Eq. 3 gave the best fit between the measured and calculated (Eq. 1) values.

The error in $\phi_{N,\lambda}$ and $\phi_{Chla,\lambda}$ depended on the error in the parameters used for their determination (i.e., pN_z , or $pChla_z$, $Q_{s,z,\lambda}$, and $a_{CDOM,\lambda}$ of Eq. 1). The coefficient of variation (CV) for the determination of $a_{CDOM,\lambda}$ was ca. 0.3% at the UV-A (315–400 nm) part of the spectrum, which was primarily responsible for the photochemical reactions. We estimated that the error of radiometer and that of the radiative transfer model resulted in <15% CV for the estimated irradiance at the UV-A range of spectrum approximating $Q_{s,z,\lambda}$ (discussed in Vähätalo et al. 2000). The error in pN_z and $pChla_z$ depended on the standard deviation associated with the determination of the concentration of bioavailable N and the DOM photochemistry-stimulus to Chl a . The CV of each component affecting the determination of ϕ_λ was summed for the total error estimate of ϕ_λ .

Chemical analyses—For dissolved nutrients, triplicate water samples were filtered through 0.2- μ m (Minisart polyethersulfone-filters, Sartorius) and frozen (–20°C). PO_4^{3-} was determined by a phenolphthorite method (Solorzano 1969). The combined concentration of NO_3^- and NO_2^- (NO_x^-) was determined with a Lachat Quik-Chem Method 31-107-04-1-A. PO_4^{3-} was determined with a Lachat QuikChem Method 31-115-04-3-A. For the total dissolved N (TDN) and P, the water sample was digested with a persulfate in boric acid–NaOH buffer, and the formed NO_x^- and PO_4^{3-} were analyzed as described above (Hansen and Koroleff 1999). The concentration of DON was calculated as $TDN - NO_x^- - NH_4^+$.

For particulate nutrients, particulate matter from triplicate 100-mL water samples was filtered on a combusted, acid-rinsed glass fiber filter (GF/F, 25 mm, What-

man, 0.7- μm) with an acid-rinsed glassware. For particulate N and P, the particulate matter on the filters were digested and determined as NO_x^- and PO_4^{3-} , respectively, as described above for dissolved nutrients.

For Chl *a*, particulate matter from triplicate 100-mL water samples was filtered on a glass fiber filter (GF/F, 25 mm, Whatman), extracted with ethanol, and stored at -20°C . The concentration of Chl *a* was determined as an emission at 670 nm induced by a 450-nm excitation with a spectrofluorometer (Shimadzu RF-5000).

Microscopy—The biomass of phytoplankton and protozoa were determined from samples preserved with acid Lugol's solution using the settling chamber technique (Utermöhl 1958). Small taxa were counted at $400\times$ magnification from 13 randomly selected fields (a total of 329–510 counted taxa per sample). For larger phytoplankton taxa and ciliates, two transects were counted at $200\times$ magnification. The cell densities of phytoplankton were converted to biomass using the measured and literature cell dimensions and assuming the density of 1 g mL^{-1} for the determined biovolume. The species were classified into auto-, mixo-, and heterotrophs according to the information presented in the literature (Jones 2000 and references therein).

Statistical methods—The statistical differences were tested with *t*-test using two-tailed distributions and equal variances (Systat 8.0).

Results

June experiment—In the water collected for the June experiment, the concentration of DIN and PO_4^{3-} indicated N-limiting conditions for primary production as is typical for our study region in summer (Table 1, the $0.58\text{ mmol PO}_4^{3-}\text{ m}^{-3}$ in the collected water resulted in the DIN to PO_4^{3-} ratio of 0.5; Kivi et al. 1993; Lignell et al. 2003). For the pretreatment, the introduced KH_2PO_4 further enhanced the N-limiting conditions and resulted in the DIN to PO_4^{3-} ratio of 0.2. During the pretreatment, the concentration of PN increased, indicating that the plankton community incorporated dissolved bioavailable N into their biomass (Table 1; Fig. 2A). Simultaneously, the DIN concentration changed little and the apparent decrease in DON was below the detection limit of method (Table 1). At the end of pretreatment, PN was filtered away, and the largest pool of N in the filtrate was biologically recalcitrant DON.

When this filtered water was exposed to solar radiation, the concentration of PN increased significantly relative to the dark control ($t = 3.5$, $\text{df} = 3$, $p = 0.04$; Fig. 2A; Table 1). The increase in PN and the absence of Chl *a* indicated bacterial regrowth during the exposure to solar radiation (Fig. 2A,B). The concentration of DIN was significantly higher in the solar radiation-exposed water than in the dark control ($t = 3.5$, $\text{df} = 4$, $p = 0.025$; Table 1). Under the exposure to solar radiation, the concentration of CDOM decreased by 41% in relation to that found initially or in the dark control, but without a significant change in the spectral slope coefficient of

0.0203 nm^{-1} (Fig. 3A). Altogether, the exposure to solar radiation photobleached the CDOM, mineralized the DON ($\text{DIN}_t - \text{DIN}_0 = 0.38\text{ mmol m}^{-3}$), and stimulated the formation of PN in the form of bacterial biomass ($\text{PN}_t - \text{PN}_0 = 0.43\text{ mmol m}^{-3}$).

After inoculation with an indigenous nanoplankton community, the waters were incubated under PAR light, where the concentrations of PN and Chl *a* were consistently higher (by up to $0.40\text{ mg Chl } a\text{ m}^{-3}$) in the water exposed to solar radiation than in the dark control (Fig. 2A,B). On day 12 of the bioassay, the biomass of autotrophic phytoplankton and that of mixo- and heterotrophic plankton was 4.2- and 3.6-fold higher, respectively, in the solar radiation-exposed water than in the dark control (Fig. 4A). Diatoms comprised 56% and 10% of the total autotrophic phytoplankton biomass in the solar radiation-exposed and the dark controls, respectively (Fig. 4A). Cyanophyceae was the dominant (58% of total biomass) autotrophic phytoplankton class in the dark control, but it composed 24% of the total autotrophic biomass in the solar radiation-exposed water (Fig. 4A). These results show that under N-limiting conditions, the solar radiation exposure of biologically recalcitrant DON increased the biomass of the nanoplankton community, including autotrophic phytoplankton.

July experiment—The July experiment was carried out under N-limiting conditions, but without a pretreatment ($0.70\text{ mmol PO}_4^{3-}\text{ m}^{-3}$ in the original water, and the introduction of KH_2PO_4 resulted in the DIN to PO_4^{3-} ratios of 0.5 and 0.2, respectively; Table 1). Under the exposure to solar radiation, the concentration of PN increased significantly more than in the dark controls ($t = 8.7$, $\text{df} = 4$, $p = 0.001$; Fig. 2C; Table 1). The N:P ratio (by atoms) of accumulated particulate matter in the solar radiation-exposed water was 10 and corresponded to that typically found in bacterial biomass (e.g., the N:P of 9 reported by Goldman et al. 1987). Under solar radiation, the absorption coefficient of CDOM decreased by 39%, without a change in the spectral slope of 0.0205 nm^{-1} (Fig. 3B). No change in $a_{\text{CDOM},\lambda}$ took place in the dark controls (Fig. 3B). These results together with those from the June experiment indicate that exposure of DOM to solar radiation increased bacterial regrowth and stimulated the uptake of DON into bacterial biomass (PN).

The subsequent bioassay was carried out with an inoculum where cyanobacteria comprised 70% of the autotrophic phytoplankton biomass. The concentrations of PN and Chl *a* accumulated faster than in the June experiment, probably because the bioassay was carried at the seasonally high temperature, without a pretreatment and with a different plankton community. During the bioassay, the concentration of PN was higher in the water exposed to solar radiation than in the dark control (Fig. 2C). During the first 5 days of bioassay, the concentration of Chl *a* increased more (by 0.32 mg m^{-3}) in the solar radiation-exposed water than in the dark control (Fig. 2D). At the end of bioassay, the biomass of the plankton community and PN were similar in the solar radiation-exposed water and in the dark control (Fig. 2C,

Table 1. Concentration of nitrogen at the different phases of June and July experiments (mean \pm SD, $n=3$).*

	Concentration of nitrogen (mmol m ⁻³)								
	Pretreatment		Exposure to solar radiation				Bioassay		
June experiment									
	Initial, 0†	End, 6†	Initial, 6†	Dark, 25†	Expo, 25†	Dark, 25†	Expo, 25†	Dark, 34†	Expo, 34†
DON	19.5 \pm 0.8	18.6 \pm 0.5	18.9 \pm 0.7	18.4 \pm 0.5	17.7 \pm 0.8	17.5 \pm 0.3	16.8 \pm 1.0	17.6 \pm 0.7	16.2 \pm 0.8
PN	0.18 \pm 0.13	0.96 \pm 0.09	0.00 \pm 0.11	0.15 \pm 0.14	0.58 \pm 0.13	0.29 \pm 0.22	0.63 \pm 0.20	0.63 \pm 0.07	1.03 \pm 0.10
NO _x ⁻	0.16 \pm 0.01	0.10 \pm 0.02	0.08 \pm 0.06	0.23 \pm 0.04	0.36 \pm 0.08	0.25 \pm 0.08	0.19 \pm 0.01	0.14 \pm 0.07	0.20 \pm 0.02
NH ₄ ⁺	0.13 \pm 0.05	0.12 \pm 0.07	0.19 \pm 0.02	0.15 \pm 0.01	0.40 \pm 0.12	0.14 \pm 0.12	0.06 \pm 0.01	0.33 \pm 0.18	0.07 \pm 0.05
DIN	0.29 \pm 0.05	0.23 \pm 0.06	0.27 \pm 0.06	0.37 \pm 0.05	0.75 \pm 0.18	0.39 \pm 0.10	0.24 \pm 0.02	0.47 \pm 0.14	0.27 \pm 0.07
July experiment									
	—	—	Initial, 0†	Dark, 12†	Expo, 12†	Dark, 12†	Expo, 12†	Dark, 21†	Expo, 21†
DON	—	—	18.6 \pm 0.2	17.5 \pm 0.1	16.7 \pm 0.2	18.0 \pm 0.4	17.2 \pm 0.4	20.2 \pm 0.9‡	18.5 \pm 0.7‡
PN	—	—	0.00 \pm 0.14	0.14 \pm 0.04	0.70 \pm 0.10	0.52 \pm 0.01	1.04 \pm 0.15	3.17 \pm 0.28‡	3.56 \pm 0.18‡
NO _x ⁻	—	—	0.09 \pm 0.02	0.25 \pm 0.14	0.24 \pm 0.01	0.19 \pm 0.01	0.22 \pm 0.04	0.05 \pm 0.06‡	0.05 \pm 0.03‡
NH ₄ ⁺	—	—	0.24 \pm 0.02	0.21 \pm 0.02	0.22 \pm 0.01	0.19 \pm 0.03	0.30 \pm 0.03	0.10 \pm 0.01‡	0.14 \pm 0.02‡
DIN	—	—	0.33 \pm 0.02	0.46 \pm 0.16	0.46 \pm 0.01	0.37 \pm 0.04	0.52 \pm 0.04	0.15 \pm 0.06‡	0.20 \pm 0.05‡

* Dark, dark control; Expo, water exposed to solar radiation (Fig. 1).

† Cumulative number of days from the start of experiment (Fig. 1).

‡ Diazotrophic filamentous cyanobacteria were present in the bioassay and the increase in DON corresponded to an increase of 1.4–2.5 mmol m⁻³ DON during a cyanobacterial bloom found earlier in the study region (Lignell et al. 2003).

Fig. 4B). At the end of the bioassay, diazotrophic cyanobacteria consisted of >98% of the autotrophic phytoplankton biomass (Fig. 4B), and the increase in DON indicated N₂-fixation during the bioassay (Table 1). These results show that the concentration of PN remained elevated in the water exposed to solar radiation and suggested that the exposure of filtered Baltic Sea water to solar radiation led to an increase in the phytoplankton biomass in the early phase of bioassay.

Experimentally determined bioavailable DON—Together, bacteria and the nanoplankton community consumed 1.39 mmol DON m⁻³ (7.1 % of initial DON) during the three phases of the June experiment when the water was not exposed to solar radiation (Fig. 5A). When the water was exposed to solar radiation, the photochemical reactions, the bacteria, and the nanoplankton community consumed or mineralized 2.40 mmol DON m⁻³ (12.3 % of initial DON, Fig. 5A). The photochemically produced bioavailable N was 0.81 mmol m⁻³ (4.2 % of initial DON) at the end of solar radiation exposure without a further significant increase during the bioassay (Fig. 5A). In the July experiment, the photochemical reactions produced 0.56 mmol m⁻³ of bioavailable N (3 % of initial DON; Fig. 5B).

Rates of DOM photochemistry in the Baltic Sea—To determine the apparent quantum yield for the photoproduction of N ($\phi_{N,\lambda}$), the experimentally determined photo-produced bioavailable N (0.56 mmol N m⁻³ or 0.81 mmol N m⁻³ from Fig. 5) was divided by the number of photons absorbed by the CDOM during exposures to solar radiation (Table 2; Fig. 6C). Analogously, the DOM photochemistry-stimulated Chl *a* (0.32 or 0.40 mg Chl *a* m⁻³ from Fig. 2) was used for the determination of the apparent quantum yield for those photochemical reactions of DOM, which stimulated phytoplankton ($\phi_{Chl a,\lambda}$; Ta-

ble 2). These ϕ_{λ} s describe the experimentally determined photochemical reactivity of DOM through 300–700 nm (Table 2; Fig. 6C). For example, ϕ_{λ} s at 350 nm (ϕ_{350}) indicate that 1 mol of photons absorbed by CDOM at 350 nm produced 7–9 μ mol of bioavailable N and stimulated the phytoplankton biomass by 4–5 μ g of Chl *a* (Table 2; Fig. 6C).

To calculate the photoproduction rate of bioavailable N at the depth of 0 m (Fig. 6D), $\phi_{N,\lambda}$ was applied to Eq. 1 together with the absorption coefficient of CDOM (the initial $a_{CDOM,\lambda}$ from Fig. 3B, Fig. 6B) and a typical daily vector photon flux density spectrum arriving to the surface of the northern Baltic Sea during summer (an estimate for $Q_{s,z,\lambda}$ of Eq. 1 at $z = 0$ m, Fig. 6A). The resulting action spectrum shows that the UV-A part of the spectrum was mostly responsible for the photoproduction of bioavailable N (Fig. 6D). More specifically, the wavelengths ≤ 330 nm induced one-half of the photoproduction; i.e., the median wavelength for the photoproduction of bioavailable N ($\lambda_{50\%}$) at the surface was 330 nm. The calculated photoproduction rate of bioavailable N was 59 μ mol N m⁻³ d⁻¹ in the July experiment (Fig. 6D; Table 2).

The photoproduction rate of bioavailable N across the entire water column was calculated according to Eq. 2, assuming that CDOM absorbed completely the photolytic photons in the water column ($a_{CDOM,\lambda}/a_{tot,\lambda} = 1$, Eq. 2; Fig. 6). In this case, the resulting action spectrum is the product of solar radiation and $\phi_{N,\lambda}$ (Fig. 6D, 0– ∞ m). The wavelengths for the photoproduction of bioavailable N were longer (with $\lambda_{50\%}$ of 350 nm) in the entire water column than at the surface (Fig. 6D). Across the entire water column, the calculated photoproduction rate of bioavailable N was 26 μ mol N m⁻² d⁻¹ (Fig. 6D; Table 2).

The rate of DOM photochemistry that stimulated the phytoplankton biomass was calculated analogous to the photoproduction of N but by replacing $\phi_{N,\lambda}$ with $\phi_{Chl a,\lambda}$

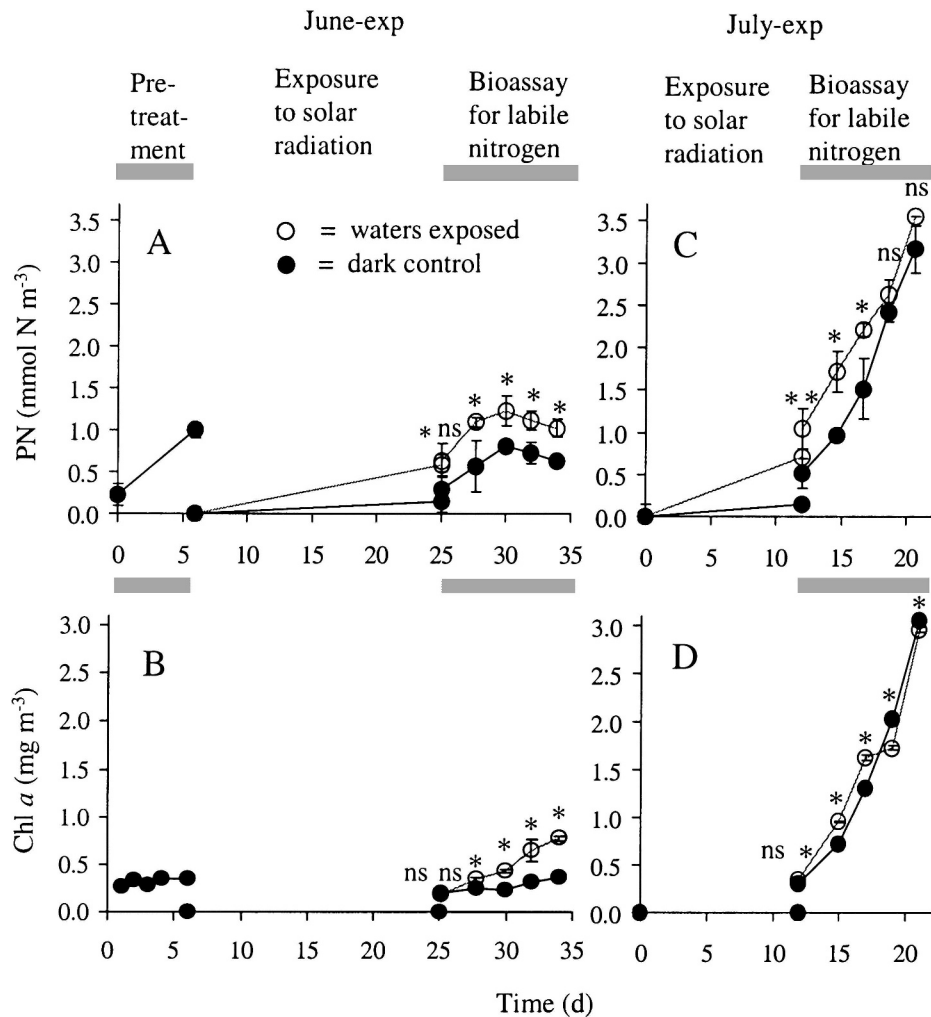


Fig. 2. Concentration of PN (A, C) and Chl *a* (B, D) in the June (panels A, B) and July (panels C, D) experiments (mean \pm SD, $n = 3$). Shading shows the incubations under PAR lamps. *Statistical difference ($p < 0.05$), and ns is no statistical difference ($p > 0.05$) between solar radiation-exposed waters and dark controls at the end of solar radiation exposure (the symbols on left) and during the bioassay (the five symbols on right).

(Fig. 6; Table 2). The spectral dependence of these photo-reactions was similar to those producing bioavailable N (Fig. 6E). DOM photochemistry was calculated to stimulate the phytoplankton biomass by $34 \mu\text{g Chl } a \text{ m}^{-3} \text{ d}^{-1}$ and $14 \mu\text{g Chl } a \text{ m}^{-2} \text{ d}^{-1}$ at the surface and across the entire water column, respectively (Fig. 6E; Table 2).

Discussion

Fate of photoproducted bioavailable N—To the best of our knowledge, this study shows for the first time that solar radiation exposure of filtered seawater can stimulate the biomass of N-limited phytoplankton. We attribute this stimulus to photochemical changes in DOM and nutrient availability. In their review, Mopper and Kieber (2002) listed 26 studies where the irradiation of biologically recalcitrant DOM produced bioavailable substrates and stimulated heterotrophic microbial metabolism. Our study

provides evidence that DOM photochemistry can also stimulate autotrophic metabolism. Gjessing and Källqvist (1991) found that the UV-irradiation of humic water resulted in reduced growth of a green alga *Selenastrum capricornutum* under P- and N-replete conditions. N-limited conditions prevailed in our study, and the photo-produced bioavailable N can explain the stimulus in the phytoplankton biomass. In this study, the yield of Chl *a* per photoproducted bioavailable N was $0.49\text{--}0.57 \mu\text{g Chl } a \mu\text{mol N}^{-1}$ when calculated from the data presented in Figs. 2 and 5 or from the $\phi_{350\text{s}}$. These values are less than the yields of phytoplankton with DIN under N-replete conditions in our study region ($1.6\text{--}3.6 \mu\text{g Chl } a \mu\text{mol N}^{-1}$; Lignell et al. 2003) or elsewhere (median of $2.2 \mu\text{g Chl } a \mu\text{mol N}^{-1}$ in the review by Tett et al. 2003). The comparison of algal yield in this study to those with DIN suggests that the amount of photoproducted bioavailable N was large enough to explain the stimulated growth of phytoplankton under the N-deplete conditions of bioassay.

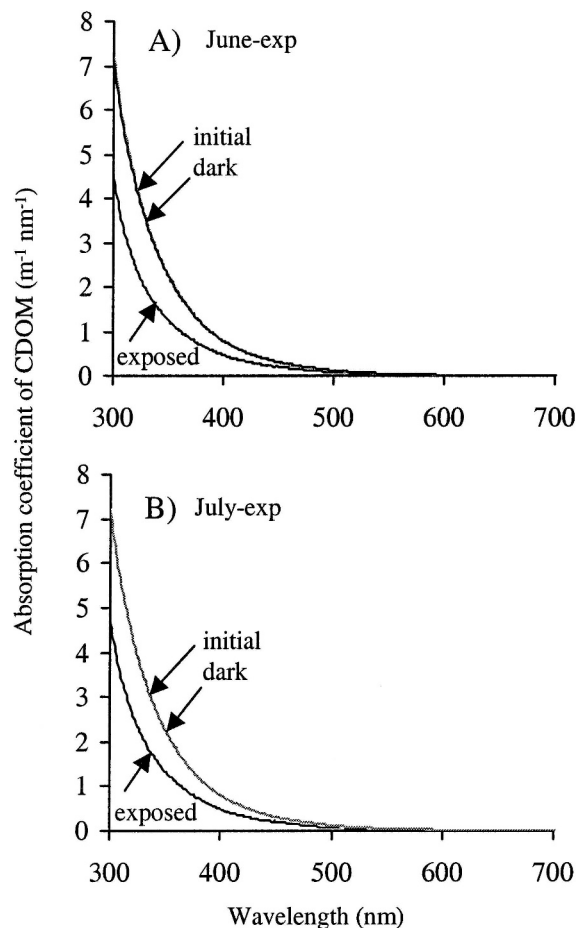


Fig. 3. Absorption spectrum of CDOM at Sta. Långskär in the (A) June and (B) July experiments. Initial, in the beginning of solar radiation exposure; exposed, exposed waters in the end of solar radiation exposure; dark, dark controls in the end of solar radiation exposure. Initial and dark controls were nearly identical and on top of each other.

Phytoplankton can directly assimilate the photoproduced DIN such as that found in the June experiment. During the solar radiation exposure of this study, the increase in PN indicated that bacteria assimilated photoproduced bioavailable N into their biomass. Mixotrophic phytoplankton can graze on bacterioplankton and assimilate N bound in bacterial biomass (e.g., Jansson et al. 1996). Heterotrophic grazers (flagellates and ciliates in our study) retain a portion of the ingested PN in their biomass, whereas a portion becomes mineralized and can supply DIN for autotrophic primary producers (e.g., Goldman et al. 1985). Thus, the photoproduced bioavailable N can be allocated into N-limited phytoplankton via several mechanisms.

Our results indicate that photoproduced N is not only allocated into heterotrophs, but also into phytoplankton. The allocation of photoproduced N into the phytoplankton can be estimated from their stimulus to DOM photochemistry assuming that the phytoplankton benefited only from DIN with the typical yield of $2.2 \mu\text{g Chl } a \mu\text{mol DIN}^{-1}$ (Tett et al. 2003). The observed stimulus of 0.40 mg Chl

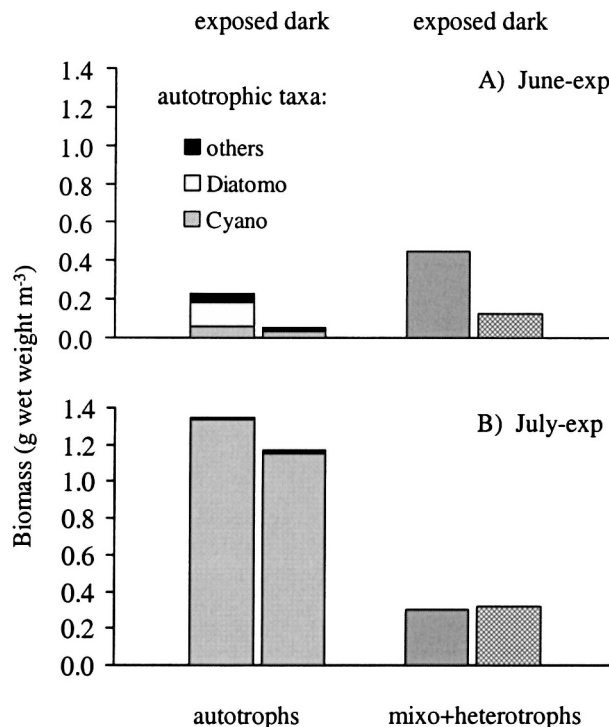


Fig. 4. The biomass of phytoplankton and that of mixo- and heterotrophic plankton in (A) June and (B) July experiments with a key for the major taxa of autotrophic phytoplankton.

$a \text{ m}^{-3}$ in the June experiment would have required $0.18 \text{ mmol DIN m}^{-3}$, which is 22% of the observed photoproduced N. According to this estimate, heterotrophic plankton took up 78% and phytoplankton 22% of the photoproduced N. This estimate should be treated with caution, because the allocation mechanisms of photoproduced N into the phytoplankton or the actual yield of Chl *a* per DIN was not known in our experiment. In surface waters, the allocation of photoproduced N between heterotrophs and autotrophs is likely to be variable depending on the strength of nutrient limitation and the composition of the plankton community (e.g., Jansson et al. 1996).

Rates and attenuation of N photochemistry in the surface waters—Our surface rates for the photoproduction of N are within the previously reported values ($\sim 310\text{--}340 \mu\text{mol N m}^{-3} \text{ h}^{-1}$ in those studies compiled by Buffam and McGlathery 2003). These rates vary considerably because they have been obtained both with natural and concentrated DON, different irradiation sources, variable concentrations of CDOM, and chemical matrices. However, the variability in the photoproduction rates is relatively small in those few studies, which have estimated the photoproduction of N throughout the water column, and the rates of this study fall into the previously reported range (Table 3).

This study, along with the earlier ones (Bushaw et al. 1996; Vähätalo and Zepp 2005), indicates that the solar UV-A radiation is primarily responsible for the photoproduction of bioavailable N. The median wavelength in-

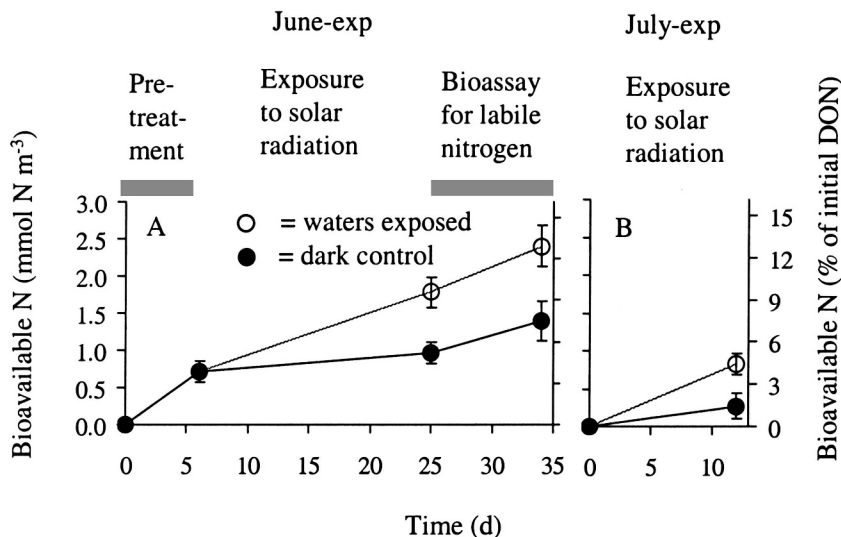


Fig. 5. Experimentally determined bioavailable N (mean \pm cumulative SD, $n = 3$) accumulating throughout the different phases of (A) June and (B) July experiments. Shading shows incubations under PAR light. The bioavailable N = the accumulated PN + the mineralized N. The accumulated PN = $PN_t - PN_0$, where PN_t and PN_0 are the concentrations of PN at the time t and at the beginning of the experiment, respectively. Mineralized N = $DIN_t - DIN_0$, where DIN_t and DIN_0 are the concentrations of DIN at the time t and at the beginning of the experiment, respectively.

ducing the photoproduction across the entire water column ($\lambda_{50\%}$ of 350 nm in this study) provides information about the depth of the photolytic layer when related to the optics of the surface waters (Vähätalo et al. 2000). Ninety percent of irradiation at 350 nm and thus also the photoproduction of bioavailable N attenuate the <1.5-m deep water column of the northern Baltic Sea (Vähätalo and Zepp 2005). Depending on the optical characteristics, the depth of the photolytic stratum for the photoproduction of N can be tens of meters in optically clear waters but only less than a few decimeters in humic-stained or turbid waters.

Photochemical reactivity of DOM—In our earlier study at Sta. LL12 closest (25 km southwest) to the site of this study, the apparent quantum yield for photoproduction of NH_4^+ ($\phi_{NH_4,\lambda}$) was similar to that for the photoproduction of bioavailable N ($\phi_{NH_4,350}$ was 5.8×10^{-6} at LL12; Vähätalo and Zepp 2005; Fig. 6C). This suggests that NH_4^+ was the major form of photoproduced N in this study, in agreement with the earlier studies where NH_4^+ accounted

for 80% of total photoproduction of amino acids and NH_4^+ (Bushaw et al. 1996; Tarr et al. 2001; Buffam and McGlathery 2003).

The apparent quantum yields for the photoproduction of bioavailable N ($\phi_{N,\lambda}$) are 1–2 orders of magnitude lower than for the photochemistry of dissolved organic carbon (DOC; $\phi_{C,\lambda}$). For example, the reported $\phi_{N,350s}$ range from 0.6×10^{-5} to 3.2×10^{-5} with a median of 0.9×10^{-5} (Gao and Zepp 1998; Vähätalo and Zepp 2005; this study), whereas the $\phi_{C,350s}$ for the photoproduction of CO_2 and labile C-substrates range from 23×10^{-5} to 229×10^{-5} with a median of 67×10^{-5} (Gao and Zepp 1998; Vähätalo et al. 2000; Vähätalo and Wetzel 2004). This difference is largely explained by the C:N ratio of DOM, but not exclusively, because photochemical reactions can decrease the C:N ratio of DOM (Buffam and McGlathery 2003).

Although photochemistry of DOM produces more C than N products, the relatively small amount of the latter can have implications for the productivity of plankton under N-limiting conditions. Heterotrophic bacterioplank-

Table 2. Apparent quantum yields for the photochemical production of bioavailable N ($\phi_{N,\lambda}$ [mol N mol photons $^{-1}$ at λ]) and for the DOM photochemistry stimulating phytoplankton ($\phi_{Chl a,\lambda}$ [g Chl a mol photons $^{-1}$ at λ]). The parameters c and d of Eq. 3 enable the calculation of ϕ_λ through 300–700 nm as exemplified at 350 nm in a separate column. CV% estimates the error for the determination of ϕ_s . The summer rates for the photochemical reactions at the surface and across the entire water column of Sta. Långskär were calculated according to Eqs. 1 and 2 (see Fig. 6).

ϕ for	Experiment	Parameters of Eq. 3		$\phi_{350} \times 10^{-6}$	CV	Rate $\mu\text{mol N}$ or $\mu\text{g Chl } a \text{ m}^{-3/2} \text{ d}^{-1}$	
		c	d			(0 m)	(0– ∞ m)
$\phi_{N,\lambda}$	June experiment	0.90	0.0335	7.4	31	51	22
	July experiment	0.89	0.0330	8.5	31	59	26
$\phi_{Chl a,\lambda}$	June experiment	0.83	0.0349	4.1	21	29	12
	July experiment	0.84	0.0345	4.8	23	34	14

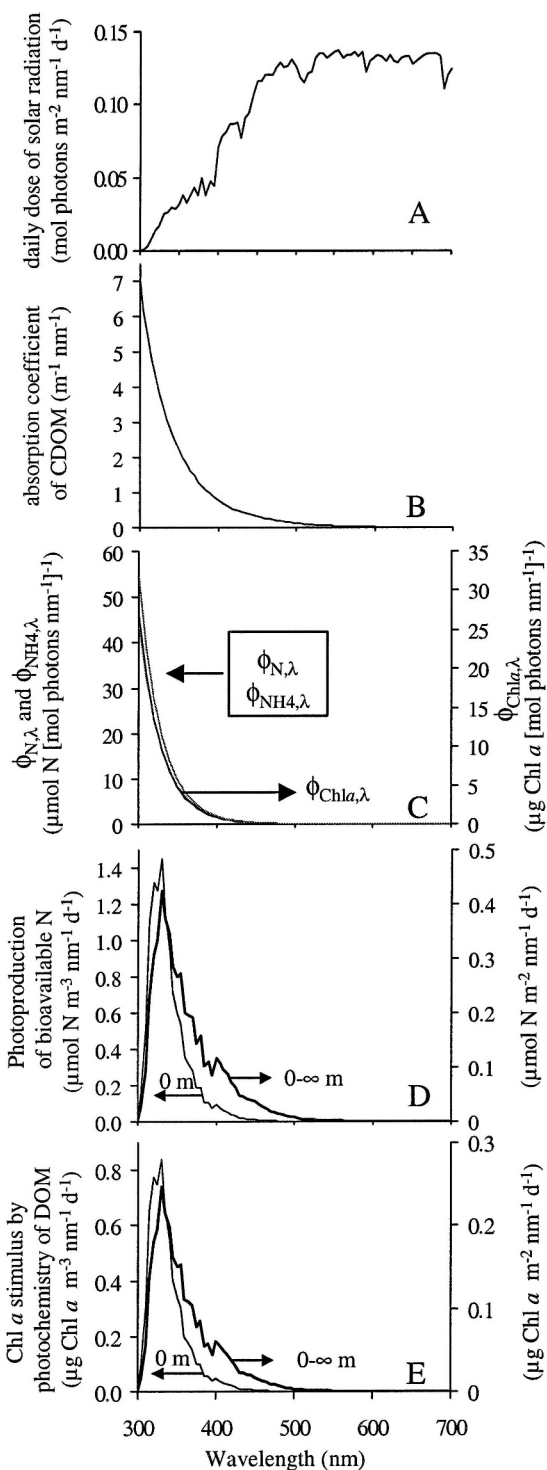


Fig. 6. Calculation of the photoproduction rate of bioavailable N and DOM photochemistry-stimulated phytoplankton at Sta. Långskär during summer. (A) The typical daily dose of downward vector photon flux density spectrum at the surface of the Baltic Sea during summer calculated according to the mean global radiation in May–August in the Baltic Sea and the solar radiation spectrum normalized to the global radiation (Vähätalo and Zepp 2005). (B) Absorption coefficient of CDOM. (C) Apparent quantum yield spectrum for the photochemical production of bioavailable N, the stimulated phytoplankton biomass (July experiment from Table 2) and the photoproduction of NH_4^+

ton gain little from the photomineralized DOC (CO_2), and their growth efficiencies with photoproduct C-substrates are low (Bertilsson and Tranvik 1998; Vähätalo et al. 2003; Smith and Benner 2005). Photoproduct NH_4^+ can serve as a nutrient for both bacterio- and phytoplankton. The bioassay results from this study indicate that the N-limited plankton community retains the photoproduct bioavailable N in their biomass (PN) in a larger extent than what was expected for the photoproduct C with large (up to 98%; Bertilsson and Tranvik 1998) respiratory losses.

Importance of photoproduct bioavailable N—The DOM photochemistry stimulus to phytoplankton ($12\text{--}14 \mu\text{g Chl } a \text{ m}^{-2} \text{ d}^{-1}$) can be transformed into the primary productivity in situ through a C:Chl *a* ratio determined during summer in our study region ($48 \text{ g C:g Chl } a$; Heiskanen and Leppänen 1995). The estimated photochemical stimulus would be $56 \mu\text{mol C m}^{-2} \text{ d}^{-1}$ or 0.17% of the typical summertime production of phytoplankton in the study region ($33 \text{ mmol C m}^{-2} \text{ d}^{-1}$; Lignell 1990). Assuming the Redfield-ratio (C:N of 6.6 by atoms) for the produced phytoplankton, the typical N-demand of phytoplankton is $5.05 \text{ mmol N m}^{-2} \text{ d}^{-1}$, and the photoproduct bioavailable N ($26 \mu\text{mol N m}^{-2} \text{ d}^{-1}$) could contribute potentially 0.5% to the N demand of phytoplankton. Thus, the importance of photoproduct bioavailable N to the total in situ rates of primary production is low, and the primary productivity must be primarily supported by other sources of N in our study region. Indeed, the recycling of N within the plankton community is the major source of N for the phytoplankton in the study region during summer (Heiskanen and Leppänen 1995; Lignell et al. 2003).

If photochemical reactions produce bioavailable N from biologically refractory DON, that process can be considered as a new source of bioavailable N contributing to the new production of phytoplankton. The proportion of new production to the total production is 14% in our study region during summer (Heiskanen and Leppänen 1995). Thus, the photoproduct N can contribute potentially 1.2% to the new primary production and 3.6% to its N demand.

If the photoproduction of bioavailable N (3–4.1% of DON) in this study is directly related to the observed (39–41%) photochemical decomposition of CDOM (as found by Buffam and McGlathery 2003), one can estimate that the complete photochemical decomposition of CDOM could potentially turn 8–10% of DON into bioavailable N. This estimate represents the lower end of earlier estimates (10–18%) for the potentially photoammonifiable DON in the northern Baltic Sea and the coastal North Atlantic Ocean (Bushaw et al. 1996; Vähätalo and Zepp 2005). This value is comparable to the consumption of

(at the station LL12, Vähätalo and Zepp 2005). (D) The calculated photoproduction rate of bioavailable N at the surface (0 m, Eq. 1) and across the entire water column (0– ∞ m, Eq. 2). (E) Calculated stimulus to phytoplankton (Chl *a*) by the DOM photochemistry at the surface (0 m, Eq. 1) and across the entire water column (0– ∞ m, Eq. 2).

Table 3. Reported rates for the photoproduction of bioavailable N across the entire water column during summer.

Site, N-species	$\mu\text{mol N m}^{-2} \text{d}^{-1}$	Reference
A coastal lagoon, U.S.A., NH_4^+	29–44†	Buffam and McGlathery 2003
Humic lake, Finland, NH_4^+	71	Vähätalo et al. 2003
Offshore northern Baltic Sea, NH_4^+	53 ± 35 (16–102)‡	Vähätalo and Zepp 2005
Coastal Baltic Sea, bioavailable N*	23–26	This study

* Bioavailable N, experimentally measured as the accumulated PN and mineralized DON.

† Calculated from the annual mean values of 6–9 $\mu\text{mol NH}_4^+ \text{m}^{-2}$ accounting for the larger solar irradiance during summer (Buffam and McGlathery 2003).

‡ Mean ± SD (range, $n =$ five sites).

DON by bacteria in darkness during a bioassay a few days in length. In the Baltic Sea, the biological lability of DON is the largest in rivers (typically 25% of DON), followed by 13% of DON close (< 13 km) to the coast, with the lowest values (3.6–5.7% of DON) farthest from the shore (Zweifel et al. 1993; Jørgensen et al. 1999; Stepanauskas et al. 2002). Because the portion of DON potentially susceptible to photoammonification can be larger than the readily bioavailable DON in the offshore Baltic Sea, the solar radiation-induced photochemical reactions are quantitatively important in the mobilization of biologically recalcitrant, but photoreactive DON into bioavailable form.

The photoproduction of N can also be compared to other new N sources into surface waters such as N_2 fixation and atmospheric inputs. In the July experiment, the increase in DON in the presence N_2 -fixing cyanobacteria exceeded the photoproduction of bioavailable N, and this finding can likely be extended to those regions of the Baltic Sea with diazotrophic cyanobacterial blooms (Wasmund et al. 2005). The estimates for N_2 fixation in the Baltic Proper and Arkona Sea range from 3.4 $\text{mmol N m}^{-2} \text{yr}^{-1}$ to 320 $\text{mmol N m}^{-2} \text{yr}^{-1}$ and are generally larger than the estimated photochemical production of NH_4^+ in May–August in the northern Baltic Sea (range of 1.9–12.2 mmol N m^{-2} with mean of 6.4; Vähätalo and Zepp 2005; Wasmund et al. 2005). The photoproduction rates of bioavailable N (Table 3) are larger than the atmospheric deposition of DIN in pristine environments (e.g., 6.8 $\mu\text{mol N m}^{-2} \text{d}^{-1}$, annual mean in Alaska), comparable to that in moderately anthropogenically effected regions (e.g., 39 $\mu\text{mol N m}^{-2} \text{d}^{-1}$, the northern Baltic Sea) but lower than the largest reported depositions (e.g., 390 $\mu\text{mol N m}^{-2} \text{d}^{-1}$, southern Baltic Sea; Hertel et al. 2003; Bergström et al. 2005; Jones et al. 2005). The role of photoproduced N as a nutrient source for plankton is likely low in the upwelling regions, under blooms of N_2 -fixing cyanobacteria, and anthropogenically affected waters, but under low or moderate new sources of N, the photoproduced bioavailable N can be an important new source of N for plankton.

Because the photochemistry of N can contribute to the production of bioavailable N (e.g., this study) and also the formation of biologically recalcitrant humic like DON (e.g., Kieber et al. 1997), its role to the aquatic ecosystems is apparently inconsistent. This inconsistency can be clarified via Wetzel's conceptual idea about the biologically

recalcitrant DOM (Wetzel 2001). In the presence of nutrients and favorable conditions, primary production is high and some of the new production is converted into biologically recalcitrant DOM. Wetzel (2001) emphasized that this DOM provides stability to the metabolism of aquatic ecosystems by being slowly used at the production site or elsewhere. Analogously, the net direction of photochemistry can be toward the formation of biologically recalcitrant DON when the concentration of bioavailable N is high (Kieber et al. 1997; Koopmans and Bronk 1999; Reitner et al. 2002). When the concentration of bioavailable N is low, the photoproduction of bioavailable N contributes to the slow biological use of the large pool of biologically recalcitrant DON (Vähätalo et al. 2003; Smith and Benner 2005; this study). Because utilization rates of recalcitrant DON are slow, one should not expect ephemeral blooms of phyto- and bacterioplankton in response to the photoproduced bioavailable N. Instead, the photoproduced N can sustain, in part, the background productivity of plankton in N-limited surface waters with low–moderate inputs of bioavailable N (Rabalais 2002; Bergström et al. 2005).

References

- BERGSTRÖM, A.-K., P. BLOMQUIST, AND M. JANSSON. 2005. Effect of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. *Limnol. Oceanogr.* **50**: 987–994.
- BERTILSSON, S., AND L. J. TRANVIK. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. *Limnol. Oceanogr.* **43**: 885–895.
- BUFFAM, I., AND K. J. MCGLATHERY. 2003. Effect of ultraviolet light on dissolved nitrogen transformations in coastal lagoon water. *Limnol. Oceanogr.* **48**: 723–734.
- BUSHAW, K. L., AND OTHERS. 1996. Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature* **381**: 404–407.
- CAESAR, D., W. GRANÉLI, E. S. KRITZBERG, AND A. M. ANESIO. 2006. Stimulation of metazooplankton by photochemically modified dissolved organic matter. *Limnol. Oceanogr.* **51**: 101–108.
- CARLSSON, P., AND E. GRANÉLI. 1993. Availability of humic bound nitrogen for coastal phytoplankton. *Estuar. Coast. Shelf Sci.* **36**: 433–447.
- DE LANGE, H. J., D. P. MORRIS, AND C. E. WILLIAMSON. 2003. Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. *J. Plankton Res.* **25**: 111–117.

- GAO, H., AND R. G. ZEPP. 1998. Factors influencing photoreactions of dissolved organic matter in a coastal river of the South-eastern United States. *Environ. Sci. Technol.* **32**: 2940–2946.
- GJESSING, E. T., AND T. KÄLLQVIST. 1991. Algicidal and chemical effect of u.v.-radiation of water containing humic substances. *Wat. Res.* **25**: 491–494.
- GOLDMAN, J. C., D. A. CARON, O. K. ANDERSEN, AND M. R. DENNETT. 1985. Nutrient cycling in a microflagellate food chain: I. Nitrogen dynamics. *Mar. Ecol. Prog. Ser.* **24**: 231–242.
- , ———, AND M. R. DENNETT. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol. Oceanogr.* **32**: 1239–1252.
- HANSEN, H. P., AND F. KOROLEFF. 1999. Simultaneous oxidation of nitrogen and phosphorus compounds with persulphate, p. 205–206. *In* K. Grasshoff, K. Kremling, and M. Ehrhardt [eds.], *Methods of seawater analysis*. Wiley-VCH, Weinheim.
- HEISKANEN, A.-S., AND J.-M. LEPPÄNEN. 1995. Estimation of export production in the coastal Baltic Sea: Effect of resuspension and microbial decomposition on sedimentation measurements. *Hydrobiologia* **316**: 211–224.
- HERTEL, O., C. AMBELAS SKJØTH, J. BRANDT, J. H. CHRISTENSEN, M. FROHN, AND J. FRYDENDALL. 2003. Operational mapping of atmospheric nitrogen deposition to the Baltic Sea. *Atmos. Chem. Phys.* **3**: 2083–2099.
- JANSSON, M., P. BLOMQUIST, A. JONSSON, AND A.-K. BERGSTRÖM. 1996. Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Östräsket. *Limnol. Oceanogr.* **41**: 1552–1559.
- JONES, J. B., K. C. PETRONE, J. C. FINLAY, L. D. HINZMAN, AND W. R. BOLTON. 2005. Nitrogen loss from watersheds of interior Alaska underlain with discontinuous permafrost. *Geophys. Res. Lett.* **32**: L02401, doi:10.1029/2004GL021734.
- JONES, R. I. 2000. Mixotrophy in planktonic protists: An overview. *Freshw. Biol.* **45**: 219–226.
- JØRGENSEN, N. O. G., L. TRANVIK, AND G. M. BERG. 1999. Occurrence and bacterial cycling of dissolved nitrogen in the Gulf of Riga, the Baltic Sea. *Mar. Ecol. Prog. Ser.* **191**: 1–18.
- KIEBER, R. J., L. H. HYDRO, AND P. J. SEATON. 1997. Photooxidation of triglycerides and fatty acids in seawater: Implication toward the formation of marine humic substances. *Limnol. Oceanogr.* **42**: 1454–1462.
- KIVI, K., AND OTHERS. 1993. Nutrient limitation and grazing control of the Baltic plankton community during annual succession. *Limnol. Oceanogr.* **38**: 893–905.
- KOOPMANS, D. J., AND D. A. BRONK. 2002. Photochemical production of dissolved inorganic nitrogen and primary amines from dissolved organic nitrogen in waters of two estuaries and adjacent surficial groundwaters. *Aquat. Microb. Ecol.* **26**: 295–304.
- LIGNELL, R. 1990. Excretion of organic carbon by phytoplankton: Its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. *Mar. Ecol. Prog. Ser.* **68**: 85–99.
- , J. SEPPÄLÄ, P. KUUPPO, T. TAMMINEN, T. ANDERSEN, AND I. GISMERVIK. 2003. Beyond bulk properties: Responses of coastal summer plankton communities to nutrient enrichment in the northern Baltic Sea. *Limnol. Oceanogr.* **48**: 189–209.
- MOPPER, K., AND D. J. KIEBER. 2002. Photochemistry and the cycling of carbon, sulphur, nitrogen and phosphorus, p. 456–507. *In* D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Academic Press.
- MORAN, M. A., AND R. G. ZEPP. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnol. Oceanogr.* **42**: 1307–1316.
- RABALAIS, N. N. 2002. Nitrogen in aquatic ecosystems. *Ambio* **31**: 102–112.
- REITNER, B., A. HERZIG, AND G. J. HERNDL. 2002. Photoreactivity and bacterioplankton availability of aliphatic versus aromatic amino acids and a protein. *Aquat. Microb. Ecol.* **26**: 305–311.
- SEITZINGER, S. P., R. W. SANDERS, AND R. STYLES. 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. *Limnol. Oceanogr.* **47**: 353–366.
- SMITH, E. M., AND R. BENNER. 2005. Photochemical transformations of riverine dissolved organic matter: Effects on estuarine bacterial metabolism and nutrient demand. *Aquat. Microb. Ecol.* **40**: 37–50.
- SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnol. Oceanogr.* **14**: 799–801.
- STEPANAUSKAS, R., N. O. G. JØRGENSEN, O. R. EIGAARD, A. ŽVIKAS, L. J. TRANVIK, AND L. LEONARDSON. 2002. Summer inputs of riverine nutrients to the Baltic Sea: Bioavailability and eutrophication relevance. *Ecol. Monogr.* **72**: 579–597.
- TARR, M. A., W. WANG, T. S. BIANCHI, AND E. ENGELHAUPT. 2001. Mechanisms of ammonia and amino acid photoproduction from aquatic humic and colloidal matter. *Wat. Res.* **35**: 3688–3696.
- TETT, P., AND OTHERS. 2003. Eutrophication and some European waters of restricted exchange. *Cont. Shelf Res.* **23**: 1635–1671.
- UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. *Mitt. Int. Verein. Limnol.* **9**: 1–38.
- VÄHÄTALO, A. V., M. SALKINOJA-SALONEN, P. TAALAS, AND K. SALONEN. 2000. Spectrum of the quantum yield for photochemical mineralization of dissolved organic carbon in a humic lake. *Limnol. Oceanogr.* **45**: 664–676.
- , K. SALONEN, U. MÜNSTER, M. JÄRVINEN, AND R. G. WETZEL. 2003. Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. *Arch. Hydrobiol.* **156**: 287–314.
- , AND R. G. WETZEL. 2004. Photochemical and microbial decomposition of chromophoric dissolved organic matter during long (months – years) exposures. *Mar. Chem.* **89**: 313–326.
- , AND R. G. ZEPP. 2005. Photochemical mineralization of dissolved organic nitrogen to ammonium in the Baltic Sea. *Environ. Sci. Technol.* **39**: 6985–6992.
- WASMUND, N., G. NAUSCH, B. SCHNEIDER, K. NAGEL, AND M. VOSS. 2005. Comparison of nitrogen fixation rates determined with different methods: a study in the Baltic Proper. *Mar. Ecol. Prog. Ser.* **297**: 23–31.
- WETZEL, R. G. 2001. *Limnology: lake and river ecosystems*, 3rd ed. Academic Press.
- ZWEIFEL, U. L., B. NORRMAN, AND Å. HAGSTRÖM. 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Mar. Ecol. Prog. Ser.* **101**: 23–32.

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