

Effects of dissolved organic matter and ultraviolet radiation on the accrual, stoichiometry and algal taxonomy of stream periphyton

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SUMMARY

1. We investigated the effects of dissolved organic matter (DOM) and ultraviolet-B (UVB) radiation on periphyton during a 30-day experiment in grazer-free, outdoor artificial streams. We established high [10–12 mg carbon (C) L⁻¹] and low (3–5 mg C L⁻¹) concentrations of DOM in artificial streams exposed to or shielded from ambient UVB radiation. Periphyton was sampled weekly for ash-free dry mass (AFDM), chlorophyll (chl) *a*, algal biovolume, elemental composition [C, nitrogen (N) and phosphorus (P)], and algal taxonomic composition.

2. Regardless of the UVB environment, increased DOM concentration caused greater periphyton AFDM, chl *a* and total C content during the experiment. Increased DOM also significantly increased periphyton C : P and N : P (but not C : N) ratios throughout the experiment. Algal taxonomic composition was strongly affected by elevated stream DOM concentrations; some algal taxa increased and some decreased in biomass and prevalence in artificial streams receiving DOM additions. UVB removal, on the other hand, did not strongly affect periphyton biomass, elemental composition or algal taxonomic composition for most of the experiment.

3. Our results show strong effects of DOM concentration but few, if any, effects of UVB radiation on periphyton biomass, elemental composition and algal taxonomic composition. The effects of DOM may have resulted from its absorption of UVA radiation, or more likely, its provision of organic C and nutrients to microbial communities. The strong effects of DOM on periphyton biomass and elemental composition indicate that they potentially play a key role in food web dynamics and ecosystem processes in forested streams.

Keywords: benthic algae, biofilm, epilithon, reverse osmosis, subsidy

Introduction

Dissolved organic matter (DOM) and ultraviolet-B (UVB) radiation are known to strongly affect important physical, chemical and biological processes in aquatic ecosystems (Williamson *et al.*, 1999). Both DOM and UVB radiation may have strong effects on

periphyton, which we define here as the organic matter attached to benthic surfaces. DOM serves as an organic substrate that fuels the growth of algae (Tuchman, 1996) and heterotrophic bacteria (Bernhardt & Likens, 2002). DOM also strongly reduces the penetration of incident solar radiation in freshwaters (Xenopoulos & Schindler, 2001), which can reduce the exposure of primary producers to damaging levels of UVB radiation (Vinebrooke & Leavitt, 1998; Kelly, Clare & Bothwell, 2001). Exposure to high levels of UVB radiation has been shown to reduce the biomass

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(Bothwell *et al.*, 1993) or have little effect (Hill *et al.*, 1997) on stream periphyton. In this study, we examined the individual and interactive effects of DOM and UVB radiation (wavelengths <320 nm) on the biomass, stoichiometry and algal taxonomic composition of stream periphyton.

Dissolved organic matter and UVB radiation may also affect the elemental composition of stream periphyton. Increasing DOM concentrations may alter the balance of carbon (C) relative to nutrients available for organism uptake, which could translate into changes in the C : nitrogen (N) and C : phosphorus (P) ratios of periphyton biomass (Cross *et al.*, 2005). In extremely high DOM systems, the reduced supply of light relative to nutrients could reduce rates of photosynthesis relative to nutrient uptake and thereby lower periphyton C : N and C : P ratios (Frost & Elser, 2002a). The UVB radiation, on the other hand, has been shown to have variable (Xenopoulos, Frost & Elser, 2002) or no (McNamara & Hill, 2000) effect on C : N and C : P ratios of aquatic producers. While DOM and UVB radiation each could have strong individual effects on periphyton stoichiometry, their interactive effects on stream periphyton C, N and P content have yet to be assessed.

The taxonomic composition of benthic algal communities may also respond to the interactive effects of DOM and UVB radiation. Some algal taxa are known to be at least partially heterotrophic and may increase in prevalence with increasing DOM concentrations (Tuchman, 1996). The increased prevalence of some algal taxa over others caused by increasing DOM may result, in part, from changes in N and P supply that can accompany increased DOM concentrations (Klug, 2002; Romani *et al.*, 2004). Algal community composition may also differ because of UVB radiation given that differential sensitivity of algal taxa to UVB radiation is well documented (Xenopoulos & Frost, 2003). However, little is known about how the taxonomic composition of benthic algal communities will respond to changes in DOM concentration in the presence and absence of UVB radiation.

Here, we examine the individual and interactive effects of DOM and UVB radiation on stream periphyton. We manipulated DOM concentrations in the presence and absence of UVB radiation and measured the responses of periphyton grown in artificial outdoor streams for 1 month. We expected strong negative effects of UVB radiation at ambient

DOM concentrations but no effects of UVB radiation at high concentrations of DOM. We also expected greater effects of DOM in the presence of UVB radiation than in its absence because of the higher lability of organic matter that results from exposure to UVB radiation (e.g. Tranvik & Bertilsson, 2001).

Methods

Artificial stream facility

We conducted our experiment in 16 outdoor circular streams at the University of Notre Dame Environment Research Center located near Land O'Lakes, WI, U.S.A. These artificial streams were arranged in three rows with each row receiving water from head boxes that was constantly fed with groundwater derived from a common source. Artificial streams (1.25-m long \times 0.5-m wide \times 0.15-m deep) held 40 L of circulating water, which was replaced approximately every other day by constant flow. Water depth in each stream was kept at 0.12 m to maintain consistent attenuation of solar radiation by the water column. Current velocity was held within artificial streams at approximately 0.1 m s^{-1} by revolving stainless steel paddles powered by electric motors. Incident solar radiation (wavelengths between 400–700 nm) was measured and recorded on every 5 min using a quantum sensor (Li-cor, LI-190SB sensor, Lincoln, NE, U.S.A.) connected to a data logger. Solar flux at the bottom of artificial streams was calculated using this integrated solar radiation data and average waveband specific [UVB, UVA and photosynthetically active radiation (PAR)] attenuation coefficients. These attenuation coefficients were measured twice during the experiment (25 June and 8 July 2003) with a fibre-optic spectrometer (S2000, Ocean Optics, Dunedin, FL, U.S.A.) connected to an underwater cosine corrected radiation sensor. Incident flux at the growing surface for each of the UV wavebands was calculated by multiplying the total PAR flux by the estimated PAR : UVA and PAR : UVB ratios and adjusting the per cent of flux attenuated at depth using attenuation coefficients following Beer's Law.

Experimental design

We manipulated DOM concentration and UVB radiation in the artificial streams using a full factorial

experimental design. The DOM treatment had two levels, ambient DOM (amb-DOM) and elevated DOM (plus-DOM). The UVB radiation was either present as full sunlight (amb-UVB) or removed by plastic filters (no-UVB) placed over the artificial streams. Each treatment combination had four artificial stream replicates. To create the plus-DOM treatment, river DOM was collected and concentrated using a portable RealSoft PROS/IS reverse osmosis system (Serkiz & Perdue, 1990). Reverse osmosis retains approximately 95–99% of DOM from water and results in relatively small changes in DOM physiochemical properties (Kilduff *et al.*, 2004). DOM was collected on five consecutive days (8–12 June 2003) from the Manitowish River (WI, U.S.A.), placed into dark amber bottles, and refrigerated at 4 °C until used in the experiments. UVB was removed from one-half of the artificial streams by placing Mylar[®] plastic (Dupont) 3 cm above the lip of the stream channel. Mylar[®] plastic removes all solar wavelengths below 320 nm (Xenopoulos *et al.*, 2002), which was verified prior to the experiment using the fiber optics spectrometer.

Periphyton was seeded in the artificial streams prior to the start of the experiment using a homogenised organic slurry collected by scraping periphyton from rocks taken from Tenderfoot Creek, Michigan. Aliquots of the periphyton slurry were added to each artificial stream and allowed to settle in the absence of the paddle-driven current onto square clay tiles, which served as the substrate for periphyton colonisation. After 24 h, the water current was initiated and the DOM and UVB treatments were applied. DOM was added thereafter to increase the raw absorbance of water at 330 nm to a specific value (0.07 cm⁻¹), estimated to correspond to a DOM concentration of 10 mg C L⁻¹. Raw absorbance at 330 nm was measured using a spectrophotometer and DOM was added twice daily to ensure DOM concentrations were held relatively constant.

Artificial stream sampling

Water chemistry and periphyton were sampled weekly during the 1 month (15 June–15 July 2003) of the experiment. Water samples for DOM and nutrient chemistry were immediately passed through 0.2-µm polycarbonate filters that were rinsed with >0.05 L of distilled water prior to use to remove potential organic contaminants. Filtered streamwater

used for DOM analysis was stored in amber bottles at 4 °C until analysis. Nutrient samples were also stored refrigerated at 4 °C and were analysed within 1 week of sampling. Periphyton was sampled from two clay tiles from all treatment combinations on each date except for the first sampling date (June 20) when three tiles per artificial stream were sacrificed. Periphyton was scraped from clay tiles with a hard-bristled toothbrush and gently homogenised in a blender. Periphyton slurries were sub-sampled for ash-free dry mass (AFDM) and elemental composition (C, N and P) by passing a fixed volume of the slurry through preashed GF/F filters (Whatman Inc., Florham Park, NJ, U.S.A.). These filters were dried for >24 h at 60 °C and then stored frozen. Chlorophyll *a* samples were also collected on GF/F filters but were frozen immediately. For assessment of algal biovolume and taxonomy, 0.02 L of the periphyton slurry was preserved with Lugol's solution and stored in the dark. At the end of the experiment, pH was measured in all artificial streams with a water quality sonde multiprobe (Hydrolab Quanta, Loveland, CO, U.S.A.).

Chemical analyses

We determined the concentration of dissolved organic carbon (DOC) with a Shimadzu TOC 5000 analyser (Columbia, ML, U.S.A.) after acidification and purging of CO₂. The UV absorption at 280 nm (*a*₂₈₀) was measured with Ocean Optics scanning spectrophotometer (USB2000, Dunedin, FL, U.S.A.). The concentration of soluble reactive phosphorus (SRP) was quantified in water taken from each artificial stream using with the molybdate-ascorbic acid technique (APHA, 1992). Ammonium (NH₄) was also determined spectrophotometrically using the phenate method (APHA, 1992). Nitrate (NO₃) was determined using a DX 600 ion chromatography system from Dionex (Sunnyvale, CA, U.S.A.) with the GS 50 gradient pump and ED 50A electrochemical detector running on Peaknet 6 (USEPA, 1993). Periphyton AFDM was determined by weighing GF/F filters used to collect periphyton before and after combustion at 550 °C for 4 h. Chl *a* was determined fluorometrically without phaeophytin correction after cold methanol extraction of filters for 24 h in the dark (Marker *et al.*, 1980). Periphyton C and N content were measured with a Costech elemental analyser (ECS 4010, Valencia, CA, U.S.A.). Following digestion with potassium

persulfate, periphyton P content was measured on the ashed AFDM filters using the molybdate-blue ascorbic acid method (APHA, 1992).

Benthic algal counting

Algal biovolume was estimated in preserved periphyton samples by counting up to 100 cells of each algal taxon under an inverted microscope. Algae were identified to genus using keys of Ward & Whipple (1959) and Wehr & Sheath (2003). In addition, for each sample, we measured the geometric dimensions of at least 20 cells of each genus following Hillebrand *et al.* (1999). Little variation in either cell size or morphology was noted within algal genera. Algal biovolume estimates, assuming a specific gravity of 1, were converted into estimates of algal C using an average conversion factor ($0.145 \mu\text{g C } \mu\text{g wet mass}^{-1}$) as performed by Hessen *et al.* (2003).

Statistical analysis

We assessed treatment effects of DOM and UVB through time using repeated measures ANOVA (RM-ANOVA) on all stream water chemistry and periphyton response variables on Systat (Systat Software Inc., Point Richmond, CA, U.S.A.). For each response variable, family-wide error rates were held below $P < 0.05$ by dividing by the sum of main effect and interaction terms ($n = 7$; Kirk, 1995). In addition, we were not explicitly interested in time effects but instead on the accumulated effects of the DOM and UVB treatments. Consequently, we conducted a two-way ANOVAs on each response variable using data from the last date of the experiment. For these two-way ANOVAs, pair-wise contrasts were completed whenever the interaction term was found to be significant. We also restricted our analysis of benthic algal taxonomic composition to two-way ANOVAs on data from the final date of the experiment.

Results

DOM and other aspects of the physico-chemical environment

We increased the concentration of DOC and the a_{280} in plus-DOM streams compared with the amb-DOM streams for the entire experiment (Fig. 1, Table 1). In

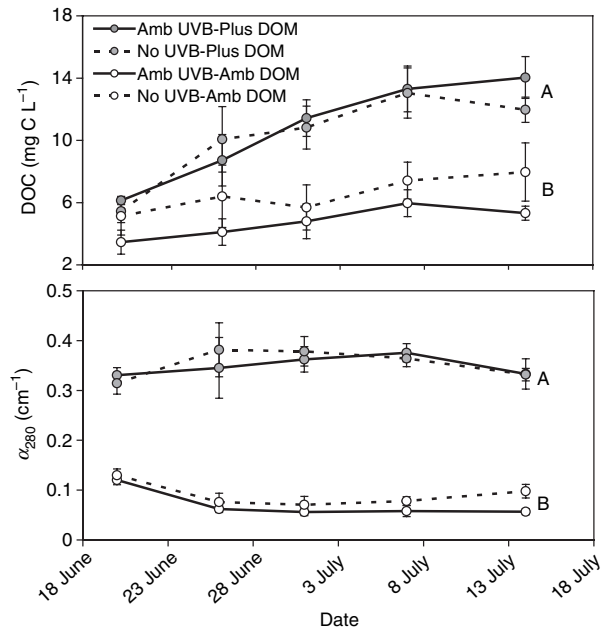


Fig. 1 Dissolved organic carbon concentrations and a_{280} in the artificial streams during the course of this study. Error bars represent ± 1 SE. Treatment levels with different letters were significantly different from each other on the last day as determined with *post hoc* pairwise comparisons. ns indicates treatment levels were not significantly different ($P < 0.05$).

contrast, we found no significant effects of UVB removal on DOC concentration or a_{280} at any point in the experiment (Fig. 1, Table 1). The addition of DOM significantly affected the ultraviolet radiation environment in the artificial streams. In artificial streams where we added DOM, UVB radiation reaching stream periphyton was reduced by approximately 99.9% of incident surface values compared with a 65% reduction in the amb-DOM treatments (Fig. 2). Consequently, total UVB flux integrated over the entire experiment was significantly lower in the plus-DOM treatments (Fig. 2). Adding DOM also reduced the total flux of UVA radiation (solar radiation in the 320–400 nm range) reaching the bottom of the artificial streams. In the plus-DOM treatments, periphyton received about approximately 15% of the surface UVA radiation whereas ambient DOM streams received about approximately 65% of incident UVA radiation. The addition of DOM did not significantly affect PAR (400–780 nm) exposure over the course of the experiment (Fig. 2). Dissolved inorganic nitrogen (DIN; $\text{NH}_4 + \text{NO}_3$) was found at very low concentrations at the start and increased

Table 1 Comparison of *P* values for repeated measures ANOVA of stream chemistry and periphyton responses to dissolved organic matter (DOM), UVB treatments (UV) and sampling date (Day)

	DOC	Abs ₂₈₀	Chl	AFDM	Alg C	PC	PN	PP	C : P	C : N	N : P
DOM	<0.001	<0.001	<0.001	<0.001	<0.007	<0.001	<0.001	<0.02	<0.007	0.20	<0.001
UVB	0.49	0.36	0.01	0.10	0.18	0.14	0.11	0.37	0.78	0.71	0.96
Day	<0.001	0.56	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
DOM × UVB	0.25	0.69	0.19	0.75	0.80	0.42	0.66	0.96	0.36	0.33	0.37
DOM × Day	0.01	0.12	<0.001	0.04	0.42	0.03	0.02	0.50	0.98	0.78	0.30
UVB × Day	0.48	0.75	0.07	0.38	0.81	0.10	0.03	0.50	0.58	0.82	0.43
DOM × UVB × Day	0.38	0.55	0.02	0.44	0.73	0.93	0.41	0.46	0.55	0.08	0.78

Significant main effects and interaction terms have been bolded.

AFDM, ash-free dry mass; Chl, chlorophyll; PC, periphyton carbon; PN, periphyton nitrogen; PP, periphyton phosphorus; C : P, carbon : phosphorus ratio; C : N, carbon : nitrogen ratio; N : P, nitrogen : phosphorus ratio.

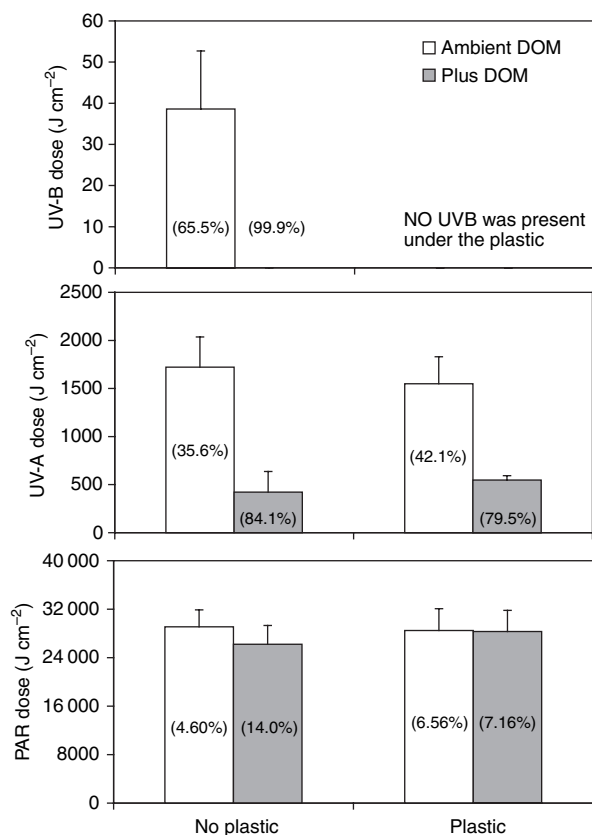


Fig. 2 Total doses of ultraviolet-B, ultraviolet-A and photosynthetically active radiation (PAR) received in each treatment reaching the periphyton over the course of the experiment. Error bars represent ± 1 SE. Also given in parentheses is the per cent of incident radiation removed prior to reaching the stream bottom. See methods for details on the calculation of total UV radiation and PAR doses received by periphyton.

over the course of the experiment (Fig. 3). SRP showed the opposite pattern by decreasing through the experiment to very low concentrations (Fig. 3). This caused the DIN : SRP ratios within the artificial

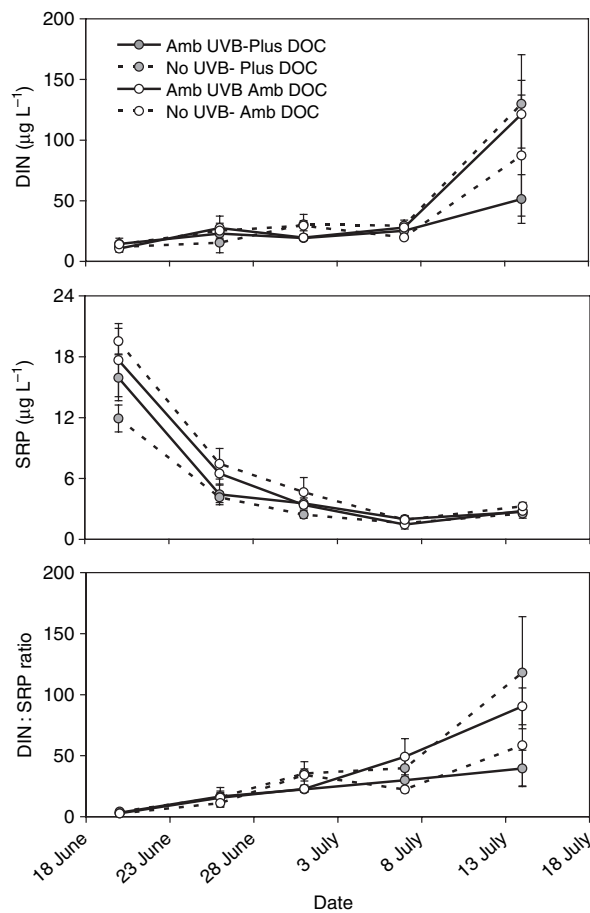


Fig. 3 Concentrations of dissolved inorganic nitrogen and soluble reactive phosphorus and their ratios in the artificial streams during the course of this study. Error bars represent ± 1 SE. No treatments were found significantly different ($P < 0.05$) from each other on the last day with a two-way ANOVA on the final day of the experiment.

streams to increase during the experiment (Fig. 3). No significant treatment effects of DOM or UVB were found on DIN, SRP or DIN : SRP ratios. We also

found no effects of DOM additions on artificial stream pH with all streams having a pH between 8.20 and 8.40.

DOM and UVB effects on periphyton biomass

Periphyton biomass (AFDM) increased significantly faster in the plus-DOM compared with amb-DOM artificial streams regardless of the UVB environment (Fig. 4, Table 1). The addition of DOM also resulted in greater chl in the presence and absence of UVB radiation for most of the experiment (Fig. 4). The removal of UVB radiation did not affect AFDM but did increase chl on the last sampling of the experiment in both the high- and low-DOM treatments (Fig. 4). While algal C increased in all artificial streams throughout the experiment (Fig. 4), we detected

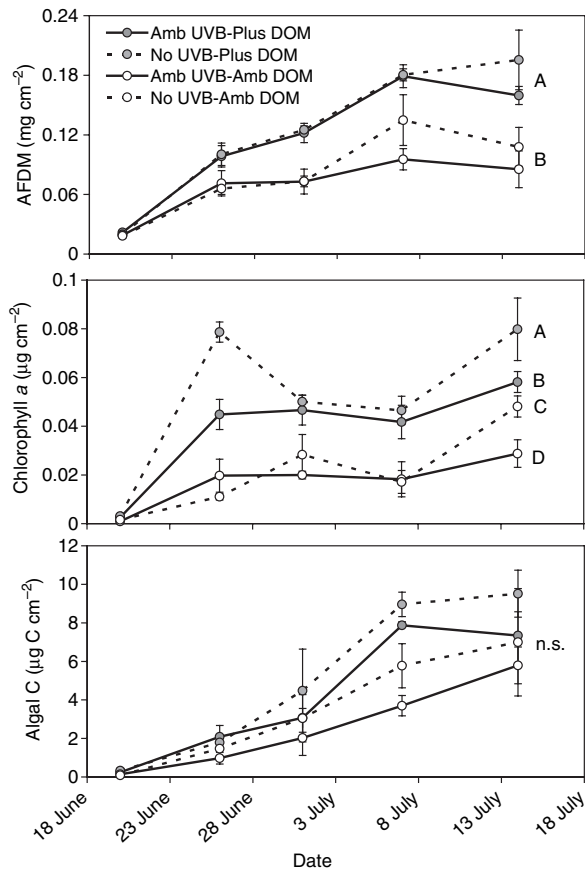


Fig. 4 Mean periphyton ash-free dry mass, chlorophyll *a*, and algal carbon in the artificial streams during the course of this study. Error bars represent ± 1 SE. Treatment levels with different letters were significantly different from each other on the last day as determined with *post hoc* pairwise comparisons. ns indicates treatment levels were not significantly different ($P < 0.05$).

no treatment effect (either by DOM or UVB) on algal C on the last day of the experiment. For AFDM, chl and algal C, we found no significant interactive effects of DOM and UVB radiation during the experiment or on the final sampling date (Fig. 4, Table 1).

Responses of periphyton stoichiometry to DOM and UVB radiation

We found significantly greater periphyton C, N and P content in plus-DOM streams for most of the experiment (Fig. 5, Table 1). The UVB removal, on the other hand, increased periphyton C and N content but not P content on the last day of the experiment. Periphyton C : N ratios were not significantly affected by the addition of DOM or the removal of UVB radiation. Adding DOM significantly increased periphyton C : P and N : P ratios for most of the experiment (Fig. 5). The removal of UVB radiation also significantly increased C : P and N : P ratios in both plus-DOM and amb-DOM streams on the last day of the experiment (Fig. 5). As for periphyton biomass, we detected no significant interactions between DOM and UVB treatments on periphyton stoichiometry (Table 1).

Effects of DOM and UVB radiation on periphyton taxonomic composition

Diatoms, chlorophytes and cyanobacteria were the primary constituents of benthic algal biomass and accounted for >90% of the biomass throughout the experiment. Diatoms were most prevalent (60–90% of total biomass) and showed rapid biomass increases from the start of the experiment (Fig. 6). Cyanobacteria biomass accumulated mostly during the first half of the experiment (Fig. 6) but constituted only 1–6% of total algal biomass at the end of the experiment. We found no significant treatment effects of either DOM or UVB radiation on diatom or cyanobacterial biomass on the last day of the experiment. Green algae (Chlorophyta) were not prevalent in the artificial streams until the last three sampling dates (Fig. 6) and after that increased to 10–30% of total biomass by the last sampling date. Biomass of Chlorophyta was significantly higher in the plus-DOM streams on the last day of the experiment (Fig. 6).

We found species specific responses of the algae to the DOM treatment. Of the four most abundant

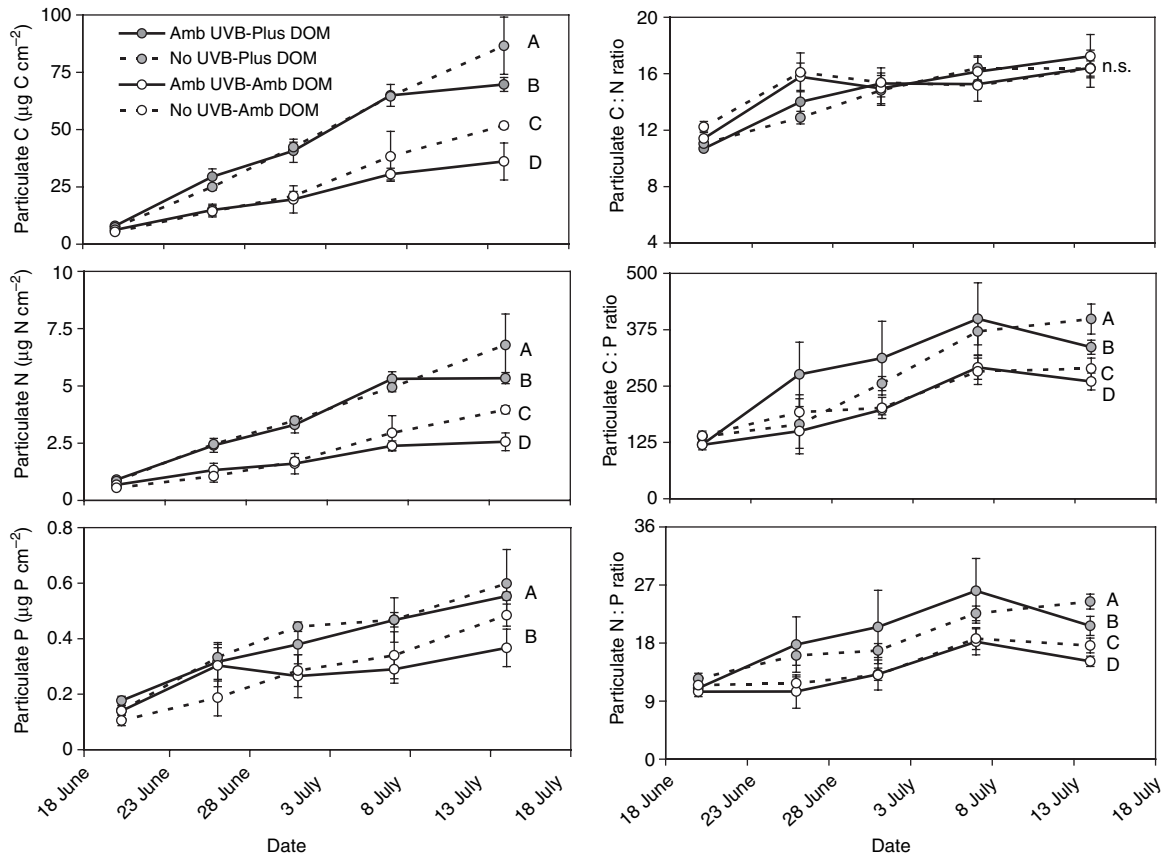


Fig. 5 Mean periphyton carbon (C), nitrogen (N), phosphorus (P), C : N ratios, C : P ratios and N : P ratios in the artificial streams during the course of this study. Error bars represent ± 1 SE. Treatment levels with different letters were significantly different from each other on the last day as determined with *post hoc* pairwise comparisons. ns indicates treatment levels were not significantly different ($P < 0.05$).

diatoms, one genus (*Cymbella*) showed significantly greater biomass in the plus-DOM treatment (Fig. 7) whereas two other genera (*Nitzschia* and *Fragellaria*) were significantly less abundant in the plus-DOM streams (Fig. 7). We found no significant effects of DOM or UVB removal on the most abundant diatom genera, *Synedra*, although there was about twice as much biomass of this genera in the plus-DOM artificial streams. The most common green alga, *Spirogyra*, which constituted >95% of the biomass of Chlorophyta (but <20% of total algal biomass), was positively affected by the addition of DOM to artificial streams (Fig. 7). The most common cyanobacteria, *Aphanothece*, showed increased biomass over the first 2 weeks and relatively constant biomass after that. No significant effects of DOM additions were found on the biomass of *Aphanothece* in the artificial streams. We found no significant effects of UVB removal on the biomass of any algal taxon (Fig. 7).

Discussion

We detected strong and direct effects of increased DOM concentrations on periphyton biomass, stoichiometry and algal taxonomic composition. In general, periphyton biomass, C : P and N : P ratios, and algal biomass all increased in the presence of increased DOM. Such DOM-mediated effects could, in turn, alter benthic food web dynamics and stream biogeochemistry. For example, higher periphyton biomass resulting from increased stream DOM concentrations may increase secondary production of invertebrate consumers (Feminella & Hawkins, 1995), alter particulate organic carbon export (Battin *et al.*, 2003), and serve as a sink for N and/or P (Bernhardt & Likens, 2002).

Previous studies that elevated DOM concentrations in streams used relatively simple and highly labile carbon substrates (e.g. sucrose or potassium acetate;

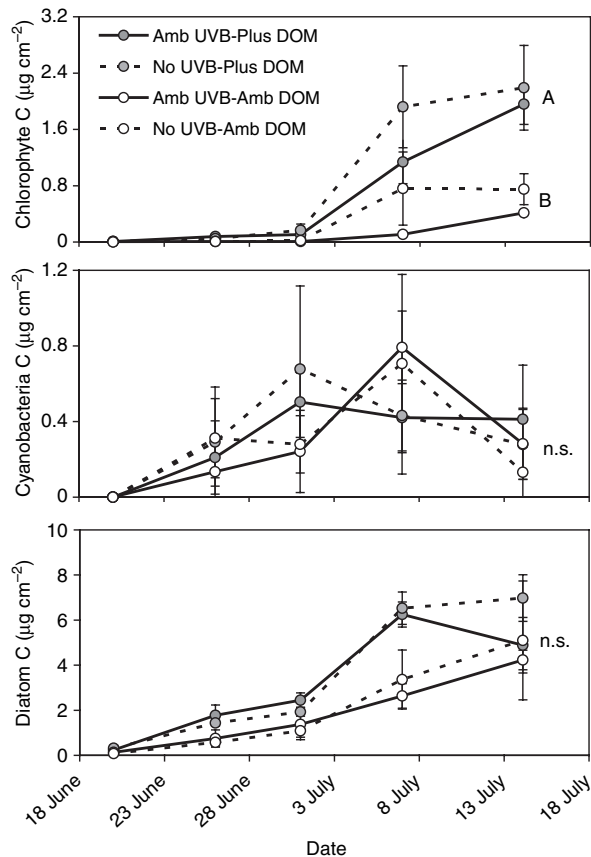


Fig. 6 Mean biomass of Chlorophyta (green algae), Cyanobacteria and diatoms in the artificial streams during the course of this study. Error bars represent ± 1 SE. Treatment levels with different letters were significantly different from each other on the last day as determined with *post hoc* pairwise comparisons. n.s. indicates treatment levels were not significantly different ($P < 0.05$).

Bernhardt & Likens, 2002). These studies found that the addition of organic carbon greatly increased the biomass of the 'sewage' bacterium, *Sphaerotilus* sp. (Bernhardt & Likens, 2002), presumably because of greater carbon supply to and uptake by these heterotrophs. Other studies of lake plankton (Klug, 2002) and littoral periphyton (Vinebrooke & Leavitt, 1998) used more natural mixtures of DOM and also found increased biomass of seston and periphyton, respectively. We also found greater periphyton biomass in artificial streams that received the concentrated DOM, which is further indication that natural sources of DOM may provide important material or energetic resources to benthic ecosystems in lakes and streams.

One explanation for increases in periphyton biomass in the DOM-augmented treatments is that

increased supplies of organic C and nutrients (N, and/or P) fuelled greater growth of algae and/or heterotrophic bacteria (Vinebrooke & Leavitt, 1998; Klug, 2002). This nutrient supply hypothesis would require, in part, a periphyton matrix consisting primarily of bacterial and algal biomass. Without a sizable contribution of these biomass types, increased growth rates of algal and/or bacterial cells (even if caused by the DOM) would be unable to account for the increased periphyton biomass that we observed. While we found a positive effect of DOM on the biomass of algal cells over the course of the experiment, we found a relatively small percentage (<20%) of total periphyton biomass to be accounted for by the algal biomass. A relatively low contribution of algae to periphyton biomass appears to be quite common in a wide range of benthic habitats (Frost, Hillebrand & Kahlert, 2005). While the relative biomass and growth responses of bacteria in the periphyton was not assessed, bacterial biomass also is often a fairly small percentage (<10%) of total periphyton biomass (Frost *et al.*, 2005). Other heterotrophs (i.e. protozoans and other microinvertebrates) can also constitute a modest proportion of the periphyton (Hillebrand *et al.*, 2002), but they were not abundant in the artificial stream periphyton (C. Cherrier, personal communication). Given these typically low relative contributions (<30–40% of total biomass) of the combined biomass of algae, bacteria, and other heterotrophs to the periphyton, it seems unlikely that increased algal or bacterial growth in the presence of increased DOM concentrations entirely accounts for the increases of periphyton biomass observed in the plus-DOM streams.

Greater periphyton biomass may have also resulted in the DOM-augmented streams because of algal and bacterial growth responses to the reduced UVA flux into these artificial streams. Plus-DOM streams received only approximately 30% of the UVA radiation that reached the periphyton growing in the amb-DOM streams. It is possible that this UVA reduction resulted in greater algal growth rates (Bothwell *et al.*, 1993; Xenopoulos *et al.*, 2002; Kelly, Bothwell & Schindler, 2003) and, thereby, increased periphyton biomass in the plus-DOM streams. However, the relatively low prevalence of algal biomass (20%) in the periphyton as described above would again indicate that UVA inhibition of algal growth does not fully account for the changes in periphyton biomass seen in our experiment.

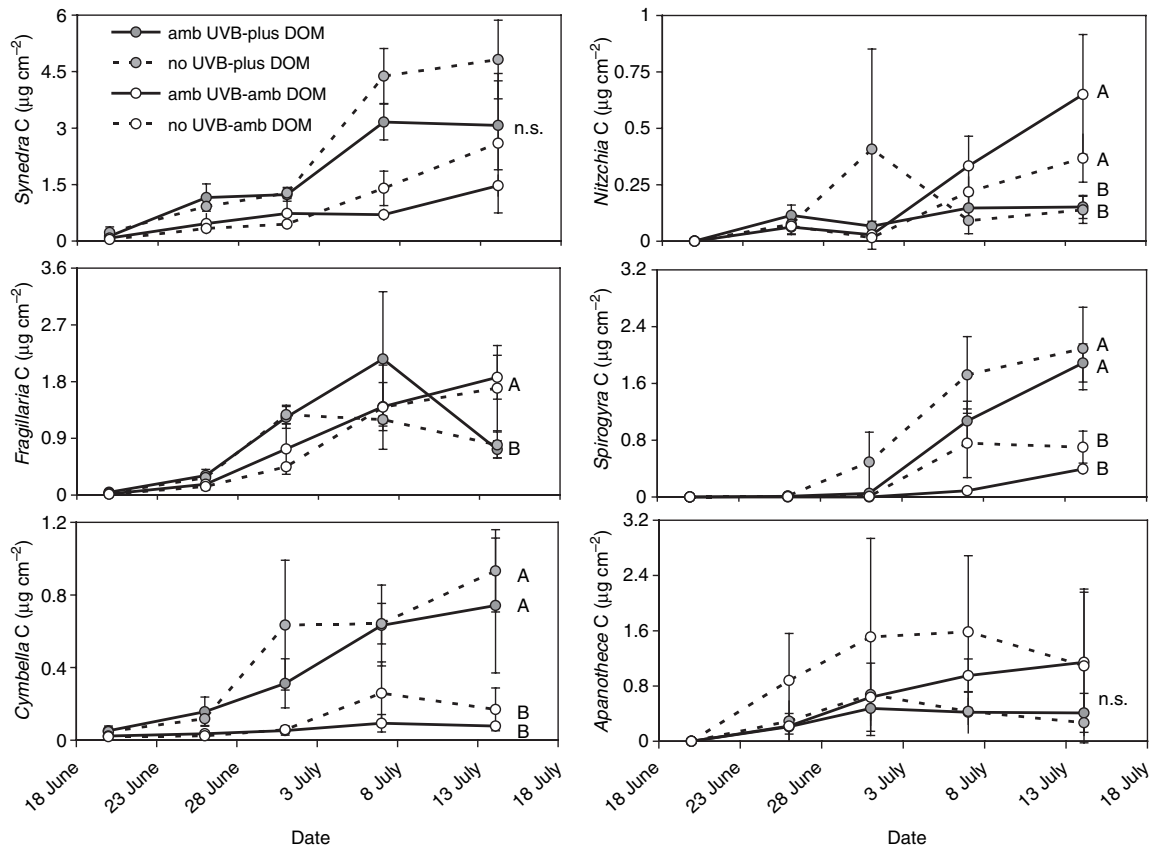


Fig. 7 Mean biomass of selected algal genera in the artificial streams during the course of this study. Error bars represent ± 1 SE. Treatment levels with different letters were significantly different from each other on the last day as determined with *post hoc* pairwise comparisons. ns indicates treatment levels were not significantly different ($P < 0.05$).

An alternative explanation for DOM effects on periphyton biomass is that changes to resource supplies or UVA shading altered the detrital biomass (non-living organic matter) in periphyton found in the plus-DOM streams. Greater detrital biomass would presumably result from changes in its rate of deposition and/or its decomposition. Increased DOM concentrations may have caused greater carbon acquisition by bacterial and/or algal cells, causing them to exude a greater amount of organic matter as extracellular polymeric substances (EPS). EPS are a well-known component of the periphyton (Hoagland *et al.*, 1993) and appears to be more prevalent under nutrient limiting conditions (Frost & Elser, 2002a). The relatively low nutrient content of the EPS would have reduced its rate of decomposition (Cebrian & Duarte, 1995) and contributed to its greater accumulation. It is also possible that the UVA protection provided by DOM increased net photosynthesis, which yielded greater carbon exudation in these

presumably P-limited algae (Sterner *et al.*, 1997). These processes involving the greater acquisition and exudation of carbon by periphytic algae and bacteria are thus a mechanism that may account for the increased periphyton biomass in the plus-DOM streams.

We also found that increased DOM concentrations significantly altered periphyton stoichiometry. Periphyton in DOM-augmented streams had higher C : P and N : P ratios but showed no difference in C : N ratios. This was likely a result of elevated DOM : P ratios in the water column in the plus-DOM streams, given the low background SRP concentrations in the groundwater feeding the artificial streams and a very low P content of the DOM. The DOC : total phosphorus (TP) molar ratio of the DOM used was about 900 : 1, which is presumably much higher than ratios found in attached algal and bacterial cells. Previous work has shown high DOM : P supply ratios, such as this, can heighten P-limitation in heterotrophic bacteria (Chrzanowski, Sterner & Elser,

1995) and increase their competition for P with algae (Elser *et al.*, 2002). Increases in DOM : P ratios in the stream water may have elevated C : P ratios of both bacterial and algal cells and increased their exudation of C-rich EPS causing C : P ratios in the periphyton to increase through the experiment. High DOM : P supply ratios thus likely accounts for the greater relative abundance of C relative to P in the periphyton. Additional work should clarify the mechanisms (bacterial-algal competition, exudation of POM, changes in cellular N and P content) underlying DOM-mediated changes in periphyton stoichiometry.

We also found that algal taxonomic composition responded strongly to DOM addition. In particular, we found greater chlorophyte biomass in the DOM-augmented streams and increased prevalence of one diatom taxon, *Cymbella*. Two other diatoms, *Fragillaria* and *Nitzschia*, declined in abundance in the presence of increased DOM concentrations. These changes in the prevalence of algal species probably resulted from altered nutrient supply rates or ratios (Stelzer & Lamberti, 2001) and/or changes to UVA exposure (Xenopoulos *et al.*, 2002; Xenopoulos & Frost, 2003). Changes to the N : P supply ratios can alter the competitive arena for benthic algal taxa (Stelzer & Lamberti, 2001) and may explain the increases in some diatom taxa but not others to increased DOM concentrations. Another explanation for changes to the periphyton algal community composition is that the DOM additions altered the pH in the artificial streams. This mechanism would not account for the changes in benthic algal community composition seen here as we found no differences in pH among the artificial streams. Nonetheless, the effects of increased DOM concentrations on algal taxonomic composition found here, whether mediated by heterotrophy, nutrient resources or UVA exposure, is further indication of important role that this material plays in aquatic ecosystems (Williamson *et al.*, 1999).

The addition of DOM led to greater periphyton biomass in the artificial streams but this organic material was relatively P-poor compared with periphyton growing under ambient DOM concentrations. Consequently, this increased food *quantity* may have lower food *quality* for benthic consumers as high C : N and C : P ratios can constrain the growth of benthic consumers (Frost & Elser, 2002b). Periphyton stoichiometry has been proposed to strongly affect population dynamics of particular consumer taxa,

alter benthic food web dynamics and change grazer-mediated ecosystem processes (Cross *et al.*, 2005). Our study shows that benthic food web stoichiometry may be responsive to changes in stream DOM concentrations given the relatively strong effects this material had on periphyton elemental composition.

Our results suggest that DOM should be considered an important chemical feature of streams in northern Michigan as we have shown it to affect periphyton growth, elemental composition and algal community structure. DOM concentrations vary in streams of this region from 3 to 40 mg C L⁻¹ (Frost *et al.*, 2006), but even a much narrower range (approximately 3–12 mg C L⁻¹) in our experiment elicited measurable changes in the periphyton. Given that the range in our experiment encompasses only the lower 50% of DOC concentrations in streams of the region, it is unclear how further increases in DOM concentration would affect periphyton communities. Higher DOM concentrations would lead to lower light and possibly light limitation of algal production in many streams. Visible solar radiation did not appear to be limiting in our experiment as little of the incident PAR was removed in the water column of plus-DOM streams (Fig. 2). Greater light limitation would probably further shift the community towards heterotrophy and lead to a greater importance of heterotrophic bacteria and algae (Williamson *et al.*, 1999). Given these complexities, it is unclear whether higher concentrations of DOM (above 12 mg C L⁻¹) would lead to even greater periphyton biomass and whether it would further increase periphyton C : P and N : P ratios. In addition, given the sometimes stark differences in DOM chemistry found among streams (Frost *et al.*, 2006) in the region, stream-specificity in the responses of periphyton and its microflora to DOM is another possible complicating factor. Future work should consider how DOM concentration and chemistry affects periphyton biomass, taxonomic composition and stoichiometry and its subsequent effects on stream food webs and nutrient cycling.

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References

- APHA (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. American Public Health Association, Washington, D.C.
- Battin T.J., Kaplan L.A., Newbold J.D. & Hansen C.M.E. (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*, **426**, 439–442.
- Bernhardt E.S. & Likens G.E. (2002) Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology*, **83**, 1689–1700.
- Bothwell M.L., Sherbot D., Roberge A.C. & Daley R.J. (1993) Influence of natural ultraviolet radiation on lotic periphytic diatom community growth, biomass accrual, and species composition: short-term versus long-term effects. *Journal of Phycology*, **29**, 24–35.
- Cebrian J. & Duarte C.M. (1995) Plant growth-rate dependence of detrital carbon storage in ecosystems. *Science*, **268**, 1606–1608.
- Chrzanowski T.H., Sterner R.W. & Elser J.J. (1995) Nutrient enrichment and nutrient regeneration stimulate bacterioplankton growth. *Microbial Ecology*, **29**, 221–230.
- Cross W.C., Benstead J.P., Frost P.C. & Thomas S.A. (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology*, **50**, 1895–1903.
- Elser J.J., Frost P.C., Kyle M., Urabe J. & Andersen T. (2002) Effects of light and nutrients on plankton stoichiometry and biomass in a P-limited lake. *Hydrobiologia*, **481**, 101–112.
- Feminella J.W. & Hawkins C.P. (1995) Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society*, **14**, 465–497.
- Frost P.C. & Elser J.J. (2002a) Effects of light and nutrients on the net accumulation and elemental composition of epilithon in boreal lakes. *Freshwater Biology*, **47**, 173–184.
- Frost P.C. & Elser J.J. (2002b) Growth responses of littoral mayflies to the phosphorus content of their food. *Ecology Letters*, **5**, 232–240.
- Frost P.C., Hillebrand H. & Kahlert M. (2005) Low algal content and its effects on the C : P stoichiometry of lake and coastal periphyton. *Freshwater Biology*, **50**, 1800–1808.
- Frost P.C., Larson J.H., Johnston C.A., Young K.C., Maurice P.A., Lamberti G.A. & Bridgman S.D. (2006) Landscape predictors of stream dissolved organic matter concentration and physiochemistry in a Lake Superior river watershed. *Aquatic Sciences*, **68**, 40–51.
- Hessen D.O., Andersen T., Brettum P. & Faafeng B.A. (2003) Phytoplankton contribution to sestonic mass and elemental ratios in lakes: implications for zooplankton nutrition. *Limnology and Oceanography*, **48**, 1289–1296.
- Hill W.R., Dimick S.M., McNamara A.E. & Branson C.A. (1997) No effects of ambient UV radiation detected in periphyton and grazers. *Limnology and Oceanography*, **42**, 769–774.
- Hillebrand H., Durselen C.-D., Kirschtel D., Pollinger U. & Zohary T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, **35**, 403–424.
- Hillebrand H., Kahlert M., Haglund A.L., Berninger U.G., Nagel S. & Wickham S. (2002) Control of microbenthic communities by grazing and nutrient supply. *Ecology*, **83**, 2205–2219.
- Hoagland K.D., Rosowskim J.R., Gretz M.R. & Roemer S.C. (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *Journal of Phycology*, **29**, 557–566.
- Kelly D.J., Clare J.J. & Bothwell M.L. (2001) Attenuation of solar ultraviolet radiation by dissolved organic matter alters benthic colonization patterns in streams. *Journal of the North American Benthological Society*, **20**, 96–108.
- Kelly D.J., Bothwell M.L. & Schindler D.W. (2003) Effects of solar ultraviolet radiation on stream benthic communities: an intersite comparison. *Ecology*, **84**, 2724–2740.
- Kilduff J.E., Mattaraj S., Wigton A., Kitis M. & Karanfil T. (2004) Effects of reverse osmosis isolation on reactivity of naturally occurring dissolved organic matter in physicochemical processes. *Water Research*, **38**, 1026–1036.
- Kirk R.E. (1995) *Experimental Design: Procedures for the Behavioral Sciences*, 3rd edn. Brooks/Cole Publishing, Pacific Grove, CA, U.S.A.
- Klug J.L. (2002) Positive and negative effects of allochthonous dissolved organic matter and inorganic nutrients on phytoplankton growth. *Canadian Journal of Fisheries and Aquatic Science*, **59**, 85–95.
- Marker A.F.H., Nusch E.A., Rai H. & Riemann B. (1980) The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusions and recommendations. *Archiv für Hydrobiologie*, **14**, 91–106.

- McNamara A.E. & Hill W.R. (2000) UV-B irradiance gradient affects photosynthesis and pigments but not food quality of periphyton. *Freshwater Biology*, **43**, 649–662.
- Romani A.M., Giorgi A., Acuna V. & Sabater S. (2004) The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. *Limnology and Oceanography*, **49**, 1713–1721.
- Serkiz S.M. & Perdue E.M. (1990) Isolation of dissolved organic-matter from the Suwannee River using reverse-osmosis. *Water Research*, **24**, 911–916.
- Stelzer R.S. & Lamberti G.A. (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnology and Oceanography*, **46**, 356–367.
- Sterner R.W., Elser J.J., Fee E.J., Guildford S.J. & Chrzanowski T.H. (1997) The light : nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. *American Naturalist*, **150**, 663–684.
- Tranvik L.J. & Bertilsson S. (2001) Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecology Letters*, **4**, 458–463.
- Tuchman N.C. (1996) The role of heterotrophy in algae. In: *Algal Ecology: Freshwater Benthic Ecosystems* (Eds R. Stevenson, M.L. Bothwell & R.L. Lowe), pp. 299–316. Academic, San Diego, CA, U.S.A.
- USEPA (1993) *Method 300.0, Test Method for the Determination of Inorganic Anions in Water by Ion Chromatography*. Environmental Monitoring Systems Laboratory, USEPA ORD, Cincinnati, OH.
- Vinebrooke R.D. & Leavitt P.R. (1998) Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web. *Limnology and Oceanography*, **43**, 1065–1081.
- Ward H.B. & Whipple G.C. (1959) *Freshwater Biology*. John Wiley and Sons Inc., New York.
- Wehr J.D. & Sheath R.G. (2003) *Freshwater Algae of North America: Ecology and Classification*. Academic, New York.
- Williamson C.E., Morris D.P., Pace M.L. & Olson A.G. (1999) Dissolved organic carbon and nutrients as regulators of lake ecosystems: resurrection of a more integrated paradigm. *Limnology and Oceanography*, **44**, 795–803.
- Xenopoulos M.A. & Frost P.C. (2003) Ultraviolet radiation changes the taxonomic composition of phytoplankton in a boreal lake. *Journal of Phycology*, **39**, 291–302.
- Xenopoulos M.A. & Schindler D.W. (2001) Physical factors determining ultraviolet flux into ecosystems. In: *Ecosystems, Evolution and UV Radiation* (Eds C.S. Cockell & A.R. Blaustein), pp. 36–62. Springer, New York.
- Xenopoulos M.A., Frost P.C. & Elser J.J. (2002) Joint effects of UV radiation and phosphorus supply on algal growth rate and elemental composition. *Ecology*, **83**, 423–435.

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