

# Positive and negative effects of allochthonous dissolved organic matter and inorganic nutrients on phytoplankton growth

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**Abstract:** Dissolved organic matter (DOM) can have both positive and negative effects on phytoplankton growth. The magnitude of these effects may vary depending on the source of DOM and the composition of the phytoplankton community. Here, I address the relative importance of the positive and negative effects of DOM extracts on phytoplankton growth. In short-term experiments with phytoplankton from West Long Lake, a small, moderately coloured lake in northern Michigan, U.S.A., the net effect of doubling ambient DOM on phytoplankton growth was positive. Increasing DOM concentrations from  $\sim 10 \text{ mg C}\cdot\text{L}^{-1}$  to  $\sim 20 \text{ mg C}\cdot\text{L}^{-1}$  had a negative effect on total phytoplankton growth by reducing irradiance and thus reducing the depth to which growth was positive. However, inorganic nutrients in the DOM extracts increased growth at each irradiance level. The positive effect on phytoplankton growth owing to the nutrients associated with DOM was greater than the negative effect caused by shading. Although the positive effects of allochthonous DOM inputs outweighed the negative effects for the nutrient-limited phytoplankton in these experiments, the net effect depends on the concentration and availability of nutrients associated with allochthonous DOM as well as the physiological status of the phytoplankton community.

**Résumé :** La matière organique dissoute (DOM) peut avoir des effets tant positifs que négatifs sur la croissance du phytoplancton. L'importance de ces effets peut varier selon la source de DOM et la composition de la communauté phytoplanctonique. La présente étude cherche à déterminer l'importance relative des effets positifs et négatifs d'extraits de DOM sur la croissance du phytoplancton. Dans des expériences à court terme sur le phytoplancton de West Long Lake, un petit lac aux eaux moyennement colorées du nord du Michigan, É.-U., le dédoublement de la DOM ambiante a un effet positif sur la croissance du phytoplancton. L'augmentation des concentrations de DOM de  $\sim 10 \text{ mg C}\cdot\text{L}^{-1}$  à  $\sim 20 \text{ mg C}\cdot\text{L}^{-1}$  a un effet négatif sur la croissance du phytoplancton total en réduisant l'irradiance, diminuant ainsi la profondeur jusqu'à laquelle la croissance est positive. Cependant, les nutriments inorganiques dans les extraits de DOM augmentent la croissance, à tous les niveaux d'irradiance. L'effet positif des nutriments associés à la DOM sur la croissance du phytoplancton est plus grand que l'effet négatif de l'ombrage. Bien que les effets positifs des apports allochtones de DOM dépassent les effets négatifs dans ces expériences chez du phytoplancton qui est limité par la carence de nutriments, l'effet net dépendra de la concentration et de la disponibilité des nutriments associés à la DOM allochtone, de même que de l'état physiologique de la communauté phytoplanctonique.

[Traduit par la Rédaction]

## Introduction

Limnologists have long recognized the importance of coloured dissolved organic matter (DOM) in aquatic systems (Birge and Juday 1927), but the net effects of DOM on primary productivity are still debated (Carpenter et al. 1998; Nurnberg and Shaw 1998; Williamson et al. 1999). DOM is a mixture of organic compounds that give brown water lakes their characteristic colour. Common constituents of DOM include organic acids such as humic and fulvic acids, and smaller molecules like amino acids and carbohydrates

(McKnight and Aiken 1998). Coloured DOM may be an important regulator of autotrophic phytoplankton growth because it alters two resources that phytoplankton depend on: light and nutrients (Jones 1992; Hessen and Tranvik 1998).

DOM absorbs light and decreases the depth to which light penetrates the water column (Kirk 1996). In some clear lakes, the light absorbing properties of DOM may benefit phytoplankton by protecting them from harmful ultraviolet radiation (Ekelund 1993). However, in most lakes, DOM is more likely to be detrimental owing to decreased light available for photosynthesis (Jones 1992; Carpenter et al. 1998). In addition, decreases in light penetration in the water column may indirectly affect phytoplankton by altering the physical structure of the lake. Small lakes with high DOM concentrations typically have thinner mixed layers than lakes with lower DOM concentrations (Fee et al. 1996). The overall effect on the light environment of the epilimnion hinges on how DOM affects the size of the photic zone relative to the size of the mixed layer. For example, Jones (1992) found that DOM did not influence the average amount of light in the epilimnion of small lakes in Finland because the photic

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zone and the mixed layer were both shallower in highly coloured lakes.

The effects of DOM on nutrient availability are similarly diverse. Some sources of DOM contain high levels of nitrogen and phosphorus. For example, surveys have found that total phosphorus concentration is higher in lakes with high water colour and organic carbon (Meili 1992; Nurnberg and Shaw 1998). However, the availability of the phosphorus and nitrogen associated with DOM is largely unknown and involves a number of separate processes. For example, DOM may form DOM-iron-phosphorus complexes, which prevent sedimentation of phosphorus but also reduce its availability to phytoplankton (Jones 1998). Phytoplankton may be able to access the bound phosphorus if it is released by photolysis (Francko and Heath 1979; DeHaan 1992) or by production of enzymes that mediate hydrolysis of the complexes (Francko 1986). Release of nitrogen may also be increased by photolysis (Carlsson and Graneli 1993). In addition, DOM is involved in the cycling of trace elements such as iron. Studies have shown that complexes of DOM with iron and phosphorus can either increase (Prakash et al. 1973) or decrease (Jackson and Hecky 1980) the availability of iron to phytoplankton.

Because DOM can have both positive and negative effects on phytoplankton, it is difficult to predict the overall impact that changes in DOM may have on phytoplankton growth. I conducted three mesocosm experiments to explore some of the mechanisms by which DOM affects phytoplankton. In addition to the experiments, I used a simple Monod model of phytoplankton growth to illustrate the relative importance of the different mechanisms by which DOM can affect phytoplankton. Because DOM can affect both light and nutrient availability, I independently manipulated DOM, light, and inorganic nutrients to evaluate their relative influence on phytoplankton growth. Each experiment followed a similar protocol (see below), although each experiment was designed to test different hypotheses about phytoplankton response to DOM and thus contained different treatments. I expected DOM to have a negative effect on the light environment experienced by phytoplankton. In addition, I expected DOM to either increase or decrease nutrient availability to phytoplankton depending on the nutrient-binding capacity and availability of inorganic nutrients associated with the DOM source.

## Methods

### Study site

Long Lake is a small seepage lake located in Gogebic County, Mich. (89°32'W, 46°13'N), at the University of Notre Dame Environmental Research Center. During the 1990s, the lake was part of a set of whole-lake experiments designed to test whether lakes with different food webs responded differently to nutrient addition. In May 1991, Long Lake was divided into three basins (east, west, and central) using plastic curtains (Christensen et al. 1996). The east and west basins were used for the experiments described here. The installation of the curtain caused DOM concentrations (measured as dissolved organic carbon, DOC) to increase in East Long Lake because of changes in basin hydrology. Following curtain installation, DOC concentration was always higher in East Long Lake than in West Long Lake

(Christensen et al. 1996). At the time of the experiments reported here, extinction coefficients in East and West Long lakes were  $3.1 \text{ m}^{-1}$  and  $\sim 1.7 \text{ m}^{-1}$ , respectively. Limnological conditions in the lake at the time of the experiments are listed in Table 1.

### Experimental protocol

I used 10-L square polyethylene cubitainers (I-Chem, Newcastle, Del.) as mesocosms. Water for the mesocosms was collected from West Long Lake using a 2.5 m long, 8 cm diameter PVC (polyvinyl chloride) integrated sampler between 7:30 and 9:00 to avoid light damage to the organisms. The sampler was lowered to 2 m to collect an integrated sample from 0 to 2 m. The thermocline depth ranged from 2.5 to 3 m at the time of the experiments, thus this method integrated most of the well-mixed epilimnion and avoided collection of phytoplankton from the metalimnion (Table 1). Water was passed through an 80 mm mesh net to remove cladocerans, copepods, and most rotifers. Very large phytoplankton were also excluded, but on average comprised only 3.1% of total phytoplankton biovolume (S.R. Carpenter, Center for Limnology, 680 N. Park St., Madison, WI 53706, unpublished data). Ciliates and other heterotrophic protozoans passed through the mesh. Treatments were mixed in 100-L plastic pails and then poured into the mesocosms. Lake water was diluted 1 part treatment liquid to 8 parts lake water. Control mesocosms were diluted with deionized water. The mesocosms were incubated in situ and were sampled every 1–2 days by inverting the mesocosms three times to mix and then pouring into a sample bottle.

### Sources of DOM

Two sources of DOM were used in experiments: commercial *Sphagnum* peat moss (Premier Pro-Moss Sogevex, Premier Horticulture, Dorval, Que.) and freshly collected *Sphagnum* peat moss. I used DOM extracted from commercial peat moss because each experiment was designed to test a different mechanism, and a standardized DOM source was necessary to ensure consistency among experiments. To assess the effects of a different DOM source, I compared DOM extracted from commercial peat moss with DOM extracted from fresh peat moss. Commercial peat moss was extracted with deionized water. Extract was made using 140 g commercial peat moss for every 1 L of deionized water. The peat moss – water slurry was mixed every other day and left to sit at room temperature for 10 days. Extract was then coarse-filtered through muslin bags and centrifuged for 10 min at 7000 rpm (revolutions per minute). The supernatant was filter-sterilized through either a 0.2-mm or 0.45-mm filter cartridge to remove bacteria, refrigerated, and used within two days of filtering. Fresh peat extract was collected from the bog mat surrounding Tuesday Lake, a small lake less than 5 km from Long Lake. I dug a shallow (ca. 25 cm) hole in the mat approximately 30 m from the edge of the lake and collected peat from just below the water surface. Water was squeezed from the peat by hand and was filtered following the same protocol as the commercial peat moss. I measured the DOC concentration of the extract using an OI TOC (total organic carbon) analyzer (College Station, Tex.) with persulfate oxidation, pH using an electrode presoaked in sample water, and phosphorus and nitrogen concentrations using a

**Table 1.** Limnological conditions in West Long Lake at the time of the experiments.

| Experiment         | Initial chl <i>a</i><br>( $\mu\text{g}\cdot\text{L}^{-1}$ ) | Initial<br>DOC<br>( $\text{mg}\cdot\text{L}^{-1}$ ) | TP and TN<br>( $\mu\text{g}\cdot\text{L}^{-1}$ ) | Thermocline<br>depth (m) | Depth of<br>1% light<br>level (m) | $k_{\text{dPAR}}$ ( $\text{m}^{-1}$ ) | Avg.<br>irradiance<br>( $\text{E}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ ) | Avg. SI<br>( $\text{E}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ ) |
|--------------------|---|---|--|--------------------------|-----------------------------------|---------------------------------------|--|---|
| High vs. low light | 6.3   | 11.5  | 27.2, 756.5                                      | 2.5                      | 2.7                               | 1.8 (West)<br>3.1 (East)              | 11.6 (West)<br>4.6 (East)  | 46.4  |
| DOM source         | 8.5   | 9.3   | 20.6, 620.1                                      | 2.5                      | 3                                 | 1.5                                   | 14.9   | 47.1  |
| Light gradient     | 4.4   | 10.2  | 22.5, 690.2                                      | 3.0                      | 2.9                               | 1.6                                   | 0.56–37.7  | 37.7  |

**Note:** Avg. irradiance, average irradiance at incubation depth; Avg. SI, average surface irradiance. DOC (dissolved organic carbon) and TP (total phosphorus) and TN (total nitrogen) refer to the ambient concentrations in the water used for the experiments.  $k_{\text{dPAR}}$  and average irradiance at incubation depth are also given for East Long Lake because containers were incubated in East Long Lake for the low light treatment of the high vs. low light experiment.

Lachat colorimetric autoanalyzer (Milwaukee, Wis.). Total phosphorus was measured following persulfate digestion and total nitrogen was measured following Kjeldahl digestion. Phosphate, nitrate, and ammonia were measured on undigested extract. The concentrations of DOC, N, and P added with the DOM extract varied because of differences in extract chemistry (Table 2). DOM treatments lowered the pH of sample water by  $<0.5$  pH units.

#### NP treatments

Fertilizer for the inorganic nutrient treatments was made from potassium nitrate ( $\text{KNO}_3$ ) and sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ). In each NP treatment, ambient P and N concentrations were elevated by  $32 \mu\text{g}\cdot\text{L}^{-1}$  and  $320 \mu\text{g}\cdot\text{L}^{-1}$ , respectively, corresponding to an N–P atomic ratio of 23:1. This ratio was chosen to maintain phosphorus limitation. Preliminary experiments in West Long Lake showed that this concentration was sufficient to saturate growth with respect to nutrients (J.L. Klug, unpublished data).

#### Limnological analyses

Chlorophyll *a* concentration was quantified to assess changes in total phytoplankton biomass and was determined fluorometrically. Samples (100–200 mL) were filtered onto a Whatman GF/F filter. Filters were placed in film canisters and frozen for at least 24 h. Chlorophyll *a* was extracted in methanol for 24 h and analyzed with a fluorometer before and after acidification to correct for pheopigments (Marker et al. 1980). For all experiments, chlorophyll *a* concentration was determined on whole water samples. For the high light vs. low light experiment, chlorophyll *a* concentration in size-fractionated water samples was also measured. Size fractions measured were  $>35 \mu\text{m}$ ,  $10\text{--}35 \mu\text{m}$ , and  $<10 \mu\text{m}$ . For the DOM source experiment, phytoplankton community composition was quantified using the Utermöhl method (Utermöhl 1958). Preserved 25-mL subsamples were gravity settled for at least 22 h and counted on an inverted microscope at  $400\times$ . At least 25 fields or 200 cells were counted per sample. Most phytoplankton were identified to genus.

Surface irradiance ( $I_0$ ) was measured using a model 3010 Weathertronics Mechanical Pyranograph (NovaLynx Corp., Grass Valley, Calif.), which was located in the middle of an open field approximately 1 km from the study site. Irradiance was measured continuously throughout the summer. Irradiance at depth ( $I_z$ ) was measured at least weekly using a model LI-193SA LI-COR Spherical Quantum Sensor (Lincoln, Nebr.) light meter.

#### Experimental design

##### High vs. low light experiment

An experiment was designed to test how DOM affected phytoplankton growth by changing the light environment. Three replicates of eight treatments were prepared: high light control, low light control, high light NP (added inorganic nitrogen and phosphorus), low light NP, high light DOM (added DOM extracted from commercial peat moss), low light DOM, high light NPDOM (added NP and DOM), and low light NPDOM. I used the difference in DOC concentration between the east and west basins of Long Lake (see Study site section) to manipulate the light environment. The mesocosms were small enough that the light environment within the mesocosms was determined by light in the incubation environment rather than the DOM concentration in the mesocosms. All replicates were incubated at 0.75 m below the water surface. Three mesocosms each of control, NP, DOM, and NPDOM were incubated in the high DOM basin (the low light treatment), and three mesocosms each of control, NP, DOM, and NPDOM were incubated in the lower DOM basin (the high light treatment). A total of 24 mesocosms was used for this experiment. Temperature at 0.75 m was the same in both basins ( $\sim 22^\circ\text{C}$ ). This was the only experiment in which treatments were incubated in the high DOM basin. The experiment was conducted 1–7 July 1998. Response variables were total chlorophyll *a* concentration over time and size-fractionated chlorophyll *a* concentration at the beginning and end of the experiment.

##### DOM source experiment

The DOM source experiment was designed to test whether DOM from different sources had similar qualitative effects on phytoplankton growth. In this experiment there were no light treatments and I used DOM extracts from both commercial and freshly collected peat moss instead of just commercial peat moss. The NP treatment was the same as in the high vs. low light experiment. A total of 18 mesocosms was used in this experiment. The experiment was conducted 23–28 June 1999. Response variables were total chlorophyll *a* concentration over time and phytoplankton community composition at the beginning and end of the experiment.

##### Light gradient experiment

The light gradient experiment was designed to identify the effects of DOM on phytoplankton growth at different irradiance levels. At each of nine depths in the epilimnion (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, and 2.5 m below the surface), I incubated one control mesocosm, one added NP

**Table 2.** Dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) added in each experimental treatment.

| Experiment         | Treatment   | DOC added (mg·L <sup>-1</sup> ) | TN added (µg·L <sup>-1</sup> ) | TP added (µg·L <sup>-1</sup> ) | C:N:P of DOM |
|--------------------|-------------|---------------------------------|--------------------------------|--------------------------------|--------------|
| High vs. low light | Control     | 0                               | 0                              | 0                              | —            |
|                    | NP          | 0                               | 320 (320)                      | 32 (32)                        | —            |
|                    | DOM dried   | 9.9                             | na                             | na                             | na           |
|                    | NPDOM dried | 9.9                             | na                             | na                             | na           |
| DOM source         | Control     | 0                               | 0                              | 0                              | —            |
|                    | NP          | 0                               | 320 (320)                      | 32 (32)                        | —            |
|                    | DOM dried   | 10.9                            | 986.6 (391.7)                  | 30.6 (19.4)                    | 352:32:1     |
|                    | DOM fresh   | 5.0                             | 190.9 (7.1)                    | 18.7 (14.6)                    | 263:10:1     |
|                    | NPDOM dried | 10.9                            | 1306.6 (711.7)                 | 62.6 (51.4)                    | 352:32:1     |
|                    | NPDOM fresh | 5.0                             | 510.9 (327.1)                  | 50.7 (46.6)                    | 263:10:1     |
| Light gradient     | Control     | 0                               | 0                              | 0                              | —            |
|                    | NP          | 0                               | 320 (320)                      | 32 (32)                        | —            |
|                    | DOM dried   | 7.9                             | 666 (368)                      | 19.1 (12.5)                    | 414:35:1     |

**Note:** Values in parentheses are the concentrations of added nutrients that were inorganic (phosphate, nitrate and (or) ammonia). Nutrient ratios are by weight. DOM, dissolved organic matter; na, data not available.

mesocosm, and one added DOM mesocosm. DOM was extracted from commercial peat moss. A total of 27 mesocosms was used in this experiment. The experiment was conducted 6–12 August 1998. The response variable was total chlorophyll *a* concentration over time.

Incubating mesocosms over a range of depths allowed me to calculate the total amount of phytoplankton growth throughout the water column (areal growth). Areal growth,  $G$ , can be calculated by integrating phytoplankton growth over depth:

$$(1) \quad G = \int_0^{z_{eu}} g(z) dz$$

where  $g(z)$  is growth at depth  $z$ , and  $z_{eu}$  is the depth of the euphotic zone. Irradiance decreases with depth according to Beer's law such that

$$(2) \quad I_z = I_0 e^{-k_d z}$$

where  $I_z$  is irradiance at depth  $z$ ,  $I_0$  is the surface irradiance, and  $k_d$  is the extinction coefficient of light. The change in irradiance over depth is

$$(3) \quad \frac{dI}{dz} = -k_d I_0 e^{-k_d z} = -k_d I_z$$

Equation 3 can be used in eq. 1 to give areal growth as a function of irradiance instead of depth:

$$(4) \quad G = \int_{I_0}^{I_{eu}} g(I) \frac{1}{-k_d I} dI = \frac{1}{k_d} \int_{I_{eu}}^{I_0} \frac{g(I)}{I} dI = \frac{1}{k_d} G_0$$

where  $g(I)$  is phytoplankton growth as a function of irradiance. Equation 4 gives areal growth as a function of extinction coefficient and an aggregate growth function  $G_0$

given by  $G_0 = \int_{I_{eu}}^{I_0} \frac{g(I)}{I} dI$ . Representing areal growth  $G$  as a

function of the extinction coefficient is useful because it allows comparison of areal growth in waters with different DOM concentrations and therefore different extinction coefficients; specifically, if

$$(5) \quad G_1 = \frac{1}{k_{d1}} G_0 \text{ and } G_2 = \frac{1}{k_{d2}} G_0, \text{ then } \frac{G_1}{G_2} = \frac{k_{d2}}{k_{d1}}$$

Therefore, if the areal growth and extinction coefficient are known for a particular lake, eq. 5 can be used to predict the change in areal growth for a given change in extinction coefficient. This method of calculating relative areal growth is only valid if the mechanism for a change in extinction coefficient (e.g., change in DOM) does not change the aggregate growth function  $G_0$ . For example, increasing DOM changes extinction coefficients but could also change the relationship between growth and irradiance by changing the spectral quality of light.

#### Model exercise

To illustrate the relative importance of the different mechanisms by which DOM can affect phytoplankton, I used the standard Monod model of phytoplankton growth modified to include two limiting resources (O'Neill et al. 1989; Huisman and Weissing 1995) where the growth rate  $\mu$  is a function of the maximum growth rate ( $\mu_{max}$ ) and the external amounts of light ( $I$ ) and phosphorus ( $P$ ) such that

$$\mu = \mu_{max} \frac{I}{K_I + I} \frac{P}{K_P + P}$$

$K_I$  is the light level at which growth is half of  $\mu_{max}$ , and  $K_P$  is the phosphorus concentration at which growth is half of  $\mu_{max}$ . Because phytoplankton growth is a function of light and phosphorus, which may both change as DOM concentration changes, this model can be used to explore how growth rates change relative to DOM concentration and source. For this exercise, I used irradiance at 1 m to calculate growth rate. I used two physiological scenarios: one scenario in which  $K_I < K_P$  and one scenario in which  $K_I > K_P$ . These parameter values were not chosen to represent particular species but rather to illustrate the effect of phytoplankton physiology on the shape of the curves. This exercise assumes that phytoplankton are obligate autotrophs.

#### Statistical analyses

For the high vs. low light and DOM source experiments, I used repeated measures analysis of variance (RM-ANOVA)

to assess time effects, treatment effects (light, DOM, and NP effects), and time  $\times$  treatment interactions on chlorophyll *a* concentration (Gurevitch and Chester 1986). I applied a logarithmic transformation to normalize the residuals and homogenize variances. The symmetry of the covariance matrix did not meet the assumptions of RM-ANOVA. However, the Huynh–Feldt (H–F) conditions were satisfied, and therefore I used H–F corrected *p* values (Potvin et al. 1990). If a significant ( $p < 0.05$ ) treatment or time  $\times$  treatment interaction was found and there was more than one level of the treatment (e.g., in the DOM source experiment, DOM had two levels, DOM fresh and DOM dried), I used pairwise contrasts to assess which treatments were significantly different.

## Results

### High vs. low light experiment

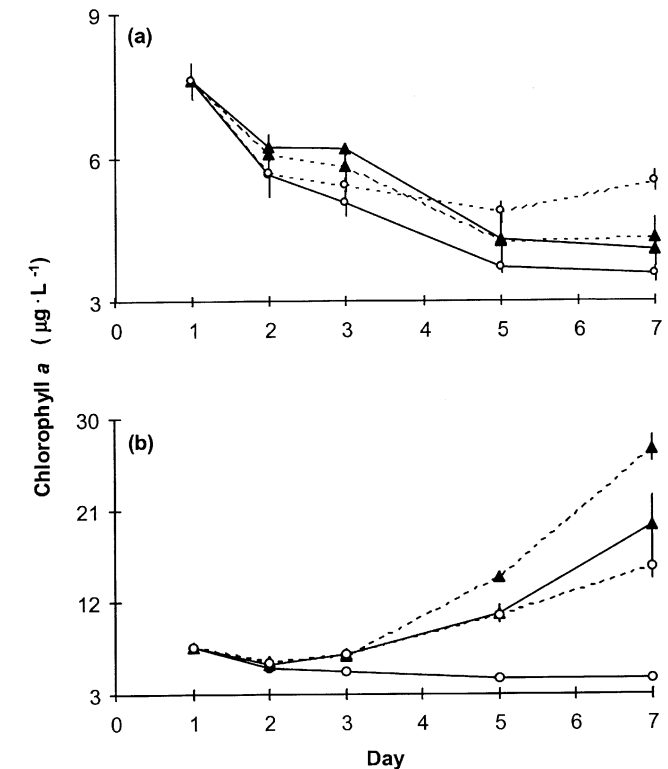
The high vs. low light experiment took advantage of different DOM concentrations in the west and east basins of Long Lake by using the difference to modulate the light environment of the incubated mesocosms. Phytoplankton biomass (estimated as chlorophyll *a* concentration) was higher in the DOM (added DOM) and NP (added nitrogen and phosphorus) treatments than in the control treatment (Fig. 1; DOM effect  $F_{1,16} = 66.69$ ,  $p = 0.0001$ ; NP effect  $F_{1,16} = 67.54$ ,  $p = 0.0001$ ). However, there was a strong NP  $\times$  light and DOM  $\times$  light interaction, suggesting that phytoplankton response to DOM extracts and NP depended on the light environment (Fig. 1; DOM  $\times$  light interaction  $F_{1,16} = 46.97$ ,  $p = 0.0001$ ; NP  $\times$  light interaction  $F_{1,16} = 24.13$ ,  $p = 0.0001$ ). Phytoplankton biomass declined slightly in all of the containers that were incubated at low light levels (Fig. 1a). The low light levels in this experiment were caused by high DOM in the water column, which suggests a strong negative effect of DOM on phytoplankton growth via shading. In the high light treatments, phytoplankton responded positively to NP and DOM (Fig. 1b), and the magnitude of the response to DOM was similar to the response to NP (Fig. 1b). There was a marginally significant negative DOM  $\times$  NP interaction, which suggests that phytoplankton response to DOM and NP in combination was less than expected based on the response to NP and DOM alone (Fig. 1; DOM  $\times$  NP interaction  $F_{1,16} = 4.31$ ,  $p = 0.054$ ).

The phytoplankton community was initially dominated by very small taxa. Measuring the chlorophyll *a* concentrations of size-fractionated water samples showed that the proportions of  $<10 \mu\text{m}$ ,  $10\text{--}35 \mu\text{m}$ , and  $>35 \mu\text{m}$  taxa were 0.84, 0.06, and 0.10, respectively. In all treatments, the proportion of very small taxa declined and the proportion of larger taxa increased by the end of the experiment such that the proportions of  $<10 \mu\text{m}$ ,  $10\text{--}35 \mu\text{m}$ , and  $>35 \mu\text{m}$  taxa averaged 0.53, 0.24, and 0.23, respectively. The increase in larger taxa may be due to the absence of macrozooplankton grazers. Although there was a shift in size structure of the phytoplankton, the community remained dominated by very small taxa ( $<10 \mu\text{m}$ ).

### DOM source experiment

To test whether the positive effect of the DOM extracts was consistent with DOM sources other than commercial peat moss, I compared phytoplankton response to DOM ex-

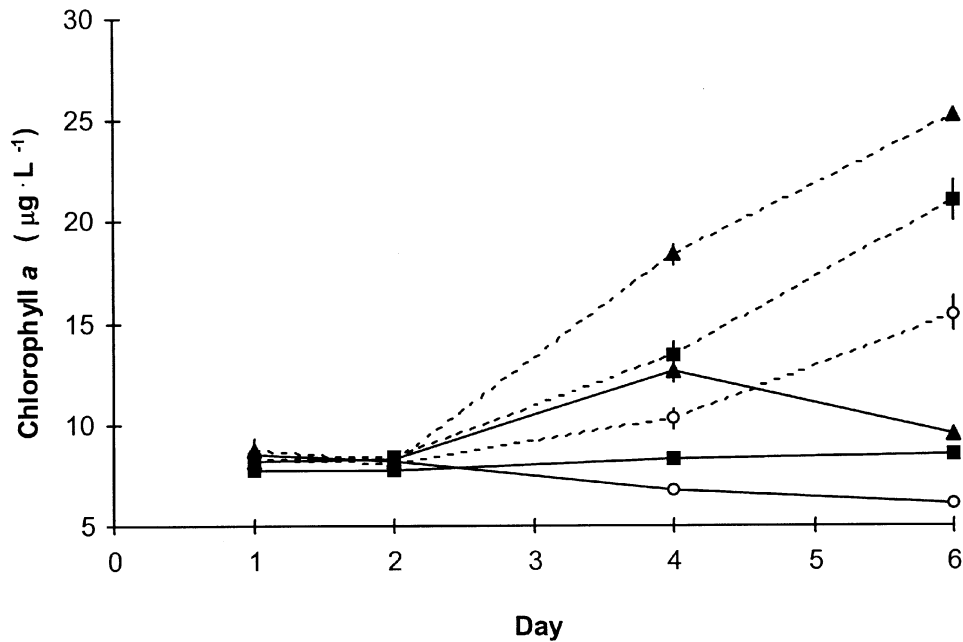
tracted from dried commercial peat moss (DOM dried) with their response to DOM extracted from freshly collected peat moss (DOM fresh). Phytoplankton biomass increased in response to both sources of DOM and to NP (Fig. 2; DOM effect  $F_{2,12} = 141.6$ ,  $p = 0.0001$ ; NP effect  $F_{1,12} = 704.95$ ,  $p = 0.0001$ ). The magnitude of the phytoplankton response to fresh peat moss was less than the response to dried peat moss (Fig. 2; pairwise contrast between dried and fresh DOM  $F_{1,12} = 48.05$ ,  $p = 0.0001$ ). Algal biomass was highest in the NPDOM dried and NPDOM fresh treatments. Unlike the high vs. low light experiment, there was no DOM  $\times$  NP interaction (Fig. 2; DOM  $\times$  NP interaction  $F_{2,12} = 1.47$ ,  $p = 0.27$ ).



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Despite large changes in phytoplankton biomass, phytoplankton community composition was not affected by the different treatments. The phytoplankton community was initially dominated numerically by *Cryptomonas*, *Closterium*, and a small, rod-shaped cyanobacteria species. However, owing to its small size, the relative biomass of the cyanobacterium was less than 5%. In all treatments, the cyanobacteria species declined dramatically, possibly because of protozoan grazers. The large increases in chlorophyll *a* concentration in the NP and DOM treatments were due to increases in *Cryptomonas*, *Closterium*, and *Cystomonas*, a nonmotile, colonial chlorophyte.

**Fig. 2.** Mean and standard error of chlorophyll *a* concentration throughout the DOM source experiment. Solid line with open circles, control treatment; solid line with solid triangles, treatment with added dissolved organic matter (DOM) extracted from dried peat moss (DOM dried); solid line with solid squares, treatment with added DOM extracted from fresh peat moss (DOM fresh); broken line with open circles, treatment with added nitrogen and phosphorus (NP); broken line with solid triangles, treatment with added DOM dried and NP; broken line with solid squares, treatment with added DOM fresh and NP.



### Light gradient experiment

The light gradient experiment was designed to compare growth in DOM extract and NP treatments at a range of irradiance levels. Growth in the control treatment was negligible at all depths. In contrast, growth in the DOM and NP treatments was positive over most of the epilimnion (Fig. 3*a*). In addition, the growth vs. irradiance curves for DOM and NP exhibited very similar shapes, and growth in these treatments was light saturated at about  $12 \text{ E} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$  (corresponding to depth of 0.25 m) (Fig. 3*b*). The similarity of the DOM and NP curves suggests that the amount of light removed by the DOM extract in the DOM mesocosms is small compared with the amount of light removed by the water above the mesocosms.

Areal growth for each of the treatments in the light gradient experiment was calculated by summing the change in chlorophyll *a* over all depths. In addition, I calculated areal growth in the epilimnion of West Long Lake over the 6 days of the light gradient experiment using the change in total epilimnetic chlorophyll *a* obtained from ongoing measurements in the lake (S.R. Carpenter, Center for Limnology, 680 N. Park St., Madison, WI 53706, unpublished data). I calculated areal growth in West Long Lake to compare with growth in the control treatment. Areal growth rates were highest in the NP and DOM treatments, negative in the control treatment, and very low in West Long Lake (Fig. 4).

### Net effects of DOM

The high vs. low light experiment showed that DOM has a negative effect on phytoplankton growth caused by a reduction in light availability (Fig. 1). The DOM source and light gradient experiments showed that once the effect of light was removed, DOM extract had a positive effect on phyto-

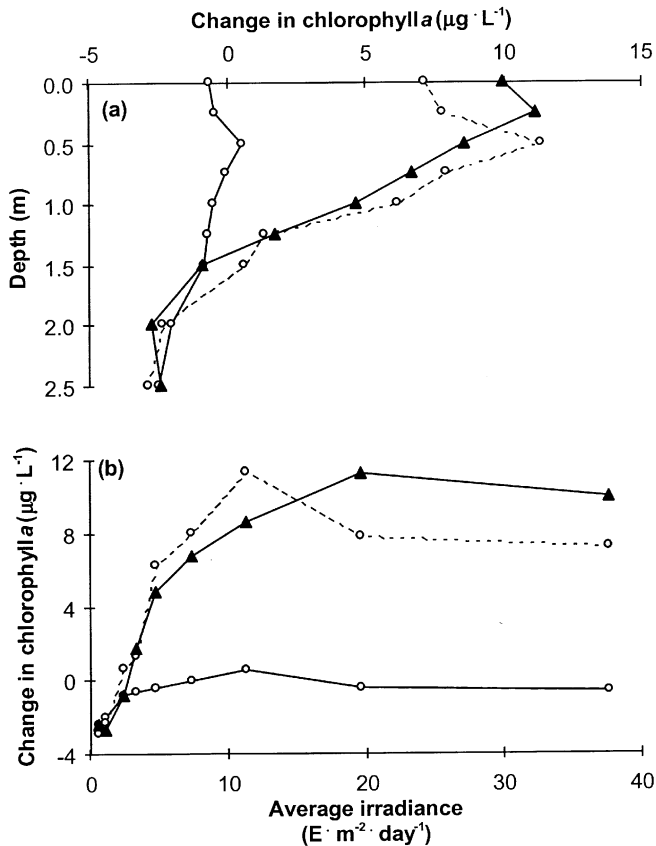
plankton growth (Figs. 2 and 3). However, none of the experiments measured the net effect of DOM because the experimental containers were small and the elevated DOM levels in the containers did not shade the phytoplankton. Therefore, I combined the positive and negative effects of DOM observed in these experiments using the equations derived in the Methods section.

Phytoplankton growth is a function of irradiance, which means that total phytoplankton growth integrated over a given depth is a function of the extinction coefficient (eq. 4). If areal growth ( $G_1$ ) is known at a particular  $k_d$  ( $k_{d1}$ ) then I can calculate the expected areal growth ( $G_2$ ) at  $k_{d2}$  (eq. 5; Table 3). The light gradient experiment was conducted in West Long Lake, which had an ambient DOC concentration of  $10.2 \text{ mg} \cdot \text{L}^{-1}$  and extinction coefficient ( $k_{d1}$ ) of  $1.59 \text{ m}^{-1}$  (Table 3). The observed areal growth in the DOM treatment was  $1298 \text{ } \mu\text{g chl } a \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ . To estimate the reduction in areal growth due to reduced irradiance caused by higher DOM, I calculated the extinction coefficient ( $k_{d2}$ ) using the DOC concentration in the DOM treatment ( $\text{DOC} = 18 \text{ mg} \cdot \text{L}^{-1}$ ) (Table 3) and used  $k_{d1}$ ,  $k_{d2}$ , and the observed growth in the DOM treatment ( $G_1$ ) in eq. 5 to calculate  $G_2$ . Accounting for the estimated reduction in growth in the DOM treatment caused by reduced light leads to a net areal growth rate ( $G_2$ ) of  $607 \text{ } \mu\text{g chl } a \cdot \text{m}^{-2} \cdot \text{day}^{-1}$  rather than the observed ( $G_1$ )  $1298 \text{ } \mu\text{g chl } a \cdot \text{m}^{-2} \cdot \text{day}^{-1}$  (Table 3). Nonetheless, after accounting for the negative effects of reduced light, DOM still has a large net positive effect compared with the control treatment (Fig. 4).

### Model results

To explore the relative importance of positive effects of DOM owing to nutrients and negative effects owing to light

**Fig. 3.** (a) Change in chlorophyll *a* at each depth in the light gradient experiment. (b) Growth (change in chlorophyll *a*) versus irradiance curve for the light gradient experiment. Solid line with open circles, control treatment; solid line with solid triangles, treatment with added dissolved organic matter (DOM) extracted from dried peat moss (DOM dried); broken line with open circles, treatment with added nitrogen and phosphorus (NP).

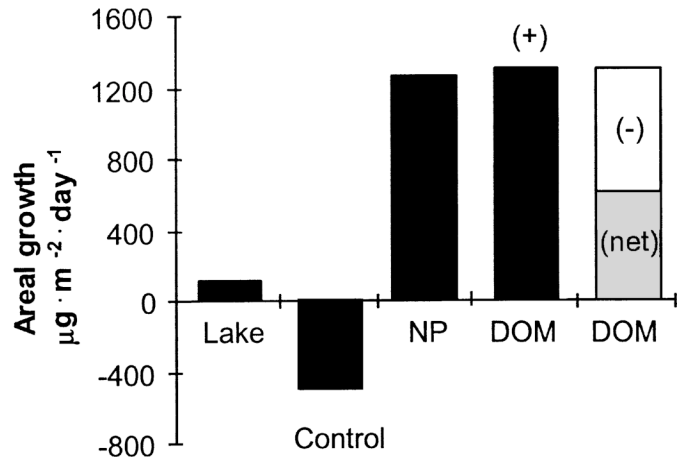


absorption, I used the Monod model of phytoplankton growth as an illustration. Model results show that phytoplankton growth rate at 1 m increases when DOM increases from low to moderate DOM concentrations and then decreases at higher DOM concentration (Fig. 5). However, the absolute effect of DOM on phytoplankton growth rate depends on the phosphorus concentration and light absorption properties of the DOM, as well as the physiological properties of the phytoplankton (Fig. 5). At low DOM, the difference in phosphorus concentration between DOM sources determines growth rate, whereas at high DOM, the difference in light absorption between DOM sources determines growth rate, suggesting that the negative effects of light become more important as DOM concentration increases. Phytoplankton that grow well at low light can better take advantage of the positive effects of DOM at higher DOM concentrations than other phytoplankton (Figs. 5a, 5b). Likewise, phytoplankton that grow well at low nutrient levels have a higher growth rate at low DOM concentrations (Figs. 5a, 5b).

## Discussion

The experiments described here show a net positive effect

**Fig. 4.** Areal growth ( $\mu\text{g chl } a \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ ) for treatments in the light gradient experiment and the lake during the experiment. NP denotes the treatment with added nitrogen and phosphorus and DOM denotes the treatment with added dissolved organic matter extracted from dried peat moss. The solid DOM bar shows the positive effect of DOM extracts on phytoplankton observed during the experiment. The open bar shows the estimated negative effect of DOM extracts owing to reduction in light penetration. Reduction in growth owing to differences in light penetration was estimated using eq. 5 (Table 3). The shaded bar shows the estimated net effect (positive – negative effects) of DOM extracts on areal growth.



of increased DOM on phytoplankton growth. The net positive effect was present despite negative effects owing to shading by high levels of DOM. The relative importance of the positive versus negative effects will likely depend on a variety of factors including the source of DOM and physiological properties of the phytoplankton community present in the lake.

## Negative effects of DOM on phytoplankton

Results from the high vs. low light experiment show that when phytoplankton were incubated in a low light environment they did not grow, regardless of nutrient availability. This is not a surprising result given that phytoplankton are photosynthetic organisms. In this case, the low light environment was caused by high concentrations of DOM in the lake in which the low light mesocosms were incubated, suggesting that DOM has a strong negative effect on phytoplankton owing to light absorption. When the same treatments were incubated at the same depth (0.75 m) in a lake with a lower concentration of DOM, and thus higher light, phytoplankton biomass increased in the NP and DOM treatments relative to the control treatment. The fact that growth at a given depth is lower in high DOM waters relative to lower DOM waters suggests that DOM has a negative effect on phytoplankton growth. These results are consistent with previous studies that have shown that the light absorption by DOM leads to reduction in phytoplankton growth (e.g., Jones 1992; Carpenter et al. 1998).

The effects of DOM caused by changes in light were further investigated using the results from the light gradient experiment in combination with the expected reduction in growth from increased  $k_d$ . In the DOM treatment of the light gradient experiment, increasing DOC concentration from

**Table 3.** Values used to calculate reduction in growth owing to effect of reduced light.

|  | $k_d^a$ ( $m^{-1}$ ) | G in DOM treatment ( $\mu g \text{ chl } a \cdot m^{-2} \cdot \text{day}^{-1}$ ) |
|--|----------------------|--|
| DOC in lake during light gradient experiment $10.2 \text{ mg} \cdot \text{L}^{-1}$ | 1.59                 | 1298 <sup>b</sup>  |
| DOC in DOM treatment $18 \text{ mg} \cdot \text{L}^{-1}$                           | 3.4                  | 607 <sup>c</sup>   |

**Note:**  $k_d$ , extinction coefficient of light; G, areal growth, DOC, dissolved organic carbon; DOM, dissolved organic matter.

<sup>a</sup> $k_d$  calculated using the relationship between  $k_d$  and DOC in West Long Lake,  $k_d = 0.23(\text{DOC} - 0.75)$ ,  $n = 32$ ,  $r^2 = 0.84$  (data are weekly summer samples from 1991 and 1992 provided by S.R. Carpenter, Center for Limnology, 680 N. Park St., Madison, WI 53706, and M.L. Pace, Institute of Ecosystem Studies, Box AB, Millbrook, NY 12545, U.S.A.).

<sup>b</sup>Observed growth in the DOM treatment  $G_1$ .

<sup>c</sup>Estimated growth in the DOM treatment if light effects are added using the relationship  $G_2 = \frac{k_{d1}}{k_{d2}} G_1$  where  $\frac{k_{d1}}{k_{d2}} = \frac{1.59}{3.4}$ .

$10.2 \text{ mg} \cdot \text{L}^{-1}$  to  $18 \text{ mg} \cdot \text{L}^{-1}$  should increase  $k_d$  from 1.59 to 3.4. This increase in  $k_d$  corresponds to a predicted 53% reduction in the growth observed in the DOM treatment.

There are several other possible mechanisms by which DOM may have a negative effect on phytoplankton growth. Mechanisms involving decreases in the availability of trace minerals and possible negative effects of phenolic compounds present in the DOM are beyond the scope of this manuscript.

### Positive effects of DOM on phytoplankton

The positive effects of the DOM used in these experiments were most likely due to the nitrogen and phosphorus present in the DOM extract. Arvola et al. (1996) reached similar conclusions in an experiment using DOM collected from the outflow of a bog. Similarly, Wehr et al. (1998) found that inputs of deciduous tree leaves or macrophytes significantly increased phosphorus concentrations in experimental containers. In the experiments described here, phytoplankton growth increased in the presence of added nitrate and phosphate (NP treatments), suggesting that phytoplankton in West Long Lake were nutrient limited at the time of these experiments. Phytoplankton responded to added DOM in the same way as they did to the inorganic nutrient treatments. Although the magnitude of the response to DOM was lower than NP in the DOM source experiment, the magnitude was the same in the high vs. low light experiment, and the growth vs. irradiance curves for DOM and NP in the light gradient experiment were similar.

Both of the DOM sources (fresh peat moss and dried commercial peat moss) used in the experiments were associated with appreciable amounts of inorganic and organic nitrogen and phosphorus, which may have been available to phytoplankton. The magnitude of phytoplankton response to DOM was higher for DOM derived from dried peat than for DOM derived from fresh peat. This could be due to differences in nutrient concentration and availability. DOM extracted from dried peat contained higher concentrations of dissolved nitrogen and phosphorus. In addition, very little of the nitrogen extracted from fresh peat was nitrate or ammonia; most was dissolved organic nitrogen, which is less readily used by phytoplankton (Turpin 1988).

The C-N and C-P weight ratios of DOM used in other ex-

perimental work on the effects of DOM on phytoplankton are usually higher than the extracts I used in these experiments (e.g., Tilonen et al. 1992; Vinebrooke and Leavitt 1998). There are several potential reasons for this. First, most experimental work with DOM uses different extraction methods than those used in this study (but see Arvola et al. 1996 for an exception). I used a plain water extraction, and therefore the extracts contained only readily leached compounds. In contrast, other studies filtered DOM into size fractions (e.g., Tilonen et al. 1992) or extracted with basic or acidic solutions and then dialyzed to remove loosely bound nutrients (e.g., Vinebrooke and Leavitt 1998), which may change the form of the nutrients supplied by DOM (DeHaan 1992). Chemical extraction or size fractionation of DOM is necessary when the objective is to collect large amounts of DOM or when particular fractions of DOM are needed.

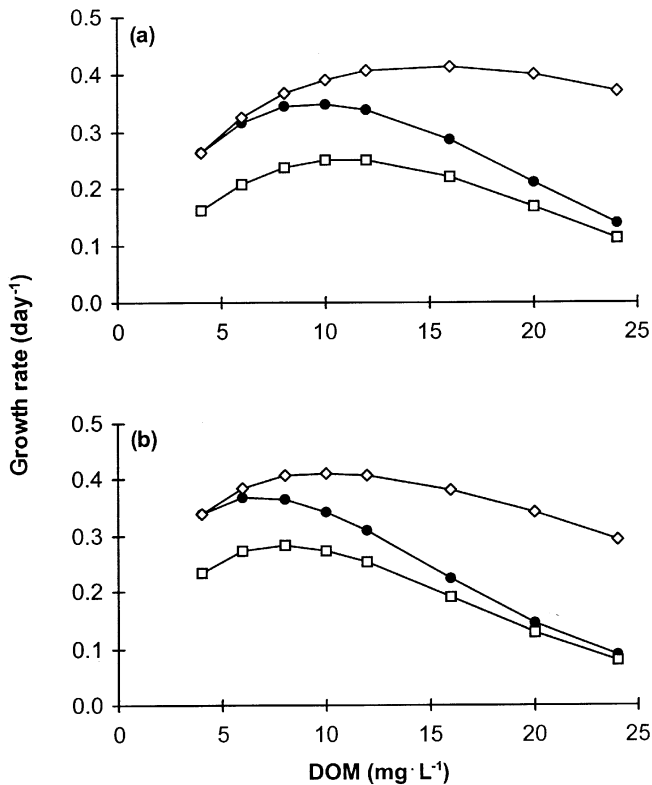
Second, this study used extracts from fresh and dried *Sphagnum* peat moss, which may be richer in nutrients than other commonly used sources (e.g., soil). Peat moss is formed from undecomposed plant material, and substantial amounts of nitrogen and phosphorus are buried in peat-forming bogs (Verhoeven et al. 1990). Other studies have shown that nutrients present in peat moss may stimulate phytoplankton growth. Guildford et al. (1987) added peat moss to limnocorrals and observed short-term increases in chlorophyll *a* concentration caused by increases in soluble nitrogen and phosphorus leached from the peat moss.

I used DOM extracted from peat moss because *Sphagnum* bogs are very common in northern Wisconsin and likely contribute much of the DOM present in these lakes. Dillon and Molot (1997) found that the extent of peatlands in a catchment was correlated with the amount of DOC and total phosphorus exported to lakes in central Ontario. Gergel et al. (1999) found a similar relationship between the proportions of wetlands in a catchment and DOC concentration in northern Wisconsin lakes. The DOM produced in peat bogs and wetlands travels to lakes via surface or groundwater flow and may undergo chemical and physical changes en route. Thus, the DOM extracted from fresh and dried peat moss was likely not the same as the DOM, which enters West Long Lake but provided a consistent DOM source for use in the experiments.

### When do the positive effects outweigh the negative effects?

Increasing DOM will increase the extinction coefficient of light and will decrease the amount of light available to phytoplankton at any given depth. However, if DOM contains nutrients that are available to phytoplankton, increasing DOM should increase the growth of phytoplankton at a given irradiance. Using the Monod model for growth for illustration, it is possible to explore the relative importance of the different mechanisms by which DOM can affect phytoplankton. The positive effect caused by nutrients is most important at low DOM concentrations, whereas the negative effect from reduced light becomes most important at high DOM. Although the qualitative shape of the curves is similar regardless of the physiological properties of the phytoplankton, it is likely that initial phytoplankton community composition will influence the net effect of DOM on

**Fig. 5.** Change in growth rate with increasing dissolved organic matter (DOM) concentration for (a) phytoplankton that grow well at low light levels ( $K_I = 5$ ,  $K_P = 10$ ) and (b) phytoplankton that grow well at low nutrient concentrations ( $K_I = 10$ ,  $K_P = 5$ ). Maximum growth rates ( $\mu_{max}$ ) are  $0.8 \text{ day}^{-1}$  in both panels. The different lines on each graph represent DOM sources with different phosphorus concentrations or different light absorption properties. The line with the solid circles represents DOM with the characteristics of DOM extracted from fresh peat moss. The line with the open diamonds represents a DOM source that is half as coloured as peat moss DOM and the line with the open squares represents a DOM source with half as much phosphorus as peat moss DOM.



phytoplankton growth. West Long Lake is a moderately coloured lake and the phytoplankton community at the time of the experiments was dominated by cryptophytes, which are common in lakes with high DOM (Jones 1998). Cryptophytes are motile and able to grow at low light levels (Klaveness 1988), which may explain their prevalence in coloured lakes. In lakes with low DOM, where phytoplankton are adapted to high light levels, the negative effects of DOM owing to reduced light may outweigh the positive effects owing to nutrients.

The simple model assumes that phytoplankton are obligate autotrophs. However, there is evidence that some phytoplankton species (including some cryptophytes) are mixotrophic (Bird and Kalff 1987; Tranvik et al. 1989). The importance of mixotrophy is debated but it has been suggested that mixotrophic species may be better adapted to high DOM lakes than are obligate autotrophs. For example, some studies have shown that known mixotrophs are more prevalent than autotrophs when organic carbon supply is high (Jansson et al. 1999; Grover 2000). It is possible that

some cryptophyte species could have responded positively to DOM additions because of their mixotrophic capabilities. However, some of the noncryptophyte species (e.g., *Cystomonas starrii*) that responded strongly to additions of nutrients and DOM have no known mixotrophic capabilities.

The dominance by cryptophytes may also help explain why growth in the control treatments was lower than growth in the lake. The increased phytoplankton growth in the nutrient treatments suggests that phytoplankton in West Long Lake were nutrient limited. Therefore, the negative growth in the control treatments may be due to lower nutrient availability in the mesocosms relative to the lake. Some cryptophyte species undergo diurnal vertical migrations, which give them access to nutrients in the hypolimnion (Salonen et al. 1984). Phytoplankton within the mesocosms did not have access to that nutrient source. In addition, the absence of nutrient recycling by zooplankton and fish could lead to lower nutrient availability in the control treatments relative to the lake.

Although there were strong treatment differences in total phytoplankton biomass, phytoplankton community composition was not substantially different among treatments. These results are likely due to the short time scale of the experiments, as longer-term studies have shown that changes in DOM do affect phytoplankton community composition (Wehr et al. 1998; Klug and Cottingham 2001). The experimental manipulations presented here represent short-term pulses of DOM as might occur following storm events or spring snow melt. Previous work has shown that DOM supplied during these short-term episodes has different characteristics than DOM supplied during baseline hydrologic conditions (Ivarsson and Jansson 1994; Jansson et al. 1999).

In summary, in many cases, it is only possible to see the net effect of DOM on organisms. However, by separating the nutrient effects from the light effects, I showed both positive and negative effects of DOM on phytoplankton growth. The relative effects of light and nutrients will depend on the characteristics of the DOM as well as properties of the phytoplankton community. Experimental results showed that in moderately coloured, nutrient-limited lakes such as West Long Lake, the positive effects exerted by inorganic nutrients associated with DOM outweighed the negative effects of decreased light. Because the observed positive and negative effects of DOM were a function of the nutrient availability and colour of DOM, different sources of DOM will have different net effects on phytoplankton growth. Distinguishing the mechanisms by which DOM affects phytoplankton growth and improving techniques for characterizing the availability of nutrients associated with DOM should help predict how phytoplankton will respond to different DOM sources.

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