UNIVERSITÉ DU QUÉBEC À MONTRÉAL (UQAM)

# EFFETS DE LA MATIÈRE ORGANIQUE DISSOUTE SUR LA CROISSANCE DES ESPÈCES DE CYANOBACTÉRIES À LA BAIE MISSISQUOI DU LAC CHAMPLAIN

# EFFECTS OF DISSOLVED ORGANIC MATTER ON THE GROWTH OF CYANOBACTERIAL SPECIES IN EUTROPHIC MISSISQUOI BAY, LAKE CHAMPLAIN

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## RÉSUMÉ

Les études récentes ont montré qu'il y aurait une forte influence de la matière organique dissoute (MOD) sur la dynamique des écosystèmes aquatiques. En particulier, certaines catégories de MOD ont des propriétés antialgues, susceptibles de réduire la biomasse des espèces de cyanobactéries. Nous avons testé les effets de différentes catégories de matières organiques dissoutes, ainsi que leurs interactions avec la lumière et les nutriments, sur la composition des communautés de phytoplancton lors d'incubations in situ (7 jours). La composition initiale en phytoplancton et les caractéristiques de la matière organique dissoute ont été des facteurs déterminants pour les modifications de la structure des communautés de phytoplancton, lors des incubations. Les résultats de notre étude montrent que la croissance du phytoplancton était fortement dépendante de la disponibilité en nutriments dans la baie Missisquoi en 2007, et que les concentrés d'extrait d'orge et de matière organique naturelle peuvent inhiber la croissance du phytoplancton, particulièrement des espèces de cyanobactéries.



**Mots-clés:** Matière organique dissoute (MOD), cyanobactérie, phytoplancton, incubations, Lac Champlain.

## ABSTRACT

Recent research shows that there is a strong influence of dissolved organic matter (DOM) in the dynamics of aquatic ecosystems. In particular, some types of DOM have antialgal properties that can decrease the biomass of cyanobacterial species. We tested the effect of different types of dissolved organic matter, and their interaction with light and nutrients, on the composition of the phytoplankton community in field incubation experiments (7 days). Initial phytoplankton composition and characteristics of dissolved organic matter added were determinant in changes in taxonomical community structure of samples in the incubation experiments. Our results demonstrate that phytoplankton growth was strongly dependent on the availability of nutrients in Missisquoi Bay in 2007, and that barley extract and natural organic matter concentrate may inhibit the growth of phytoplankton, particularly Cyanobacterial species.



**Key words:** Dissolved organic matter (DOM), cyanobacteria, incubations, phytoplankton, Lake Champlain.

# CHAPTER I: INTRODUCTION

## 1.1. Characteristics of dissolved organic matter in freshwater systems

There is an increased interest in the role of dissolved organic matter (DOM) in aquatic ecosystems (Kosakowska et al. 2007; Steinberg 2008; Stets et al. 2008). DOM can be an important element in physical, chemical, biochemical and whole lake ecosystem processes. Evidence has shown that dissolved organic matter (DOM), sometimes considered as an inert substance in the water, can have a major influence on ecological dynamics at different trophic levels.

Sources of lake water DOM are diverse. DOM comes principally from degradation products of vegetal material in the watershed soils on terrestrial environment. This fraction is termed allochthonous to reflect its origin exterior to the lake system. The remaining fraction is produced within the lake (autochthonous) from algal and aquatic plant excretion and other forms of aquatic organism metabolism. Allochthonous DOM consists principally of humic substances (HS) or fulvic acid-like material (Larson 1978; Thurman et al. 1981; Jones 1992). This material is polar, straw-coloured, and principally composed of organic acids that are derived from soil humus and terrestrial and aquatic plants and generally comprise one-third to one-half of the dissolved organic carbon (DOC) in water. Aquatic fulvic acids derived from plant litter and soils generally contain a significant content of aromatic carbon (25%–30% of total carbon), reflecting the contribution of lignin degradation to their formation (Thurman et al. 1981; Brooks et al. 2007). Quinones were characterized by electrochemical studies as the dominant redox-active moieties associated with DOM (Nurmi et al. 2002). All these substances are characterized by important acid-base properties as well as metal and nutrient binding and complexing abilities (Thurman et al. 1981; De Haan 1992). These properties confer an active role on DOM in aquatic chemical, physical and biological dynamics (Wetzel 1992).

There is a strong correlation between DOM and water color (Cuthbert et al. 1992). For example, humic and fulvic acids strongly absorb the UV part (200-365 nm) of the light spectrum (De Haan 1992). Because of their lower aromaticity, microbially derived fulvic acids absorb less visible and ultra-violet light than plant- or soil-derived fulvic acid (McKnight et al. 2001). Differences in DOM composition reflected in light absorption patterns can be helpful to understand its principal origin, composition and influence on food webs.

The origin and composition of different sorts of DOM influence its ecological role (Amon et al. 1996; Wehr et al. 1998; Crump et al. 2003; Kosakowska et al. 2007). In some cases, the fraction of the DOC pool that can be effectively used by microorganisms in aquatic environments changes in relation with particular DOM characteristics (Del Giorgio et al. 1994). On one hand, DOM may counteract eutrophication, for example by binding phosphate (De Haan 1992) . On the other, humic substances (HS) as part of DOM might contribute to eutrophication, being mineralized more rapidly in eutrophic waters in the presence of labile organic substrates, and increased levels of inorganic nutrients.

Different fractions of DOM have different biodegradability properties. These properties depend on abiotic factors such as light climate, pH and chemical composition of the water. In this way, alterations at the global scale in the environment can influence biodegradability. Increased UV radiation intensity from natural sunlight may stimulate photodegradation (Geller 1985), that can render humic substances (HS) more susceptible to microbial degradation, liberate cofactors for metabolism or affect the binding and release of biologically important substances from aquatic humic substances as nutrients modifying its availability (De Haan 1992; Wehr et al. 1998). By surface special properties (Campbell et al. 1997) humic substances can act as modulators of the bioavailability of key nutrients through the formation of binding complexes of trace metals such Cu (Brooks et al. 2007). Complexation or solubilization of pesticides and hydrocarbons in the aqueous environment with HS (Thurman et al. 1981), or formation of complexes between humic and fulvic organic acids and extracellular enzymes (Wetzel 1992) are the best known effect of HS on

phosphorus availability by binding and sequestering phosphate in the presence of ferric iron (De Haan 1992).

### 1.2. Effects of DOM on phytoplankton

DOM can contain alellopathic substances that can be easily released or transformed by interaction with environmental factors such as light or PH or by bacterial or chemical degradation (Jasser 1995; Gross et al. 1996; Nakai et al. 2000; Körner et al. 2002; Gross et al. 2003) . Polyphenolic compounds originating from decomposition of wetland and littoral macrophytes, can result in major modifications of nutrient availability and metabolic pathways in aquatic ecosystems (Wetzel 1992). Polyphenolic-enzyme complexes can be formed, which modify or inhibit enzyme activities. These compounds can subsequently be fractured by mild UV radiation, as would be found in fresh waters, reconstituting the enzyme activity. Furthermore, activated oxygen products of photochemical reaction of humic substances can directly induce damage to intracellular catalase and act as important factor for the cell lysis as showed for *Anabaena circinalis* (Sun et al. 2006).

Humic substances (HS) are active environmental chemicals. Damages caused by several fish pathogens, such as bacteria and parasites, can be repaired more quickly in the presence of HS. Some parasites – mainly fungi – appear to be directly affected by HS (Meinelt et al. 2008). The quantitative expression of these effects depends on the concentrations of quinoid structures in the humic materials (Steinberg et al. 2001; Steinberg et al. 2003). Quinones can interfere with photosynthetic electron transport, an effect for which cyanobacterial species can be more sensitive. Humic substances have the potential to act as electron acceptors for microbial respiration, provoking the same inhibitory mode of action on photosynthesizers as does the allelopathic compound tellimagrandin II (Steinberg et al. 2006; Prokhotskaya et al. 2007; Steinberg 2008).

Growth promotion as well as growth inhibition of algae and bacteria create trade-offs between specific and non-specific effects at different ecological levels (Steinberg et al. 2001). Allochthonous DOM can act indirectly by promoting the heterotrophic component of aquatic

ecosystems, leading to important changes in the principal energy pathway in lakes. DOM as direct carbon source in the food web can be selectively degraded by microbiota (bacteria) (Kirchman 1990; Jones 1992; Wetzel 1992; Del Giorgio et al. 1994; Lindell et al. 1995; Wehr et al. 1998; Klug 2005; Steinberg et al. 2006) that become an important competitor of phytoplankton for nutrients, expanding the size of the effect of organic matter at different trophic levels (Carpenter et al. 1998). This process can be enhanced by photolytic activity of UV in DOM (Lindell et al. 1995; Wetzel et al. 1995; Moran et al. 1997; Obernosterer et al. 1999; Maurice et al. 2002). In addition, there can be established a new energy pathway from DOM to macrozooplankton via heterotrophic flagellates, and by the stimulus of mixotrophy or heterotrophy in some algal species (Jones 1992; Granéli et al. 1999; Tuchman et al. 2006). Interaction of DOM with nutrients can have also a negative effect in bacterial populations. For example, eutrophication leads to the dominance of cyanobacteria which are known to excrete some compounds that can act as toxic substances with antimicrobial activity (De Haan 1992). Other photoproducts formed (via solar radiation) from DOM (such as toxic gases) might inhibit bacterioplankton activity as well (Wetzel 1992; Obernosterer et al. 1999).

Different relations have been studied in the interaction between DOM and phytoplankton. There is an important positive effect on phytoplankton growth owing to the nutrients associated with DOM (Larson 1978; Klug 2002; Frost et al. 2007). Autochthonous DOM can also promote the growth of algae by regulation of inorganic nutrients, especially when phosphorus and humic substances are in excess (Arvola et al. 1996). There is also release of some growth promoting substances by microbial or photochemical processing of the DOM or by remineralization of nutrients by bacteria using DOM (Granéli et al. 1999; Prokhotskaya et al. 2007). Following DOM or nutrient addition total phytoplankton biovolume can vary and taxonomic composition is altered directly or indirectly via interaction with other groups (Arvola et al. 1996; Vinebrooke et al. 1998; Wehr et al. 1998; Klug et al. 2001; Klug 2002; Klug 2005). Recent studies show that growth of *Microcystis aeruginosa* (Imai et al. 1999) and *Anabaena circinalis* (Sun et al. 2005) can be inhibited by iron deficiency caused by iron complexation with fulvic acid. In contrast, humic substances (HS) can stimulate biomass production in cultures of *Microcystis aeruginosa*, depending on their source and properties

(Kosakowska et al. 2007). Clear differences in sensitivity to humic substances between groups and species have been found. Direct effects on growth of algae by humic substances can be achieved with a lesser quantity of organic matter than has been proposed for antialgal vegetal leachates (rice, barley) (Steinberg et al. 2006; Karasyova et al. 2007).

### 1.3. Barley extract and phytoplankton growth inhibition

Cyanobacterial blooms have become of global concern and have both economical and ecological implications. Currently, effort is directed to understanding the environmental dynamic that is involved in their development, in order to identify the key factors that may allow the natural restoration of damaged ecosystems. Solutions, however, are not immediately available and it will take time to achieve the implementation of environmental measures to attenuate eutrophication processes and try to recover healthy ecosystems. Meanwhile, principally for economical reasons, there is a major effort in the search for quick, environmentally-friendly solutions for achieving control of cyanobacterial blooms.

There has been an important research effort directed toward natural compounds, isolated from a wide range of terrestrial and aquatic plants, that are reported to have inhibitory effects on growth of phytoplankton species (Pillinger et al. 1995; Barret et al. 1999; Park et al. 2006). The objective was to find substances that constitute a selective (Barret et al. 1996), cheap, fast-acting, long-lasting 'slow release' (Barret et al. 1999) and low ecological impact solution for the growth and proliferation of cyanobacterial species responsible for blooms. Since 1980, when accidental addition of rotting hay to a lake appeared to reduce growth of algae, there has been a growing interest in the alellopathic properties of compounds derived from barley straw and their effects. In 1990 Welch provided the first report of the use of barley straw in reservoirs (Welch 1990). From the addition of the straw Welch achieved long term effect on the filamentous alga *Cladophora*.

Since his study inhibition of growth of selected algal species by barley straw application has been show in field trials (Gibson et al. 1990; Welch et al. 1990; Pillinger et al. 1992; Pillinger et al. 1994; Barrett et al. 1996; Caffrey et al. 1999; Ridge et al. 1999; Ball et al. 2001), in

reservoirs (Barrett et al. 1996; Everall et al. 1996; Everall et al. 1997; Barrett et al. 1999), in marine water against specific dinoflagellate species (Terlizzi et al. 2002; Grover et al. 2007), and in brackish systems (Brownlee et al. 2003). The results showed reductions in algal abundance and cyanobacterial blooms or dominance. All experiments converge to demonstrate that the effect is algistatic rather than algicidal. In some cases application of barley straw had no effect on algal growth in experimental ponds (Kelly et al. 1996; Ferrier et al. 2005; Grover et al. 2007).

Laboratory assays have provided contradictory results. Negative effects of barley straw in the growth of algal species (including green algae, diatoms, dinoflagellates and chrysophytes) (Ridge et al. 1996; Martin et al. 1999; Terlizzi et al. 2002; Brownlee et al. 2003; Ferrier et al. 2005), and fungal species (Cooper et al. 1997) have been reported. On the other hand, barley straw can produced a stimulation in growth (Larson 1978; Martin et al. 1999; Terlizzi et al. 2002; Brownlee et al. 2003; Ferrier et al. 2005; Bird et al. 2007). In some cases, the use of commercial barley straw extract has been reported to have no effect against the growth of *Anabaena* (Bird et al. 2007) and *Prymnesium* (Grover et al. 2007).

It has been proposed that different sensitivity of algal species to barley straw inhibitors could certainly influence their relative abundance (Ridge et al. 1999; Brownlee et al. 2003). Although taxonomic differences may account for results in the action of barley straw, other factors and unique conditions from each experiment are also important, including the age and condition under which rotted straw is prepared, the type (cultivar) of barley used, the conditions under which the barley was grown and the straw dosage (Gibson et al. 1990; Brownlee et al. 2003; Ferrier et al. 2005).

The concentrations of barley straw required for algal inhibition in laboratory studies were larger than those which were reported in field experiments, suggesting that organic chemicals would be more toxic under field dynamic conditions (Martin et al. 1999; Jancula et al. 2007). Growth conditions can make a difference in the growth response produced from antialgal compounds. For example, it has been suggested that unicellular green algae is harder to

inhibit than blue-green algae because blue green algae is more vulnerable due to a more rapid growth rate and a shorter life span (Choe et al. 2002).

There are many theories concerning the mode of action of barley straw against algae. Some of them suggest barley straw is a substrate for microflora and other organism that eventually can retain and immobilise nutrients thus limiting the growth of algae Studies have shown that microbial decomposition of the straw is essential for the inhibition of growth (Gibson et al. 1990; Garbett 2005). Sunlight was suggested to be important in the liberation and production by decomposition of antialgal compounds from the straw, increasing the photo-oxidation of phenolics, as well as the formation of phytotoxic hydrogen peroxide, singlet oxygen, superoxide radicals, and/or quinones (Pillinger et al. 1995; Pillinger et al. 1996; Schrader et al. 1999; Geiger et al. 2005; Bird et al. 2007; Drábková et al. 2007; Drábková et al. 2007), but contradictory results showed that phototransformation (presumably photooxidation) of straw decomposition products into phytotoxic compounds maybe is not important for photoautotrophic species (Megharaj et al. 1992; Martin et al. 1999), and that peroxide does not necessarily have an antialgal effect at natural levels (Bird et al. 2007).

Field experiments demonstrated that when straw is employed for restrictions of algae growth, suitable surface properties allow microorganisms and fungi to adhere and decompose the straw (Wisniewska et al. 2003). It appears that the nature (type and quantity) of the inhibitory substances in decomposing straw may vary over the course of the straw's decomposition (Ferrier et al. 2005). Microflora per se could metabolize compounds responsible for the alellopathic activity.

On the other hand (Pillinger et al. 1992) demonstrated that the production of algal inhibitors by specific fungi cannot explain fully the antialgal effects of rotting barley straw. Other hypotheses mention that barley straw can provide a carbon source for carbon-limited microbial growth. With the carbon availability secure, the microbial community production soars - the non-cyanobacteria populations - and phosphorus uptake is shunted through the non-cyanobacterial microbial loop ecosystem. The presence of decaying barley straw

therefore results in phosphorus limitation for algae, not inhibition by a released chemical compound (Geiger et al. 2005).

Many studies have tried to identify the biologically-active chemical (or chemicals) released from the decomposing straw. There are clues that indicate that phenolic compounds may be implicated in the inhibitory effects on specific algal species. Parks et al. (1969) found that a range of phenolics from decomposing plant material, including gallic acid, inhibited cultures of *Lyngbya* and *Anabaena*. Pillinger et al. (1994) implicated quinones, produced from the oxidation of phenolic hydroxyl groups and tannins, principally from the lignin portion of the plant material, that is in high proportion in barley (Pillinger et al. 1995; Stewart et al. 1995). Under the right conditions of increased aeration, these quinones were 10 times more toxic towards *Microcystis* and *Chlorella* than were phenolic acids. Ferrulates (the major low molecular weight phenolic compound in barley) have also been demonstrated to have important antialgal properties, enhanced by light, when applied to ponds to control excess growth (Schrader et al. 1999). For *Microcystis* and *Scenedesmus*, ester compounds were found to be antialgal chemicals, while a phenol compound was identified as a subagent (Choe et al. 2002). Protein synthesis associated with photosynthesis, cell metabolism, and membrane function in cyanobacteria are major targets of tannin compounds (Zhao et al. 1998).

Lignin seems to be the potential source of anti-algal precursory oxidised phenolics, its potential action is not restricted to barley straw, and it can be the most promising source of antialgal inhibition by the synergistic action of one or more compounds from its decomposition (Everall et al. 1997). Other materials have also been found to be anti-algal including brown-rotted wood, some leaf litters, in particular oak leaves (*Quercus robur*) (Pillinger et al. 1995; Ridge et al. 1996; Ridge et al. 1999), mugwort, rice straw (specially salicylic acid (Park et al. 2006)) and chrysanthemum (Choe et al. 2002), and members from the family *Papaveraceae* (Jancula et al. 2007).

This study investigated how an increase of organic matter and the addition of barley extract can influence the aquatic environment by changing phytoplankton populations, and

specifically whether there is an inhibition of cyanobacterial species, that allows the use of allochthonous organic matter against blooms. Concentration of allochthonous organic matter from Pike River (that goes directly to Champlain Lake and reflect natural input of organic matter from watershed), and commercial barley extract were used to achieve the effect against Cyanobacteria. A factorial experiment to asses the effect of dissolved organic matter and barley extract modulated by environmental factors (light level, nutrient status) was designed.

### 1.4. Research problem

Algal blooms are a considerable threat to the quality of surface waters, limiting their use for drinking water, recreation or fishing, and afecting ecosystems. Missisquoi Bay of Lake Champlain, situated across the US-Canada border in the province of Quebec, has developed in the last decade massive cyanobacterial blooms. Dissolved organic matter (DOM) may affect phytoplankton growth, especially cyanobacterial bloom forming species. Factors as source of DOM and light regime may be important in modulating this effect. This study tried to determine the influence of dissolved organic matter (DOM) on the cyanobacterial bloom, assessing effect of DOM source, light regime and nutrient status on the dynamic of phytoplankton.

### 1.5. Working Hypothesis

Considering the growing evidence of effect of dissolved organic matter (DOM) on phytoplankton, I hypothesize that DOM will control cyanobacterial bloom species growth in Lake Champlain. To assess the hypothesis, different kinds of DOM were tested as controlling factors for the growth of cyanobacterial bloom forming species. The experimental design evaluated the effect of light intensity and nutrient status, known to modulate the interaction between DOM and phytoplankton.

## CHAPTER II: METHODOLOGY

### 2.1. Site description

Lake Champlain is situated along the US-Canada border, between Vermont and the Adirondack Mountains of New York, and covers an area of 700 square kilometers with 13,250 square kilometers drainage basin. The field study was conducted in the Missisquoi Bay near the town of Philipsburg, in the province of Quebec.

### 2.2. General limnological characterization

We followed limnological variables at two stations in Missisquoi Bay of lake Champlain, at weekly or biweekly intervals, between May and November in 2006 and 2007. The first station, called "littoral", was located near the Philipsburg dock (average depth 2 m) (Figure 1). The other one, called "pelagic" (4 m average depth) was located approximately 2 km from the shore in the open water of the lake.

**Figure 1**. Placement of sampling stations in Missisquoi Bay, Lake Champlain. The littoral site was located within the protection of the Philipsburg quay.

Water physical and chemical parameters, such as depth, temperature, conductivity, pH, and dissolved oxygen were determined *in situ* near the lake surface with a Thermo 3 star meter for pH and an YSI 600 XLM Multi-parameter water quality monitor for the other parameters. Integrated water samples were collected in a Van Dorn sampler from surface until the photic zone limit determined from Secchi disk depth. Samples for chlorophyll a and nutrients ($NO_3$, TN, TDN, $NH_4$, TP, TDP, DOC) were taken from the depth integrated sample. All samples were taken in duplicate; samples for ammonium analysis were taken in triplicate. Ammonium ($NH_4$-N) was determined colorimetrically (APHA et al. 1998). Concentrations of total phosphorus (TP) and total dissolved phosphorus (TDP, Whatman GF/F filtered water) were measured by the molybdenum blue method after persulfate digestion. All colorimetric and absorbance measures were taken using an Ultrospec 2100 Pro spectrophotometer. Concentrations of total nitrogen (TN) and total dissolved nitrogen and nitrate (TDN - $NO_3$, Whatman GF/F filtered water) were measured as nitrates after alkaline persulfate digestion using an Alpkem Flow Solution IV autoanalyzer. Concentrations of DOC (Whatman GF/F filtered sample water) were measured by high temperature oxidation on O/I Analytical 1010 Total Organic Carbon Analyzer after acidification. Samples for chlorophyll a were filtered (Whatman GF/F) and analysis was made by hot ethanol extraction, followed by spectophotometric determination of the extracts absorption (Lorenzen 1967). Photosynthetically available radiation (PAR) was measured in μmol $m^{-2}$ $s^{-1}$ using a Li-Cor light meter LI-250. A second sensor served as a reference, measuring PAR simultaneously above the water surface.

### 2.3. Phenolic compounds determination

Lake water filtered with Whatman GF/F was analyzed for total phenolic compounds using the Folin–Ciocalteau colorimetric assay with tannic acid (Sigma) as standard (Box 1983). Total phenolics are given as tannic acid equivalents. Phenolic acids were measured in the context of the experiments.

## 2.4. Preparation of XAD 8 resin and concentration of organic matter

The use of the non-ionic macroporous XAD 8 resin allowed the isolation of the fraction of the hydrophobic DOM (Thurman et al. 1981), consisting principally of macromolecular humic substances.

### *2.4.1. Resin preparation*

Supelite™ XAD-8 resin (Supelco) was extracted in a beaker with 0.1 N NaOH. Fines were decanted off after each daily rinsing of NaOH for 5 successive days. Next, the resin was soxhlet-extracted sequentially for 24 h with methanol, diethyl ether, acetonitrile, and methanol and stored in methanol until used. Before column packing, methanol was rinsed from the resin with distilled water until free of methanol, using approximately 50 bed volumes , the packed column was rinsed three times with three pore volumes alternating of 0.1 N NaOH and 0.1 N HCl. This cleaning sequence was repeated three times (Thurman et al. 1981).

### *2.4.2. Preparation of water samples and resin extraction*

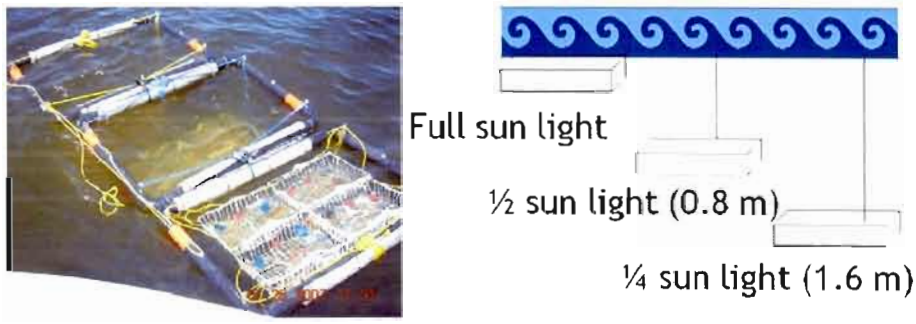
Because Pike River is a natural affluent to Lake Champlain we decided to use Pike River water to make the concentration of natural incoming DOM to the lake. Between 17 and 20 July 2007, for experiment 2 made in July, and between 20 and 23 August, for experiment 3 made in September, approximately 30 L of Pike River filtered water were acidified to pH 2.0 with concentrated HCl. The water samples were then pumped with a Cole-Palmer Masterflex pump at a rate of 15 bed volumes per hour. The hydrophobic acids adsorbed were eluted from the resin in reverse direction with 0.1 N NaOH at a flow rate of 5 bed volumes per hour, eluates derived from the procedure were desalted primarily to remove sodium and chloride ions that were added during pH adjustments, reapplied onto the respective columns at about four pore volumes per h (approximately one-fourth the flow rate used during the initial isolation step). The columns were then flushed with Milli-Q water to remove chloride ions, until the electrical conductivity of the column effluent was <750 mS/cm. Retained organics were re-eluted using 0.1 N NaOH. Sodium was removed from the (chloride-free) eluates by passage through a column containing hydrogen-saturated cation exchange resin (AG-MP 50, Biorad).

Approximately 70 ml were obtained from the concentration process in each case, different tests were conducted (spectophotometry concentration and DOC) to find the correct amount of extract necessary to increase DOM in the experiments (between 2 and 5 ml). Concentrate was stored at 4°C until utilization (Thurman et al. 1981; Moran et al. 1994; Quanrud et al. 2003).

### 2.5. Experimental setup for *in-situ* incubations

Three experiments were run in summer 2007 to investigate the effects of the addition of DOM on phytoplankton communities. First experiment was conducted between June 11 and 15, second experiment between July 23 and 27 and a final experiment between September 17 and 21. Three structures were placed at Missisquoi Bay of lake Champlain, at different depths corresponding to different light levels calculated by PAR light attenuation coefficient (Figure 2), corresponding to full sun light level (at the surface), half sun light (at 0.8 m from surface), and quarter of sun light (at 1.6 m from surface). Each light level contained closed 600 ml plastic containers with half content of whole lake water and other half with filtered lake water (to decrease the effect of grazing).

**Figure 2**. Experimental setup for incubations




For each different condition of exposure to light, three different treatments were considered: control (without any addition), plus barley dose (commercially recommended barley extract (Microbe lift CBSE) dose 15.67 µl/L for eradication of Cyanobacteria in lakes), and plus

concentrated organic matter from XAD-8 resin extraction. Each treatment was carried with a nutrient surplus replicate (using BG11 medium, (Rippka et al. 1979)) with sodium nitrate for experiments one and two and ammonium chloride for experiment three, to avoid nitrate photochemistry (Zepp et al. 1987). All the treatments were conducted in duplicate. General limnological characterization of the lake at the site of the incubations was made on day one and day four of each experiment. On day four, samples for Chlorophyll a, nutrients ($NO_3$, TN, TDN, $NH_4$, TP, TDP, and DOC), total phenolic compounds, color from filtered water, and organic matter were taken from the bottles and were analyzed as described before. Samples for taxonomical characterization were taken in triplicate from each bottle on day four and were preserved in Lugol's solution.

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### 2.6. Characterization of dissolved organic matter

Absorbance at 440 nm (color) of filtered water was measured as index to assess the concentration of humic substances in natural waters (Cuthbert et al. 1992). Measures of absorbance of filtered water at 254 and 272 nm in experiment 3 (September 2007) were included to achieve a better characterization of characteristics of DOM, due the strong capacity of humic and fulvic acids to absorb the UV part (200-365 nm) of the light spectrum. Absorbance at 254 nm is considered a good proxy for aromatic content in dissolved organic carbon, and absorbance at 272 usually reflects the proportion of humic substances in DOM (De Haan 1992).

### 2.7. Taxonomical characterization and carbon biomass determination

Phytoplankton samples for enumeration were examined from day 4 of each incubation experiment. One replicate was examined for each one of the treatments from the incubations on day 4 (Experiment 1 – 16 samples, Experiment 2 – 21 samples), for experiment 3 two replicates were examined (Replicate 1 – 21 samples, Replicate 2 – 18 samples). All the phytoplankton were identified and counted at species level. Counts were done under an inverted microscope by Utermöhl's method (Lund et al. 1958), cell size was determined by

the measurement of linear dimensions of a number of cells under high magnification using an ocular micrometer fitted into one eyepiece. Algal biovolume was calculated from single cells (Hillebrand et al. 1999; Sun et al. 2003) and converted to carbon biomass (Verity et al. 1992; Menden-Deuer et al. 2000).

### 2.8. Statistical methods

Correlation analyses were carried out to determine the relations between the different variables studied. The effects of treatments in incubations experiments were analyzed with a full factorial ANOVA (p. 0.05 level of significance). All statistical analyses were performed using the JMP 7.0 statistical software (SAS Institute). Canonical correspondence analyses were run with Canoco for Windows 4.5 and visualized by ordination diagrams in Canodraw 4 for windows (Biometris-Netherlands).

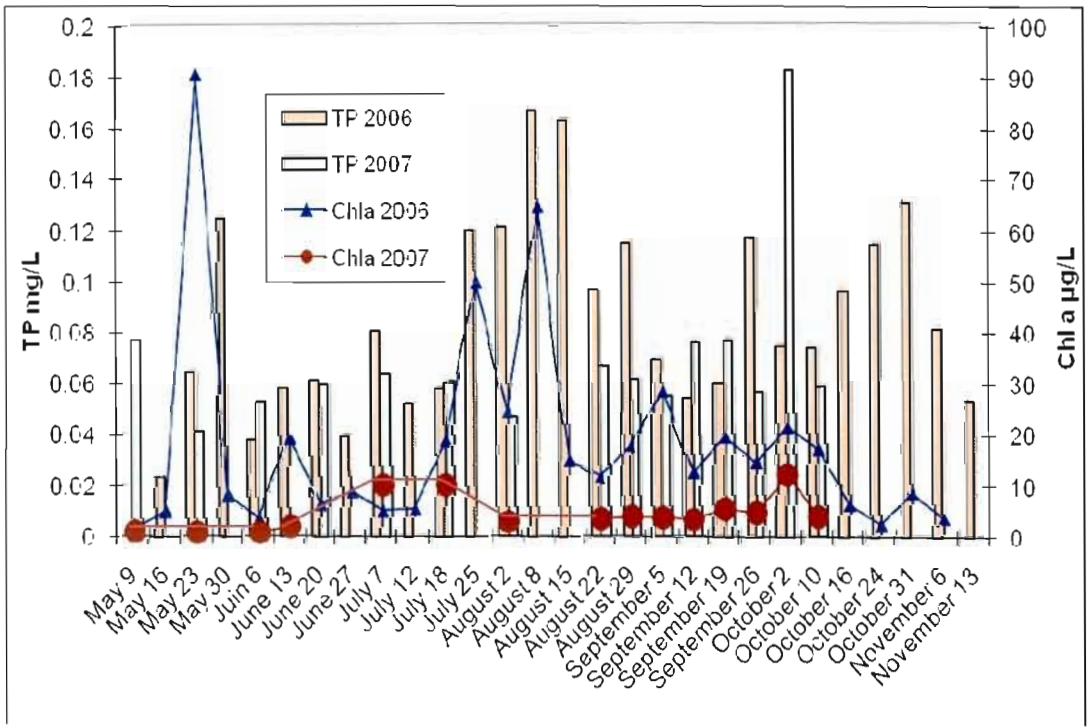
## CHAPTER III: RESULTS

### 3.1. Missisquoi Bay - Lake Champlain 2006 and 2007

**Table 1.** Mean, range and variability of nutrient parameters at the two sampling stations of Missisquoi Bay, Lake Champlain, in 2006 and 2007 (There was a tendency for mean and maximum values to be lower in 2007)

| | | TN mg/L | TDN mg/L | $NO_3$ mg/L | NH4 mg/L | TP mg/L | TDP mg/L |
|---|---|---|---|---|---|---|---|
| 2006 | Max. | 2.0157 | 1.855 | 1.6819 | 0.1935 | 0.1975 | 0.0821 |
| | Min. | 0.2973 | 0 | 0.0128 | 0.0252 | 0.0208 | 0.0132 |
| | Mean | 0.9943 | 0.6908 | 0.3911 | 0.0735 | 0.0857 | 0.0313 |
| | St. Dev. | 0.3751 | 0.3873 | 0.4030 | 0.0352 | 0.0385 | 0.0148 |
| | N. | 54 | 54 | 54 | 56 | 54 | 53 |
| 2007 | Max. | 1.0939 | 2.5309 | 0.7894 | 0.1289 | 0.1875 | 0.0444 |
| | Min. | 0.4423 | 0.2806 | 0 | 0.0122 | 0.0257 | 0.0121 |
| | Mean | 0.7104 | 0.6201 | 0.1613 | 0.0480 | 0.0691 | 0.0248 |
| | St. Dev. | 0.2363 | 0.5479 | 0.2122 | 0.0316 | 0.0350 | 0.0080 |
| | N. | 30 | 30 | 30 | 40 | 30 | 30 |

**Figure 3.** Phosphorus and chlorophyll a concentration for Missisquoi bay - Lake Champlain in 2006 and 2007



Regarding the dynamics of phytoplankton, a cyanobacterial bloom dominated by the genus *Microcystis* was observed from the middle of July until the middle of September in 2006. The only clear relation observed between physicochemical and biological parameter (Table 1) was a significant and strong positive correlation between chlorophyll a concentration and total phosphorus concentration (R2 = 0.45 – 0.60, F Prob. < 0.0001) for different stations (Figure 3), and a positive correlation between chlorophyll a and organic matter in 2007 ( R2 0.7 – 0.75; Prob > F <.0001).

### 3.2. Natural dissolved organic matter experimental extraction and addition

Treatments based on the addition of DOM supplement from XAD 8 resin extraction resulted in a 3-fold increase in natural dissolved organic carbon, and 3 to 6 fold increases in total phenolic compounds (Figure 4) compared with lake values at the time of the experiments. Concentrations of total phenolic compounds in the lake when the experiments were done (between 0.56 and 1.53 mg/L) were near that reported in the literature for other lakes in the world (between 0.24 and 0.55 mg/L) (Box 1983; Hilt et al. 2006).

With the addition of BG11 medium (Rippka et al. 1979) between 2 and 3-fold increase in total nitrogen and total phosphorus concentration was obtained (Figure 5). Treatment with the addition of organic matter did not cause a significant increase in the amount of nutrients. As supplement of nitrogen we used different sources: in experiment 1 and 2 made in June and July nitrate was used, and in experiment three ammonium was added to avoid nitrate photochemistry (Zepp et al. 1987).

**Figure 4.** Increases in dissolved organic carbon and total phenolic compounds measured at time zero for treatments in the experiments

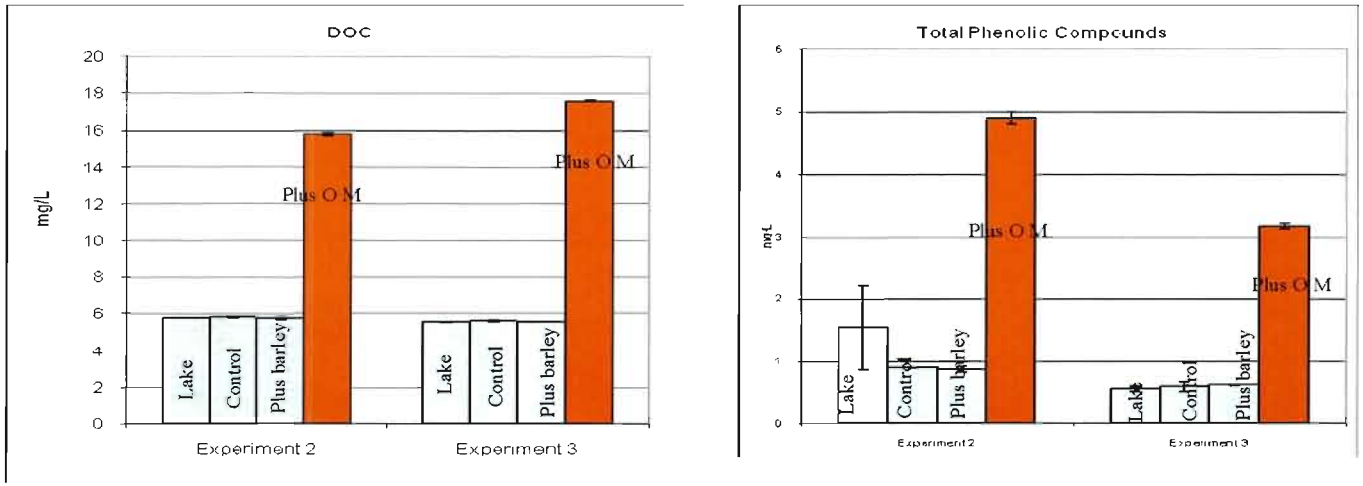
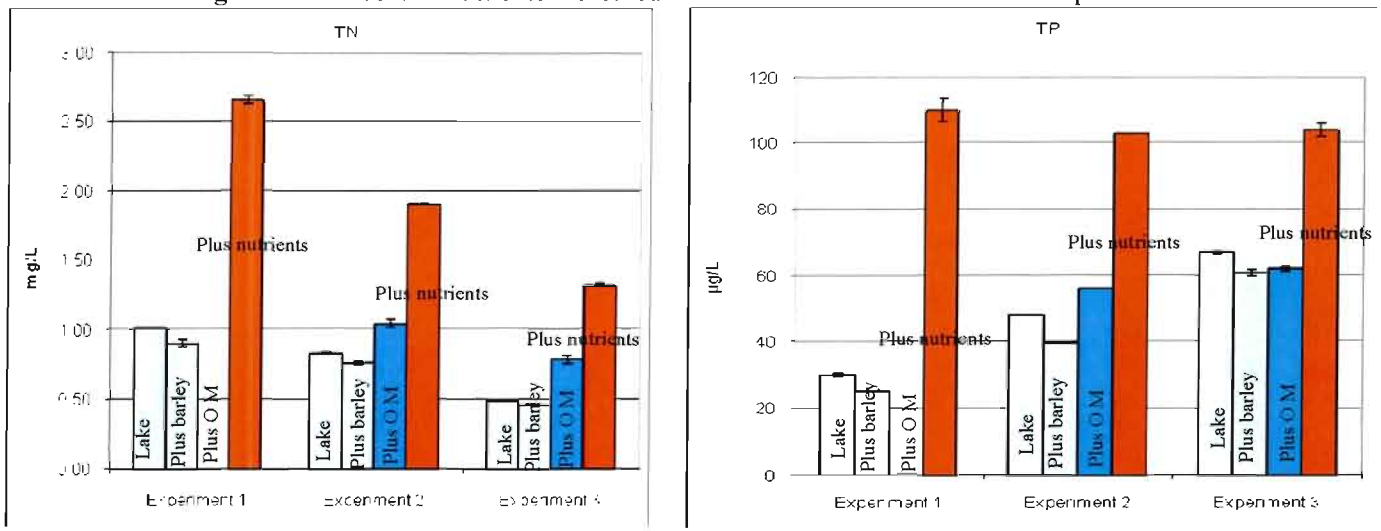



**Figure 5.** Increases in nutrients measured at time zero for treatments in the experiments



### 3.3. Incubation experiment results

**Table 2.** Correlations between variables for all the incubation experiments (monitoring data not included)

| Variable | by Variable | Correlation | Number of samples | Signif Prob |
|---|---|---|---|---|
| NOx (mg/L) | Chla (µg/l) | 0.46 | 134 | <.0001 |
| PT (mg/L) | Chla (µg/l) | 0.46 | 134 | <.0001 |
| PTD (mg/L) | NOx (mg/L) | 0.47 | 136 | <.0001 |
| PT (mg/L) | NOx (mg/L) | 0.51 | 137 | <.0001 |
| TDN (mg/L) | Chla (µg/l) | 0.54 | 130 | <.0001 |
| PTD (mg/L) | TDN (mg/L) | 0.58 | 132 | <.0001 |
| TN (mg/L) | Chla (µg/l) | 0.62 | 134 | <.0001 |
| PT (mg/L) | TDN (mg/L) | 0.68 | 133 | <.0001 |
| PT (mg/L) | TN (mg/L) | 0.77 | 137 | <.0001 |
| NOx (mg/L) | TN (mg/L) | 0.86 | 137 | <.0001 |
| NOx (mg/L) | TDN (mg/L) | 0.90 | 134 | <.0001 |
| DOC (mg/L) | Total Phenolic Compounds [µg/L] - Tannic Acid Units | 0.93 | 95 | <.0001 |
| TDN (mg/L) | TN (mg/L) | 0.94 | 133 | <.0001 |
| Absorbance 272 nm | Total Phenolic Compounds [µg/L] - Tannic Acid Units | 0.95 | 50 | <.0001 |
| Absorbance 440 nm | Total Phenolic Compounds [µg/L] - Tannic Acid Units | 0.95 | 92 | <.0001 |
| Absorbance 254 nm | Total Phenolic Compounds [µg/L] - Tannic Acid Units | 0.96 | 50 | <.0001 |
| Absorbance 440 nm | DOC (mg/L) | 0.98 | 92 | <.0001 |
| Absorbance 440 nm | Absorbance 272 nm | 0.98 | 52 | <.0001 |
| Absorbance 272 nm | DOC (mg/L) | 0.99 | 50 | <.0001 |
| Absorbance 254 nm | DOC (mg/L) | 0.99 | 50 | <.0001 |
| Absorbance 272 nm | Absorbance 254 nm | 0.99 | 52 | <.0001 |
| Absorbance 440 nm | Absorbance 254 nm | 0.99 | 52 | <.0001 |

All correlations were positive for the parameters included for the incubations experiments. There were strong positive correlations between chlorophyll a and nutrients, between different nutrients and between absorbance at different wavelengths, DOC and total phenolic compounds (Table 2).

Samples for absorbance at 254 and 272 nm were only for experiment 3 – September 2007 and samples for absorbance at 440 nm were only for experiments 2 – July 2007 and experiment 3 – September 2007.

**Table 3.** ANOVA probabilities for algal growth rate in incubation experiments

**Experiments with barley and organic matter extract addition (July, September)**

| | Growth Rate |
|---|---|
| **R Squared** | 0.976 |
| **R Squared Adj** | 0.952 |
| **Root Mean Square Error** | 0.048 |
| **Mean of Response** | 0.334 |
| **Observations** | 72 |
| **F Ratio** | 41.836 |
| **Prob > F** | <.0001 |
| **Effect Tests Prob > F** | |
| Barley | 0.266 |
| Light Level | 0.114 |
| Light Level*Barley | 0.995 |
| Light Level*Nutrients | 0.221 |
| Light Level*Nutrients*Barley | 0.518 |
| Light Level*Nutrients*OM | 0.314 |
| Light Level*OM | 0.558 |
| Month | <.0001 |
| Month*Barley | 0.111 |
| Month*Light Level | 0.057 |
| Month*Light Level*Barley | 0.801 |
| Month*Light Level*Nutrients | 0.101 |
| Month*Light Level*Nutrients*Barley | 0.110 |
| Month*Light Level*Nutrients*OM | 0.860 |
| Month*Light Level*OM | 0.365 |
| Month*Nutrients | 0.021 |
| Month*Nutrients*Barley | 0.000 |
| Month*Nutrients*OM | 0.020 |
| Month*OM | 0.023 |
| Nutrients | <.0001 |
| Nutrients*Barley | 0.211 |
| Nutrients*OM | 0.000 |
| OM | 0.016 |

**Experiments with barley addition (June, July, September)**

| | Growth Rate |
|---|---|
| **R Squared** | 0.967 |
| **R Squared Adj** | 0.932 |
| **Root Mean Square Error** | 0.060 |
| **Mean of Response** | 0.265 |
| **Observations** | 68 |
| **F Ratio** | 27.333 |
| **Prob > F** | <.0001 |
| **Effect Tests Prob > F** | |
| Barley | 0.497 |
| Light Level | 0.029 |
| Light Level*Barley | 0.799 |
| Light Level*Nutrients | 0.080 |
| Light Level*Nutrients*Barley | 0.372 |
| Month | <.0001 |
| Month*Barley | 0.378 |
| Month*Light Level | 0.0014 |
| Month*Light Level*Barley | 0.884 |
| Month*Light Level*Nutrients | 0.207 |
| Month*Light Level*Nutrients*Barley | 0.226 |
| Month*Nutrients | <.0001 |
| Month*Nutrients*Barley | 0.001 |
| Nutrients | <.0001 |
| Nutrients*Barley | 0.005 |

To evaluate whether the different treatments used at different light levels had an effect in the growth of phytoplankton we calculated the growth rate as $\mu = \frac{\ln chla_{final} - \ln chla_{init}}{incubation\_time}$.

Analyses of variance were conducted for growth rate values from the experiments with barley addition and for the experiments with barley and organic matter extract addition (Table 3). Time of experiment (indicate as month) had a significant effect on the growth rate of phytoplankton for different light and nutrient levels. The response for the addition of nutrients and different light level was as expected, increase in phytoplankton growth for all the treatments. The effect was strongest considering only experiments with barley addition.

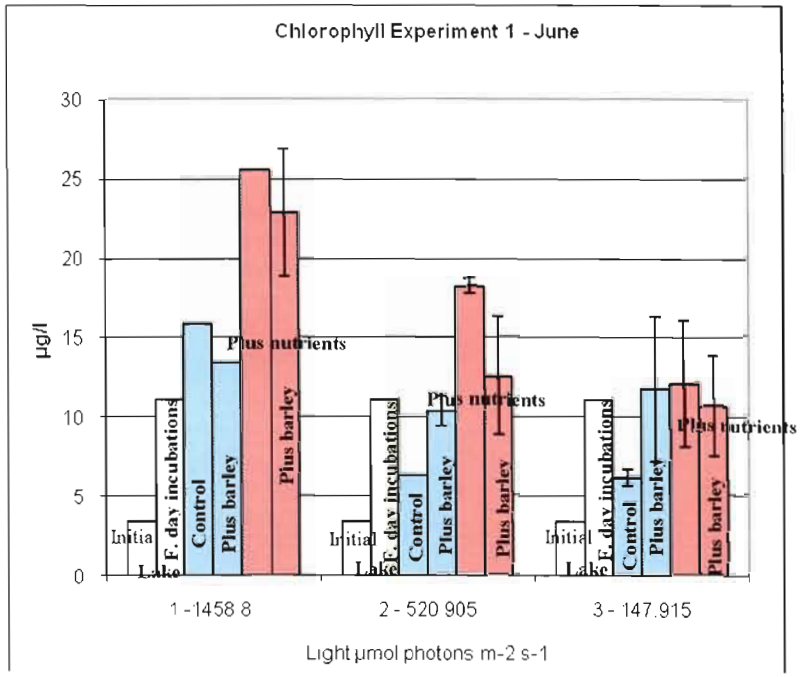
There was a significant response for the addition of barley and organic matter only in the presence of nutrient supplement. The response was significantly different for experiments in distinct months. Effect of the addition of organic matter was stronger than the effect of barley. Concerning the scaled estimates (all factors –light, nutrients, DOM and barley addition- at all levels) there was a significant interaction between nutrients and the strongest light intensity; between nutrients, barley addition and the intermediate light intensity and between the lowest light intensity and barley addition.

**Table 4.** ANOVA probabilities for growth rate in incubation experiments by month (in red significant differences, in green values near to be significant)

| | Growth Rate Prob > F | | |
|---|---|---|---|
| | **Exp. 1 (June)** | **Exp. 2 (July)** | **Exp. 3 (September)** |
| **Light Level** | **0.038** | 0.766 | 0.008 |
| **Nutrients** | 0.154 | **<.0001** | **<.0001** |
| **Light Level*Nutrients** | 0.569 | **0.061** | 0.868 |
| **Barley** | 0.984 | 0.091 | 0.695 |
| **Light Level*Barley** | 0.801 | 0.912 | 0.865 |
| **Nutrients*Barley** | **0.061** | 0.090 | **0.000** |
| **Light Level*Nutrients*Barley** | 0.528 | 0.144 | 0.598 |
| **OM** | | 0.923 | **0.001** |
| **Light Level*OM** | | 0.760 | 0.227 |
| **Nutrients*OM** | | 0.335 | **<.0001** |

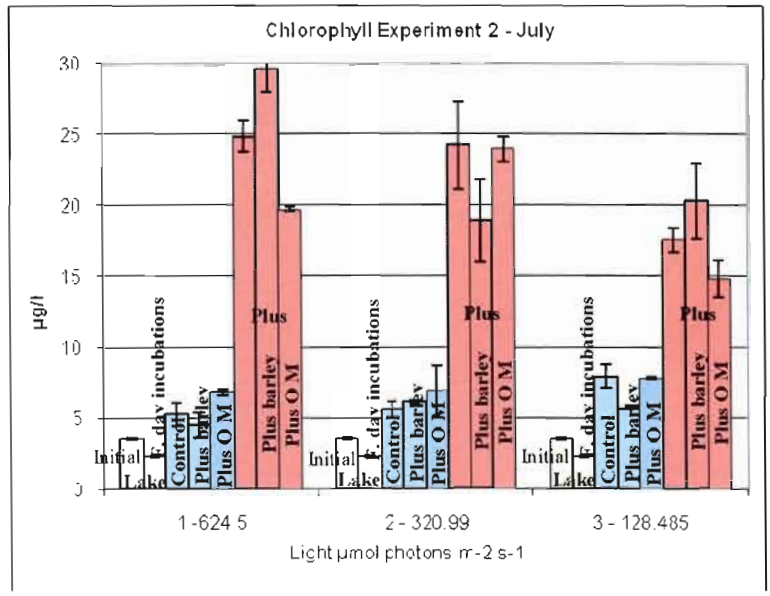
Considering each experiment there was a strong effect of light on growth rate in June. Nutrient addition had the strongest effect in July and September experiments. In September there was a significant effect of DOM addition and from the interaction between nutrient and DOM or barley addition (Table 4).

**Figure 6.** Chlorophyll a concentration for different treatments in experiment 1 – June 2007



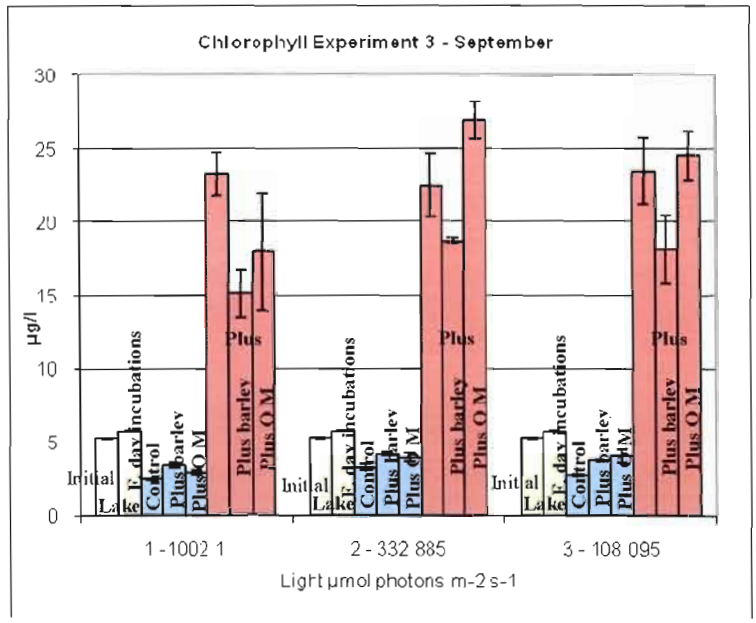

Phytoplankton in the first experiment was strongly light limited; growth was significantly higher for the highest light exposure (Figure 6). There was high variability between replicate values in this experiment making it difficult to evaluate the effect from the treatments, especially for the experiment with the lowest light intensity. The addition of nutrients increased growth of phytoplankton especially for the highest and the intermediate light levels. For the intermediate light level there was a significant decrease in the growth of phytoplankton in the treatment with barley and nutrient addition.

**Figure 7.** Chlorophyll a concentration for different treatments in experiment 2 – July 2007




Phytoplankton growth in the second experiment was strongly nutrient limited (Figure 7). Growth of phytoplankton was significantly lower with the addition of organic matter and nutrients for the highest and the lowest light levels. For the intermediate light level there was a decrease in the growth of phytoplankton with the addition of barley and nutrients.

**Figure 8.** Chlorophyll a concentration for different treatments in experiment 3 – September 2007



The experiment conducted in September showed that the phytoplankton community was still strongly nutrient limited (Figure 8). Growth of phytoplankton was significantly decreased with the addition of barley and nutrients for all light levels, especially for the highest one. For the intermediate light level there was an important increase in the growth of phytoplankton with the addition of organic matter and nutrients.

### 3.4. Responses at taxonomic level

Because of the absence of significant levels of cyanobacteria in the first two experiments, less emphasis was placed on the taxonomical characterization of experimental results. Only one replicate per treatment was counted in experiments 1 and 2. For experiment 3 we used observations from one replicate from each incubation bottle, which meant two true replicates per treatment.

Cryptophyceae was dominant in the lake at the beginning of the experiment in June 2007 (Figure 9). In the incubations the biggest proportion of biomass was from green algae (Chlorophyceae) followed by Chrysophyceae and Bacillariophyceae. In general there was an increase in Chlorophyceae with the addition of nutrients, an increase in the proportion of Chrysophyceae at highest light intensity with the addition of barley and an increase in the proportion of Cyanophyceae with the addition of nutrients and barley at the same high light level. Bacillariophyceae was the most important group for the control samples in the intermediate and lowest light intensity. The addition of barley at the highest light intensity increased the proportion of Dinophyceae. For Cyanophyceae the lowest proportions were at low and intermediate light intensities with barley addition (Figure 9, Figure 10, Figure 11).

**Figure 9.** Distribution of major algal classes at different light levels for incubation experiment 1 – June 2007

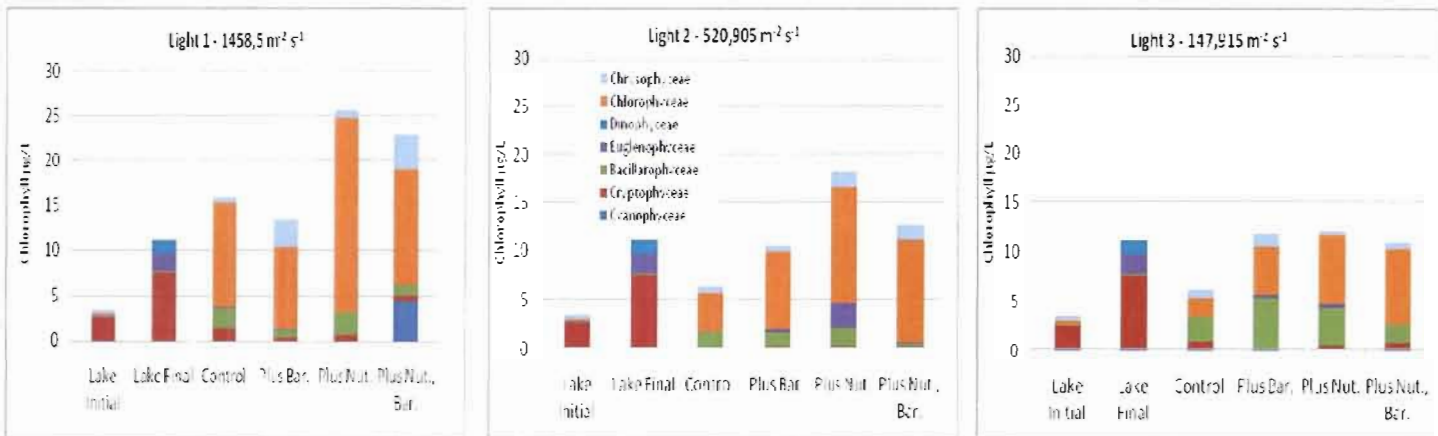
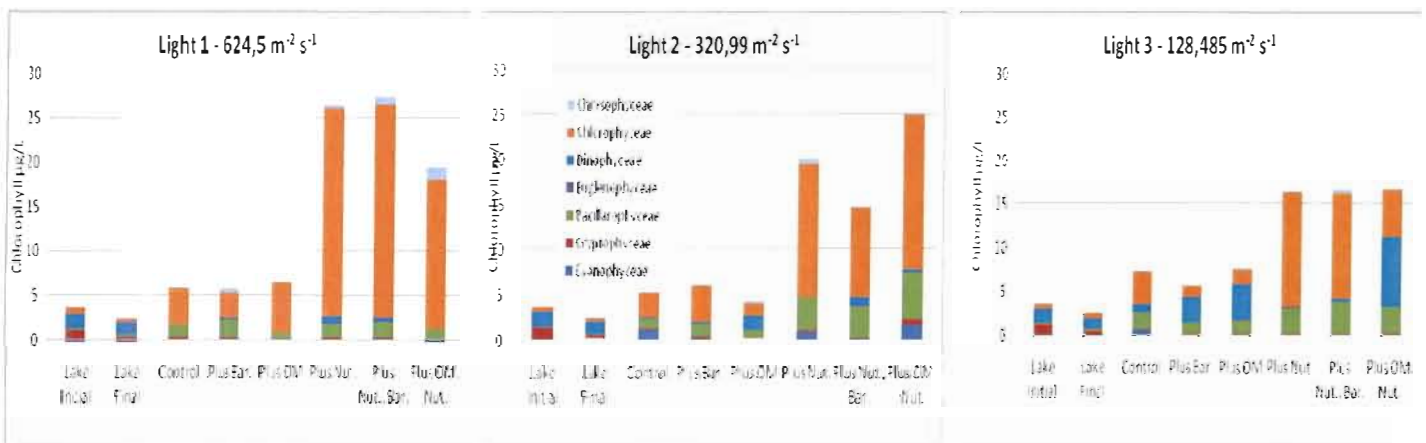



**Figure 10.** Distribution of major algal classes at different light levels for incubation experiment 2 – July 2007

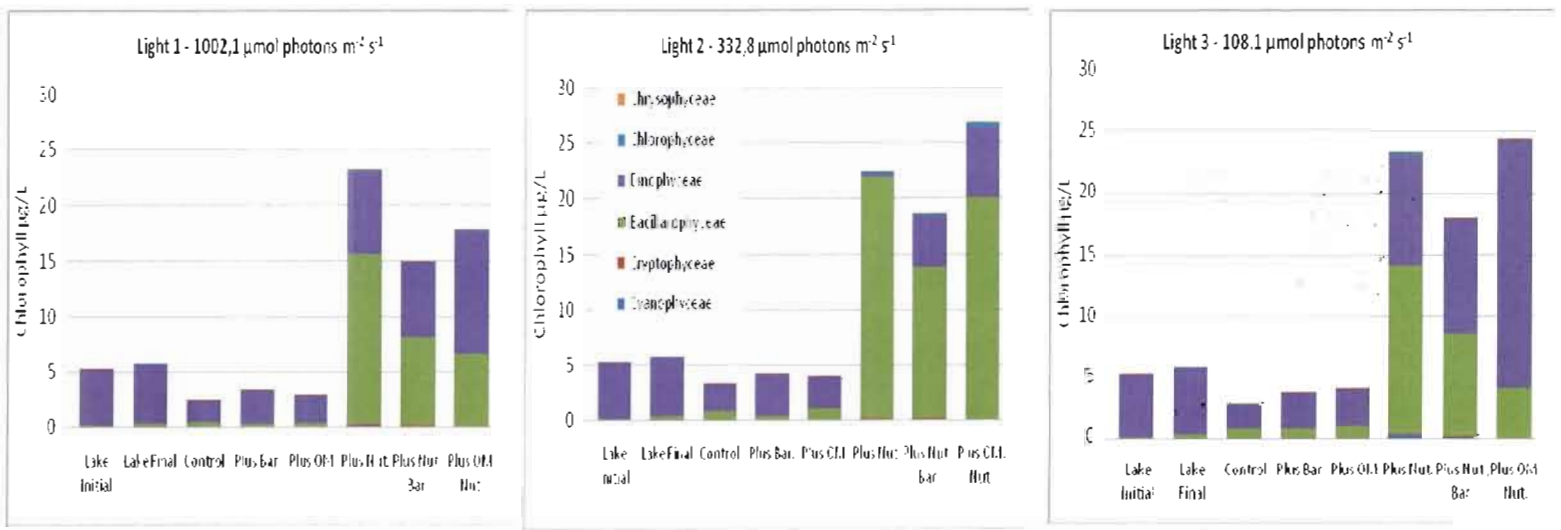


Cryptophyceae, Dinophyceae and Chlorophyceae were predominant for the lake at the beginning of the experiment in July 2007. In the incubations Chlorophyceae, Bacillariophyceae and Dinophyceae increased their proportions at the expense of nutrients. There was an important increase in the proportion of Dinophyceae with the addition of organic matter especially for the lowest and highest light intensities. There was a small increase in the proportion of Cyanophyceae at the intermediate light level for the control and with the addition of nutrients except for the samples with nutrients and barley (Figure 10).

**Table 5.** Percentage of taxonomical group composition for all the treatments in experiment 3. For each light level values in red represent highest proportions and values in green represent lowest proportions

| % | | Cyanophyceae | Cryptophyceae | Bacillariophyceae | Dinophyceae | Chlorophyceae | Chrysophyceae |
|---|---|---|---|---|---|---|---|
| Lake D 0 | | 0.044 | 0.023 | 2.300 | 97.25 | 0.296 | 0.078 |
| Lake D 4 | | 0.001 | 0.043 | 5.241 | 94.33 | 0.381 | 0.000 |
| High light level | - | 0.201 | 0.002 | 17.28 | 82.36 | 0.080 | 0.068 |
| | N | 1.203 | 0.041 | 65.34 | 31.92 | 1.423 | 0.063 |
| | B | 0.046 | 0.003 | 5.857 | 94.00 | 0.050 | 0.034 |
| | NB | 0.281 | 0.065 | 47.70 | 51.52 | 0.359 | 0.063 |
| | MO | 0.143 | 0.005 | 10.51 | 88.53 | 0.481 | 0.320 |
| | MON | 0.097 | 0.031 | 34.06 | 64.96 | 0.810 | 0.024 |
| Intermed. light level | - | 1.054 | 0.015 | 20.92 | 75.76 | 1.721 | 0.519 |
| | N | 0.694 | 0.049 | 97.12 | 0.588 | 1.501 | 0.044 |
| | B | 0.057 | 0.002 | 6.505 | 93.21 | 0.153 | 0.062 |
| | NB | 0.475 | 0.049 | 73.41 | 24.65 | 1.305 | 0.102 |
| | MO | 0.139 | 0.005 | 24.83 | 74.86 | 0.025 | 0.130 |
| | MON | 0.125 | 0.029 | 73.43 | 24.72 | 1.655 | 0.030 |
| Low light level | - | 0.622 | 0.004 | 27.32 | 71.72 | 0.101 | 0.220 |
| | N | 2.034 | 0.003 | 59.60 | 36.73 | 1.600 | 0.021 |
| | B | 0.680 | 0.009 | 20.08 | 78.90 | 0.229 | 0.089 |
| | NB | 1.268 | 0.022 | 51.74 | 46.08 | 0.858 | 0.025 |
| | MO | 0.121 | 0.006 | 24.69 | 74.57 | 0.384 | 0.221 |
| | MON | 0.088 | 0.005 | 17.73 | 81.93 | 0.211 | 0.022 |

**Figure 11.** Distribution of major algal classes at different light levels for incubation experiment 3 – September 2007



Dinophyceae, Bacillariophyceae, Cryptophyceae and Cyanophyceae were dominant in the lake at the beginning of the experiment in September 2007 (Figure 11). There was increased proportion of Bacillariophyceae with nutrient addition particularly for the intermediate light intensity. Dinophyceae proportion increased with barley addition especially for the high and intermediate light levels (Figure 11).

It is important to note that there was a change in proportion between Bacillariophyceae and Dinophyceae when nutrients and barley were added. The proportions of the taxonomical groups were similar with organic matter and barley addition. When nutrients were added with barley or organic matter, proportions of Bacillariophyceae and Dinophyceae were similar for the highest and the lowest light intensities, for the intermediate light intensity proportions were highest for Bacillariophyceae as in the samples with nutrients (Table 5).

The proportion of Cyanophyceae increased with the addition of nutrients, especially at intermediate light level. There was a decrease in the proportion of Cyanophyceae with the addition of barley, organic matter, and organic matter and nutrients, particularly for the highest and lowest light intensities.

### *3.4.1. Analysis of variance*

**Table 6.** Significant probability values for ANOVA in incubation experiments for all months

| | Growth rate – Taxonomical Group | Growth Rate – Barley addition | Growth Rate – Barley addition (Log) | Final biomass - July and September 2007 (Log) |
|---|---|---|---|---|
| **R Squared** | 0.369 | 0.378 | 0.049 | 0.040 |
| **R Squared Adj** | 0.118 | 0.068 | 0.026 | 0.017 |
| **Root Mean Square Error** | 0.542 | 0.560 | 4.33E-05 | 5.09E-05 |
| **Mean of Response** | 0.075 | 0.070 | -0.000 | -0.000 |
| **Observations** | 1134 | 756 | 1469 | 1452 |
| **F Ratio** | 1.47 | 1.220 | 2.127 | 1.721 |
| **Prob > F** | <.0001 | 0.031 | 0.000 | 0.005 |
| **Effect Tests Prob > F** | | | | |
| Experiment | 0.008 | | | |
| Experiment*B*Light Level | | | 0.045 | |
| Experiment*Group | <.0001 | | | |
| Experiment*N | | | 0.000 | 0.002 |
| Group | <.0001 | | | |
| Light Level | | | | 0.001 |
| N | | | 0.000 | |
| N*Light Level | | | | 0.025 |
| Species | | <.0001 | | |

There was a significant effect of the treatments on the growth rate at species and group levels. Time of experiment (indicated as month) and the interaction with the taxonomical group had a significant effect on the growth rate of phytoplankton (Table 6, Table 9). Considering only experiments with barley addition, there was an effect of nutrient addition, the interaction between nutrients and the month of the experiment, and the month of the experiments, nutrient and barley addition. Taking into account only experiments with barley and organic matter addition (July and September 2007), there was a significant effect in final biomass of phytoplankton at different light levels and for the interaction between nutrient addition, month and light levels (particularly for July at highest light levels). At the species level there was a significant difference in the growth rate for *Aphanocapsa, Aphanothece, Cyclotella, Stephanodiscus, Cryptomonas, Rhodomonas, Katablepharis, Carteria* and *Chlamydomonas*.

**Table 7.** Significant probability values for ANOVA regarding Cyanobacteria in incubation experiments for all months

| | Growth rate - Cyanobacteria | Biomass – Cyanobacteria Barley addition (Log) | Biomass – Microcystis July and September 2007 |
|---|---|---|---|
| **RSquare** | 0.532 | 0.249 | 0.670 |
| **RSquare Adj** | 0.297 | 0.124 | 0.359 |
| **Root Mean Square Error** | 0.479 | 0.124 | 1.94E-05 |
| **Mean of Response** | 0.091 | 0.552 | 2.04E-05 |
| **Observations** | 213 | 247 | 36 |
| **F Ratio** | 2.263 | 2.002 | 2.156 |
| **Prob > F** | <.0001 | 0.0015 | 0.057 |
| **Effect Tests Prob > F** | | | |
| B | | | 0.008 |
| N | | 0.000 | |
| N*B*Light Level | | | 0.053 |
| Species | <.0001 | | |
| Experiment | | 0.000 | |
| Experiment*N | | 0.036 | |

There was a significantly higher growth rate at species level for Cyanobacteria in the incubation experiments, particularly for *Aphanocapsa* and *Aphanothece*. In the experiments 2 and 3 (June and July 2007) there was a positive effect on the final biomass of Cyanobacterial species for different months and nutrient addition treatments, principally for the highest and intermediates light levels in June, and in general for the highest light level and the interaction with nutrient addition. At the species level there was an effect on the final biomass of *Microcystis* in the experiments with barley and organic matter addition (July and September 2007), in particular with the addition of nutrients (positive effect) and barley extract (negative effect) (Table 7, Table 9).

Considering only final biomass of *Microcystis* after the incubation experiments (Figure 12) there was a significant effect of barley addition and the interaction between barley, light level and nutrients. With the addition of nutrients there was an increase in biomass of *Microcystis* particularly for highest light level. With the addition of barley there was a decrease in the final biomass of *Microcystis*, particularly with nutrient addition. The response for the addition of organic matter was similar to the response for barley addition.

**Figure 12.** Interaction profile for final biomass of *Microcystis* (carbon biomass, g/L) with different treatments. Microcystis final biomass was higher with the addition of nutrients at high light levels and lowest with the addition of barley (Blue lines indicates treatment presence, red line treatment absence).

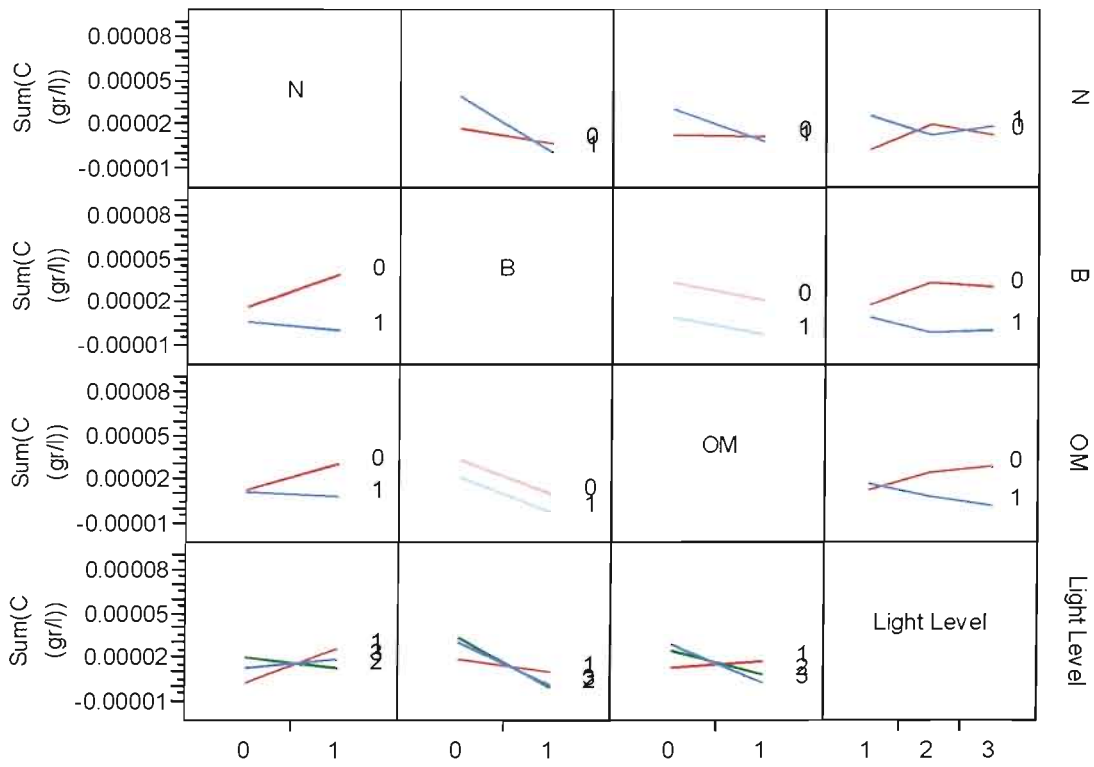


**Table 8.** ANOVA probability values in experiment 3 - September 2007

| | Growth rate – Experiment 3 species | Biomass - Taxonomical group Experiment 3 (Log) | Growth rate – Cyanobacteria Experiment 3 | Biomass – Microcystis Experiment 3 (Log) |
|---|---|---|---|---|
| **RSquare** | 0.773 | 0.628 | 0.584 | 0.732 |
| **RSquare Adj** | 0.548 | 0.574 | 0.172 | 0.464 |
| **Root Mean Square Error** | 0.267 | 0.090 | 3.51E+08 | 0.055 |
| **Mean of Response** | 0.225 | -0.468 | 3.05E+08 | 0.593 |
| **Observations** | 1080 | 844 | 216 | 35 |
| **F Ratio** | 3.429 | 11.641 | 1.418 | 2.731 |
| **Prob > F** | <.0001 | <.0001 | 0.035 | 0.0227 |
| **Effect Tests Prob > F** | | | | |
| B | 0.004 | | 0.086 | |
| Light Level | 0.017 | | | |
| N | 0.001 | | | 0.001 |
| N*Light Level | 0.008 | | | |
| B*N*Light Level | | | | 0.078 |
| Species | <.0001 | | <.0001 | |
| B*Species | 0.005 | | | |
| Species*N | <.0001 | | | |
| Species*OM | 0.030 | | | |
| Group | | <.0001 | | |
| Group*N | | 0.000 | | |

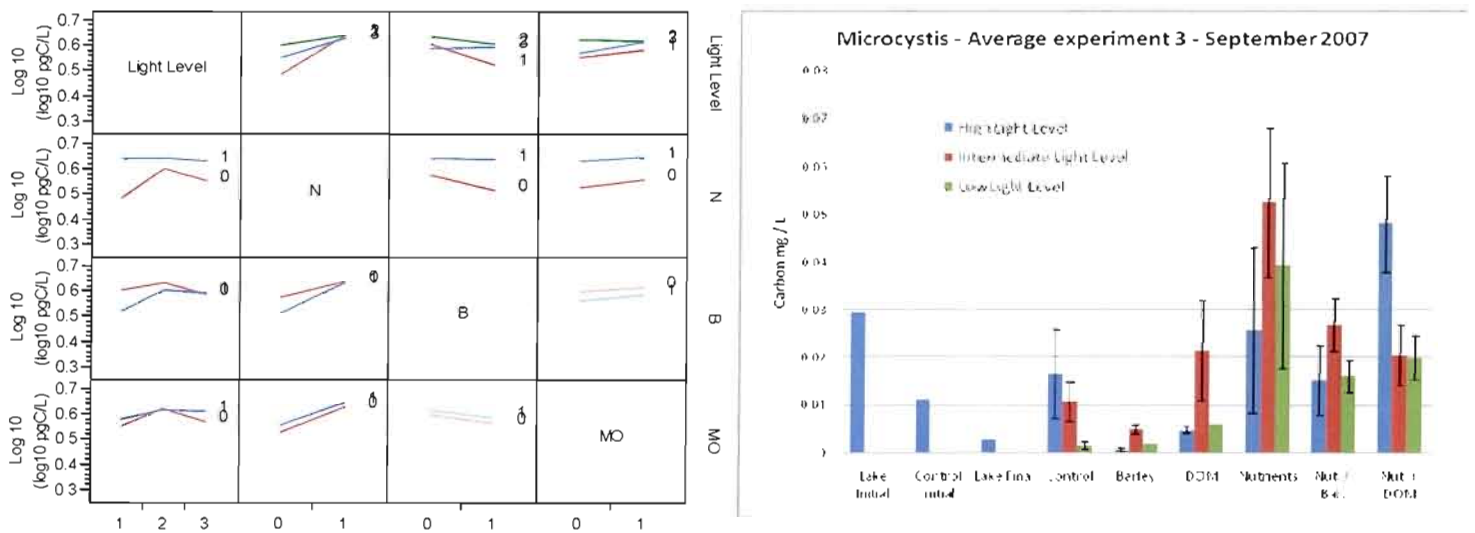
The September experiment had largest proportion of Cyanophyceae in the phytoplankton community of all the experiments. There was an effect at the species level in the growth rate for barley and nutrient addition, for different light levels particularly for the highest and the intermediate light levels. At the group level there were significant positive effects in the final biomass for the interaction between group and nutrient addition, particularly for Chlorophyceae at intermediate light level, and significant negative effects for Cyanophyceae with the addition of barley and organic matter at the highest light intensity. In the group of Cyanobacteria there was a significant negative effect at the species level especially for barley addition (Table 8, Table 9).

**Table 9.** Direction of significant effect for different parameters in the experiments

| Parameter | Experiments species and groups | Experiments Cyanophyceae | Experiment 3 | | Total phenolic compounds | OM - DOC |
|---|---|---|---|---|---|---|
| | | | Cyanophyceae | Chlorophyceae | | |
| **Light Level** | | | | | Negative | Negative |
| **Nutrients** | Positive | Positive | | Positive (Intermediate light level) | | |
| **Light Level*Nutrients** | Positive (High light level) | Positive (High light level) | | | | Positive (High light level) |
| **Barley** | | | Negative (High Light level) | | Positive | |
| **Light Level*Barley** | Positive (High light level and high nutrients) | | | | Negative | |
| **OM** | | | Negative (High light level) | | | |
| **Light Level*OM** | | | | | | |
| **Nutrients*OM** | | | | | | |
| **Light Level*Nutrients *OM** | | | | | | |

Considering only the final biomass of *Microcystis* there was a significant effect with the nutrient addition and for the interaction between nutrient, barley and light level. With the addition of nutrients there was an increase in *Microcystis* biomass principally under the highest light intensity, with the addition of barley there was a decrease in the final biomass of *Microcystis* (Figure 13).

**Figure 13.** Interaction profile and biomass plot for *Microcystis* in experiment 3 – September 2007



### *3.4.2. DOM and nutrients*

**Table 10.** ANOVA of parameters related with characteristics and composition of DOM for the incubations.

| | **Total Phenolic Compounds [µg/L] - Tannic Acid Units** | | **DOC (mg/L)** | | | **Organic matter (mg/L)** | **Absorbance 254 nm** | **Absorbance 272 nm** | **Absorbance 440 nm** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Exp. 2** | **Exp. 3** | **Exp. 1** | **Exp. 2** | **Exp. 3** | **Exp. 3** | **Exp. 3** | **Exp. 3** | **Exp. 2** | **Exp. 3** |
| RSquare | 0.971 | 0.994 | 0.559 | 0.998 | 0.999 | 0.722 | 0.996 | 0.995 | 0.536 | 0.999 |
| RSquare Adj | 0.960 | 0.988 | 0.075 | 0.996 | 0.999 | 0.461 | 0.993 | 0.990 | 0.235 | 0.998 |
| Root Mean Square Error | 207.784 | 145.859 | 0.225 | 0.272 | 0.110 | 1.421 | 0.012 | 0.062 | 0.082 | 0.004 |
| Mean of Response | 1409.464 | 1578.455 | 5.370 | 8.835 | 9.726 | 14.113 | 0.286 | 1.428 | 0.132 | 0.126 |
| Observations (or Sum Wgts) | 30 | 36 | 22 | 36 | 36 | 36 | 35 | 35 | 29 | 35 |
| **Effect Tests Prob > F** | | | | | | | | | | |
| Light Level | 0.001 | <.0001 | | | 0.001 | | | | | 0.0001 |
| Nutrients | | 0.058 | | 0.002 | 0.002 | | <.0001 | <.0001 | 0.000 | <.0001 |
| Light Level*Nutrients | | | | 0.009 | | | | | | 0.001 |
| Barley | 0.909 | | | | | <.0001 | | | | |
| Light Level*Barley | 0.936 | | | | | | | | | |
| OM | <.0001 | <.0001 | | <.0001 | <.0001 | 0.000 | <.0001 | <.0001 | | <.0001 |
| Light Level*OM | 0.108 | <.0001 | | | 0.000 | | | | | 0.001 |
| Nutrients*OM | | 0.004 | | 0.021 | | | <.0001 | <.0001 | | <.0001 |
| Light Level*Nutrients*OM | | | | | | | | | | <.0001 |

Regarding the effect of different treatments in the parameters related with DOM, there was a significant negative effect of light level and the interaction between barley and light level in the final amount of total phenolic compounds. The addition of barley had a significant positive effect in the final amount of total phenolic compounds, as might be expected. The addition of organic matter had an effect on almost all the parameters related. There was an important effect of the interaction between DOM and nutrients, for the total DOC concentration in experiment 2 (Table 10).

We observed an important effect of light for experiment 2 on the concentration of total phenolic compounds at the end of the incubations. Increasing light intensity produced a decrease in the concentration of total phenolic compounds when they were abundant (DOM, and DOM and nutrient treatments); the effect was minor for the treatment with DOM and nutrients (Figure 14, Figure 15). The addition of barley did not contribute significantly to the increase of DOC values for the incubation experiments (Figure 15).

Regarding the effect on nutrients at the end of the incubations, there was a significant effect of light level in the final concentration of almost all the nutrients for experiment 2, despite final concentration of total phosphorus. For experiment 3 the effect was significant for total nitrogen and phosphorus. For the final concentration of nitrate and ammonium there was a significant effect from practically all the treatments for experiment 2, the final concentration of ammonium was significantly affected for all the treatments in experiments 2 and 3. Organic matter significantly affected the final concentration of all the nutrients apart from nitrate. There was an important effect of the interaction between OM and light for experiment 3, and between DOM and nutrients for experiment 2 (Annex 1).

**Figure 14.** Total phenolic compounds concentration for the experiments

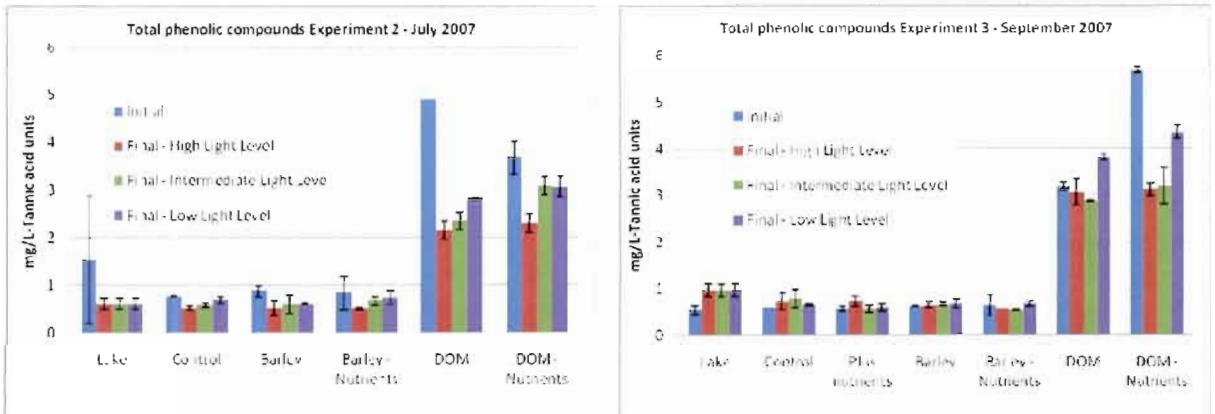
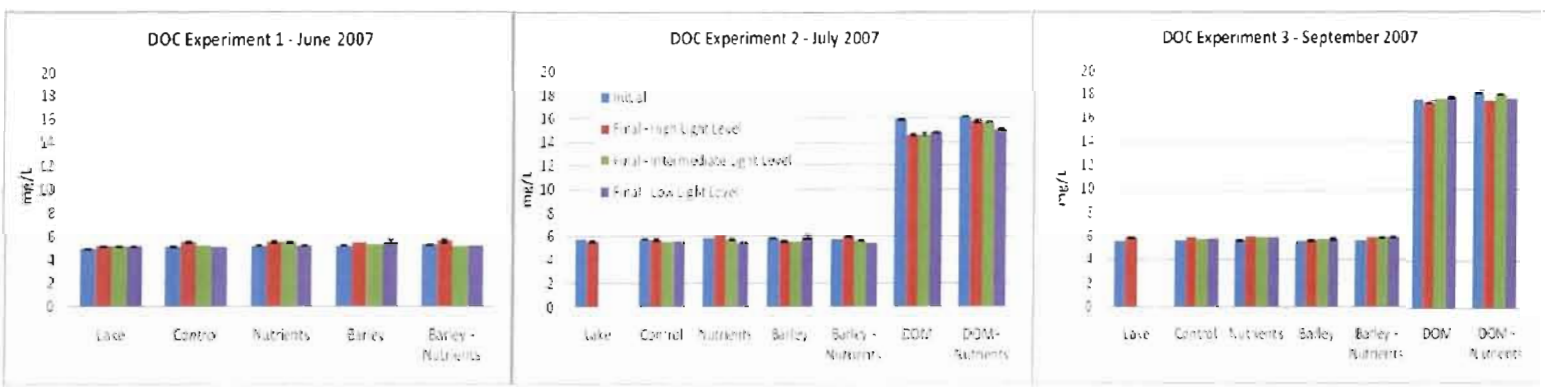



**Figure 15**. Dissolved organic carbon for the experiment.



There was an increase in the concentration of all nutrients after the addition of dissolved organic matter except for nitrate and ammonium. Total nitrogen concentration decreased with the increase of light levels in experiments 2. Total dissolved nitrogen concentration decreased with the increase of light levels for experiment 1 and 2. The decrease in the concentration of this nutrient is significantly higher after the incubations for experiment 3 regardless of the level of exposition to light, suggesting greater nutrient limitation in the fall experiment. For the treatments where nitrate was added, the final concentration decreased with the increase of light levels. Final ammonium concentration decreased with increasing of light level for experiments 1 and 2, for experiment 3 there was a significant decrease (more than 50%) regardless of the level of exposition to light (Annex 3)

Despite a significant decrease in the amount of total phosphorus after the incubations in control for experiment 1, there were no significant differences between treatments or light levels in final total phosphorus concentration. Total dissolved phosphorus decreased more than 50%, especially in experiments 2 and 3; the nutrient concentration decreased with the increase of light levels for experiment 1 (Annex 3).

Final TDN and ammonium concentrations for the treatment with dissolved organic matter and nutrient addition showed significant decrease at the highest light intensity. There was also a significant decrease in the concentration of both nutrients with the addition of nutrients for the intermediate light level. Otherwise final TDP increased in the highest light intensity, and there was a significant increase with the addition of nutrients for the intermediate light level (Annex 3).

### 3.5. Correspondence analysis for parameters related with phytoplankton in incubation experiments

**Table 11.** Summary of correspondence analysis for parameters related with phytoplankton in incubation experiments

| | Species | | | | Species-Environment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cumulative percentage variance of species data | | | | Species-environment correlations | | | | Cumulative percentage variance of species data | | | | Cumulative percentage variance of species-environment relation | | | |
| Axes | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Taxonomical Groups | 37.0 | 64.5 | 80.0 | 88.7 | 0.9 | 0.9 | 0.6 | 0.6 | 30.2 | 51.0 | 55.7 | 60.2 | 47.3 | 79.9 | 87.2 | 94.3 |
| Experiment 1 Groups | 40.6 | 64.0 | 80.3 | 89.8 | 0.9 | 0.9 | 1.0 | 0.9 | 34.3 | 52.3 | 67.3 | 75.2 | 42.0 | 64.0 | 82.3 | 92.0 |
| Experiment 2 Groups | 51.1 | 73.3 | 85.6 | 92.6 | 0.9 | 0.8 | 0.9 | 0.9 | 43.0 | 56.2 | 65.5 | 71.3 | 57.5 | 75.3 | 87.7 | 95.5 |
| Experiment 3 Groups | 41.8 | 75.8 | 87.0 | 96.9 | 0.9 | 1.0 | 0.9 | 0.7 | 35.2 | 67.9 | 76.3 | 81.6 | 42.0 | 81.1 | 91.1 | 97.5 |
| All experiments Anabaena, Microcystis | 43.0 | 64.1 | 80.5 | 94.0 | 0.9 | 1.0 | 0.8 | 0.5 | 36.5 | 55.2 | 65.8 | 69.1 | 51.0 | 77.1 | 91.9 | 96.5 |
| Experiment 3 Anabaena, Microcystis | 43.8 | 77.8 | 90.9 | 100.0 | 1.0 | 0.7 | 0.9 | 0.6 | 38.9 | 58.7 | 69.0 | 72.9 | 53.3 | 80.5 | 94.6 | 100.0 |
| Sp. All Experiments | 10.1 | 17.0 | 23.6 | 28.8 | 1.0 | 1.0 | 0.9 | 0.9 | 9.4 | 15.6 | 19.4 | 22.2 | 24.9 | 41.6 | 51.6 | 59.0 |
| Experiment 1 Species | 15.5 | 28.3 | 39.8 | 48.3 | 1.0 | 1.0 | 1.0 | 1.0 | 14.8 | 26.0 | 36.1 | 43.9 | 20.2 | 35.5 | 49.3 | 59.9 |
| Experiment 2 Species | 14.2 | 25.1 | 32.7 | 39.7 | 0.9 | 1.0 | 1.0 | 0.9 | 11.4 | 20.6 | 26.9 | 32.2 | 18.5 | 33.5 | 43.7 | 52.2 |
| Experiment 3 species | 12.0 | 21.9 | 30.6 | 38.1 | 1.0 | 1.0 | 1.0 | 1.0 | 11.7 | 21.1 | 29.6 | 36.0 | 15.4 | 27.7 | 39.0 | 47.5 |
| Experiment 3 Cyano | 19.3 | 34.7 | 47.6 | 58.2 | 1.0 | 0.9 | 1.0 | 0.9 | 17.7 | 28.0 | 37.3 | 44.8 | 25.1 | 39.7 | 52.9 | 63.6 |
| Cyano All experiments | 15.3 | 27.4 | 37.0 | 45.9 | 0.9 | 0.8 | 0.8 | 0.7 | 11.8 | 17.6 | 22.9 | 26.8 | 31.7 | 47.4 | 61.7 | 72.1 |

The most important taxonomical groups in all the experiments were Dinophyceae (especially at high light intensities), Bacillariophyceae, Cyanophyceae and Chlorophyceae. Environmental data (nutrients, DOC, phenolic compounds, absorbance at different wavelengths) from incubation experiments collected at day 4 explained most of the variation in species biomass (g C/L) (Figure 16) especially *Anabaena* and *Microcystis*.In all cases, variance explained by the analysis is highest when we consider species and environment relation for taxonomical group rather than species biomass data (g C/L) (Table 11).

Regarding the relation between taxonomical groups and the environmental variables there is a strong positive relation between Cryptophyceae and Chrysophyceae and high light intensities and total dissolved phosphorus concentration. High phosphorus concentration was positively related with the presence of Chlorophyceae. High values of absorbance related with high contents of organic matter were positively related with the presence of Dinophyceae. Low organic matter content was positively related to the presence of Bacillariophyceae and low concentration of total phenolic compounds and dissolved organic matter was positively related to the presence of Cyanophyceae (Figure 16).

Taxonomical group composition of the samples in experiment 1 (June) was more related to total dissolved nitrogen and phosphorus concentration and light (Figure 16). Taxonomical group composition for experiment 2 (July) was more related to total nitrogen, phosphorus and organic matter concentration, particularly samples with barley addition strongly were related to high total phosphorus concentration (Figure 16). For the experiment 3 (September), taxonomical composition of the samples was strongly related to high values of absorbance (related to high dissolved organic carbon content). Taxonomical groups in the samples with nutrients, barley and organic matter addition in the highest light level (Experiment 3 – September) were related to high concentration of DOC, and, taxonomical group composition for samples with nutrient addition at all light levels and samples with nutrient and barley addition in the intermediate light level (Experiment 3 – September) were strongly related to high ammonium concentration (Figure 16). Dinophyceae was evidently related to samples with barley addition in the highest and intermediate light level for the experiment 3 in September (Figure 16).

**Figure 16.** Correspondence analysis for principal taxonomical groups for different months. Between groups c: Cryptophyceae, x: Chrysophyceae, d: Bacillariophyceae, p: Dinophyceae, v: Chlorophyceae, b: Cyanophyceae. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level.

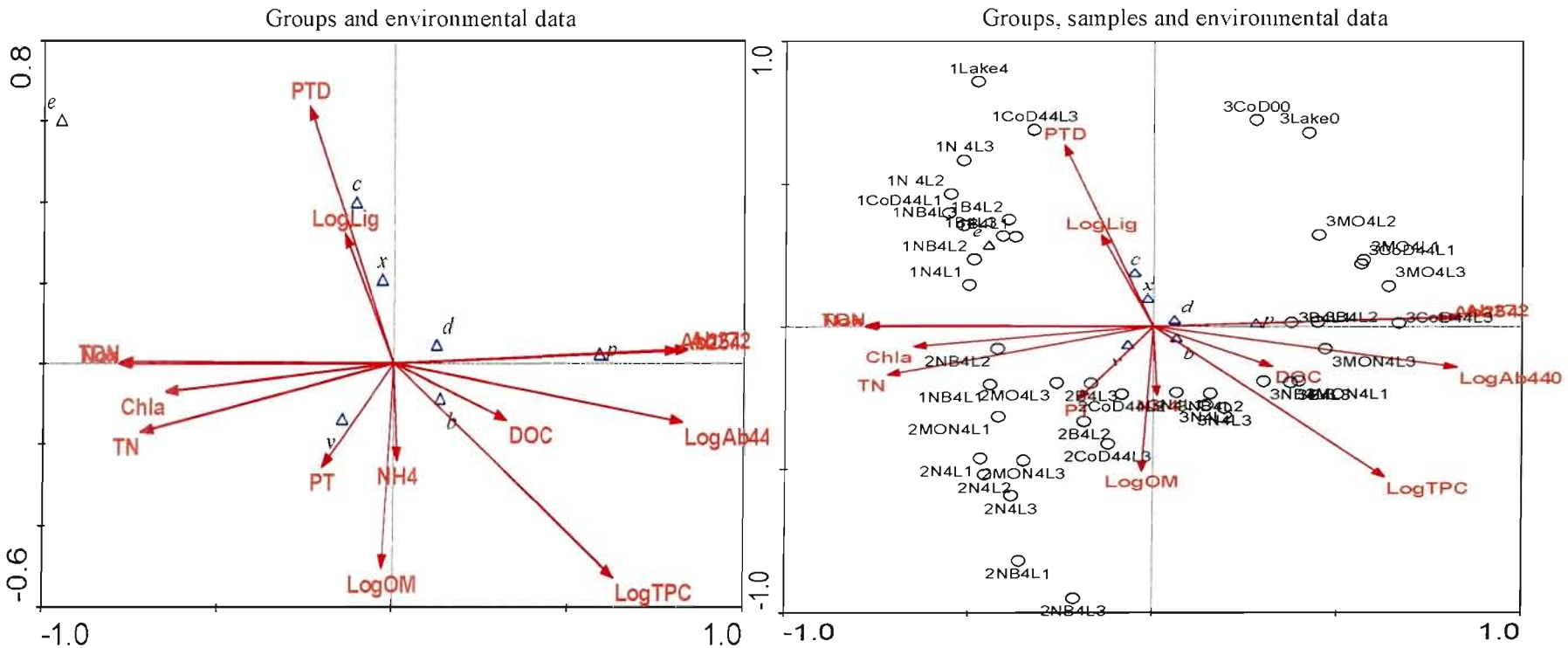
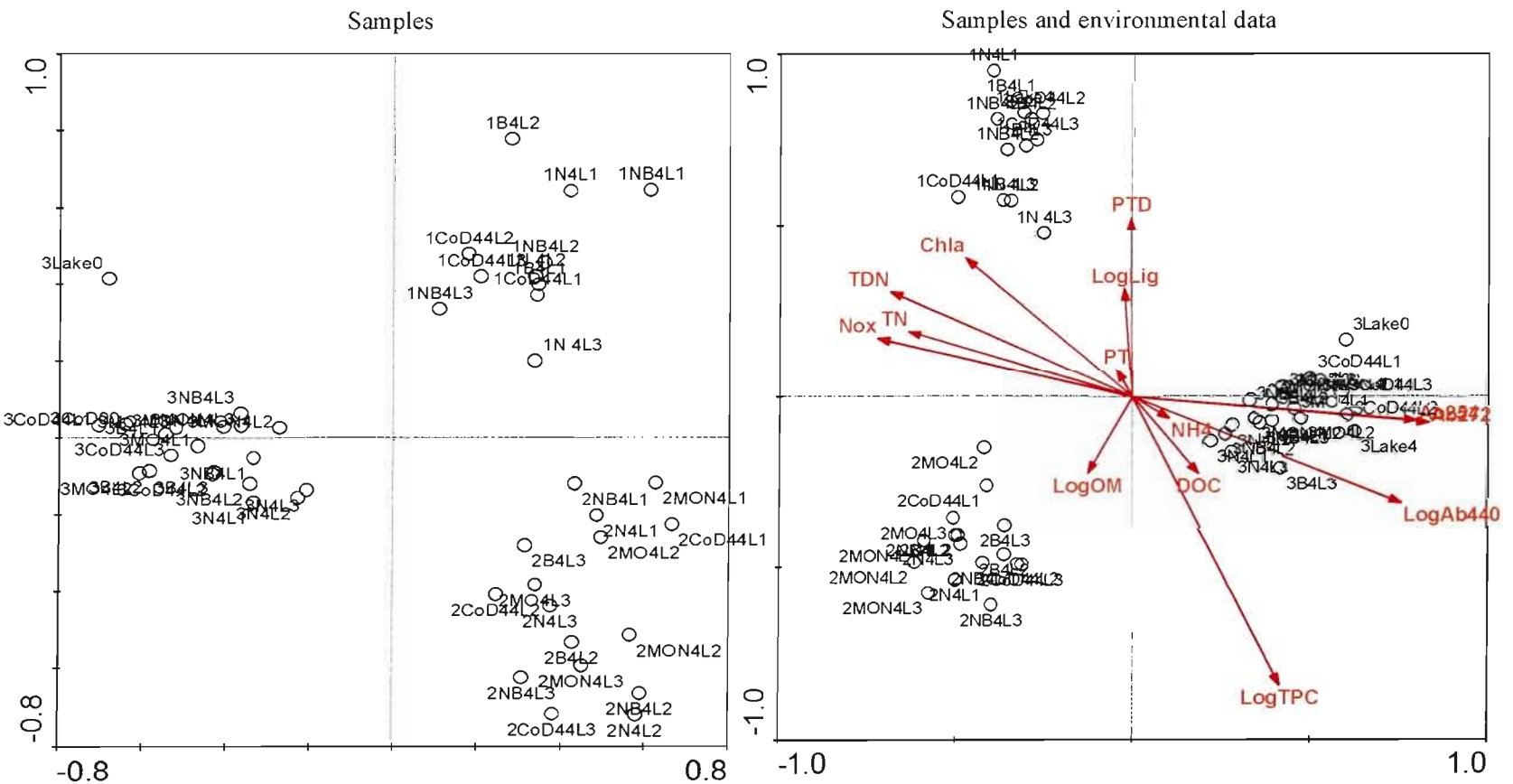


**Figure 17.** Correspondence analysis for all the species in different months. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level.



Regarding species composition of the samples there was a big variability that couldn't be explained by the correspondence analysis (Table 11). The composition of the samples relating to species was more similar between samples in experiment 3 (September) (Figure 17). Presence of species in experiment 1 was more related to the concentration of nutrients as nitrogen and phosphorus and light (Figure 17). Presence of species in experiment 2 was more related to the concentration of organic matter. Presence of species in experiment 3 was more related to absorbance at different wavelength, ammonium, DOC and low total phenolic compounds concentration (Figure 17).

The presence of Cyanobacterial species was strongly positively related to nutrient concentration for experiments 1 and 2 and negative related to total phenolic compounds, absorbance at different wavelength, and dissolved organic carbon concentration for experiment 3 (Figure 18). Presence of Cyanobacterial species at the lowest light level (experiment 3 – September) were more positively related to high phosphorus concentration. Concerning specific species relation with environmental variables, the presence of *Microcystis holsatica* and *Anabaena circinalis* was strongly positively related to low concentration of total phenolic compounds.

For experiment 3, in September, when *Microcystis* and *Anabaena* were more abundant, the presence of both species was strongly positively related to high concentrations of ammonium and phosphorus and low values of absorbance at different wavelengths (Figure 19).

Regarding species and environmental data relations, the presence of *Microcystis* was positively related to high concentration of total nitrogen and chlorophyll a, and presence of *Anabaena* was positively related to high phosphorus and ammonium concentration and low absorbance of the samples at different wavelengths.

**Figure 18.** Correspondence analysis for all cyanobacterial species in different months. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C-Co: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level.

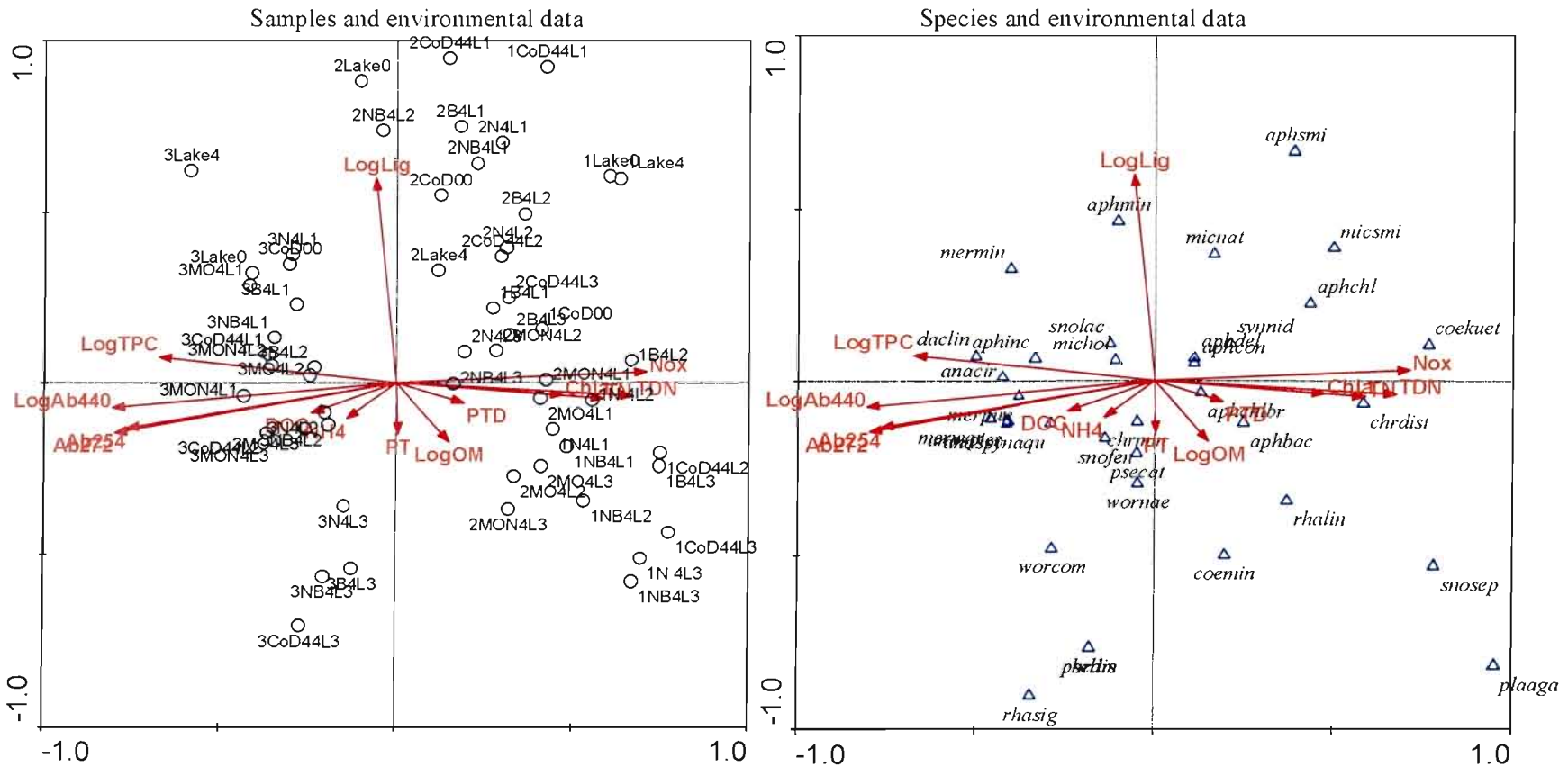
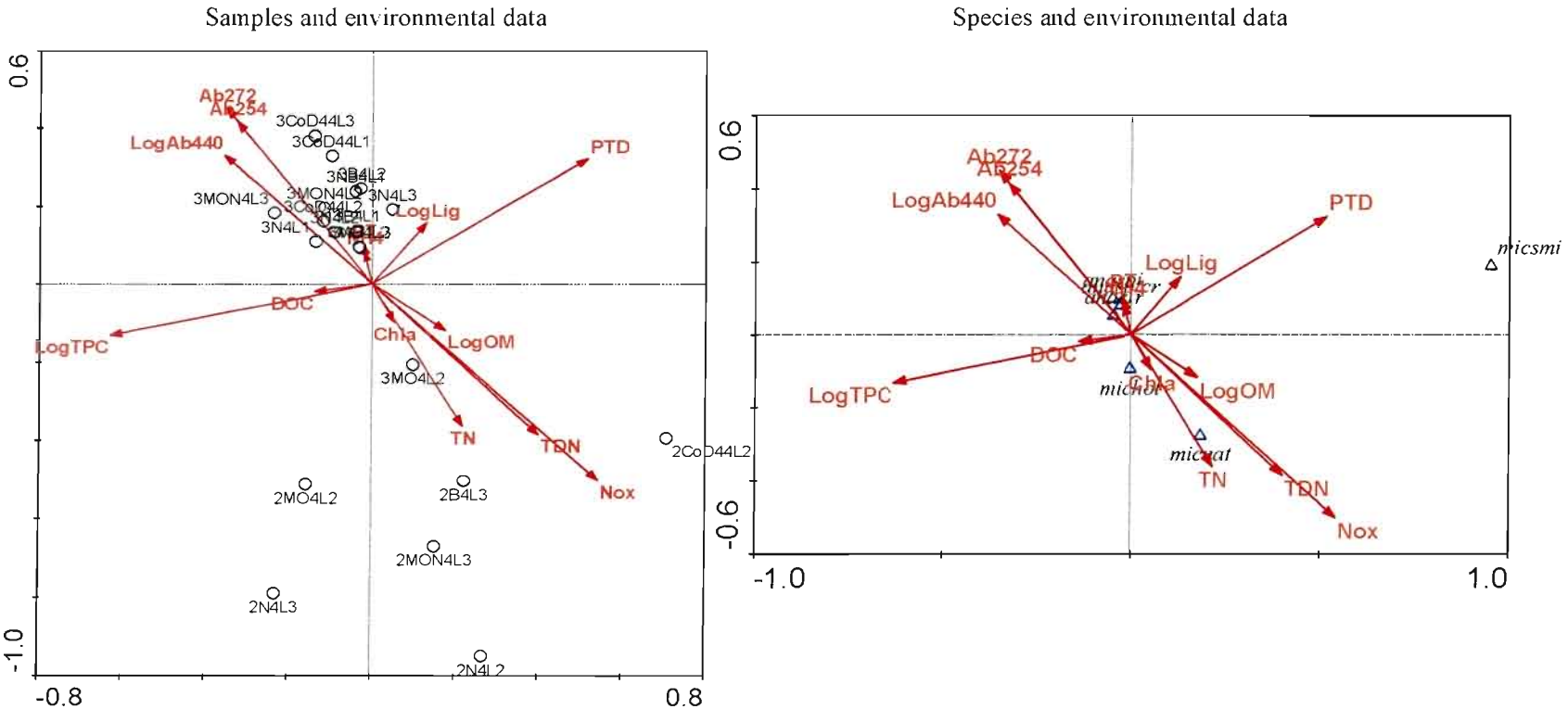


**Figure 19.** Correspondence analysis *Microcystis* and *Anabaena* in different months. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C-Co: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level.



Species from the group Chrysophyceae, Cryptophyceae and Chlorophyceae constituted the groups with the highest biomass in all the experiments. Concerning species composition for experiment 1 samples at lowest light level were positively related to high nitrate, ammonium and total dissolved phosphorus concentration, excepting samples with barley addition. Species in samples at the highest and intermediate light level were positively related to total phosphorus and nitrogen concentrations (Figure 20). Presence of species from the group Dinophyceae was positively related to barley addition at high light levels. Experiment species composition was dominated by individuals belonging to the group Bacillariophyceae followed by Chlorophyceae. Taxonomical distribution in groups for intermediate and lowest light level was positively related to organic matter concentration in the samples (Figure 20).

In experiment 2, light was an important factor for species variability. Abundance of species in the samples at the highest light intensity was positively related with ammonium concentration (Figure 21). Species distribution was similar for samples with organic matter and barley addition, and for both additions plus nutrients, but they were joined in different clusters for the highest light intensity. For the intermediate and lowest light intensity, species were similar for all the treatments. Abundance of species in the samples with barley addition at the intermediate light level was positively related to high total phenolic compounds and DOC concentrations. Abundance of species in the samples at the lowest light intensity was positively related to high values of absorbance at 440 nm (Figure 21).

**Figure 20.** Correspondence analysis for all species and groups in experiment 1 – June 2007. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C-Co: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level. Between groups c: Cryptophyceae, x: Chrysophyceae, d: Bacillariophyceae, p: Dinophyceae, v: Chlorophyceae, b: Cyanophyceae.

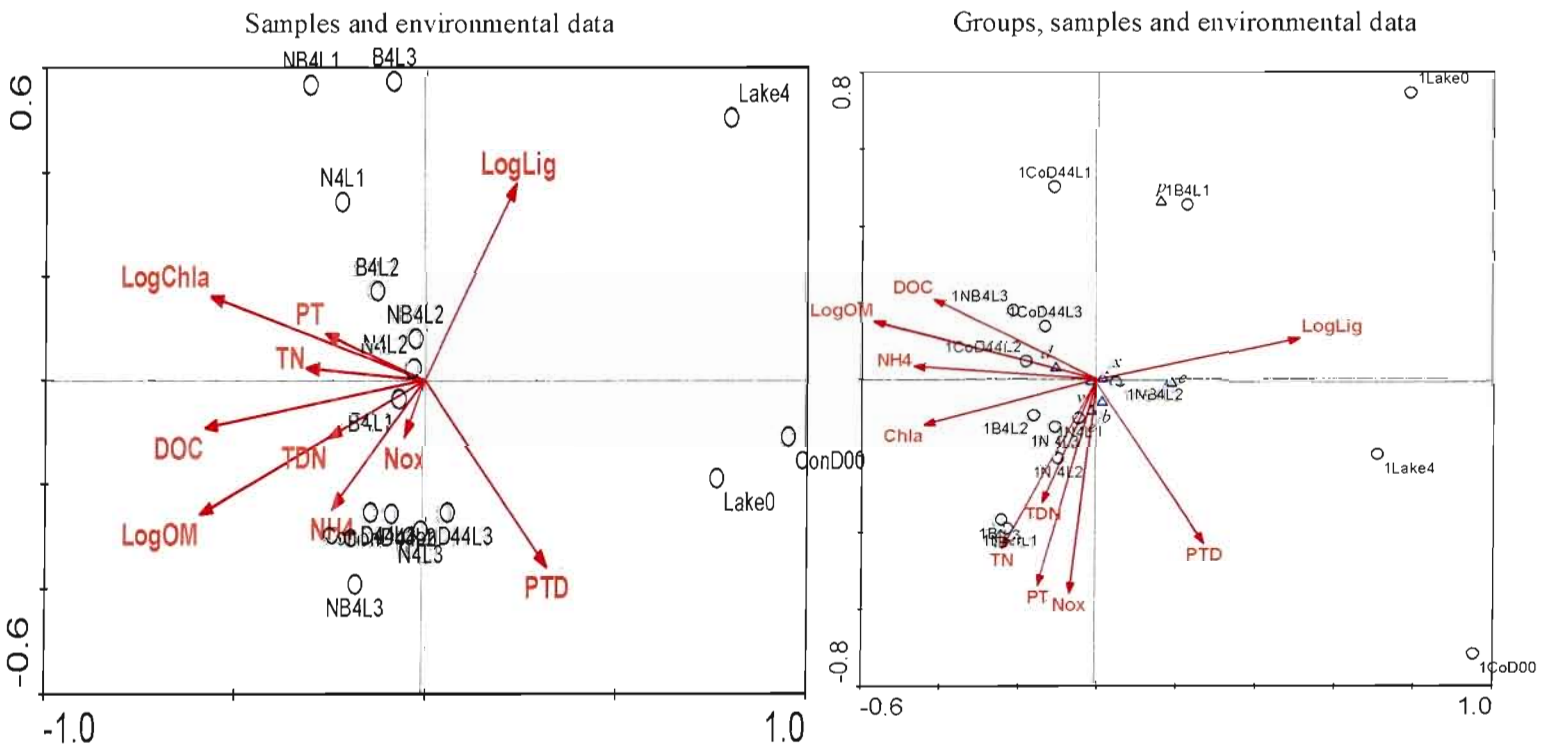
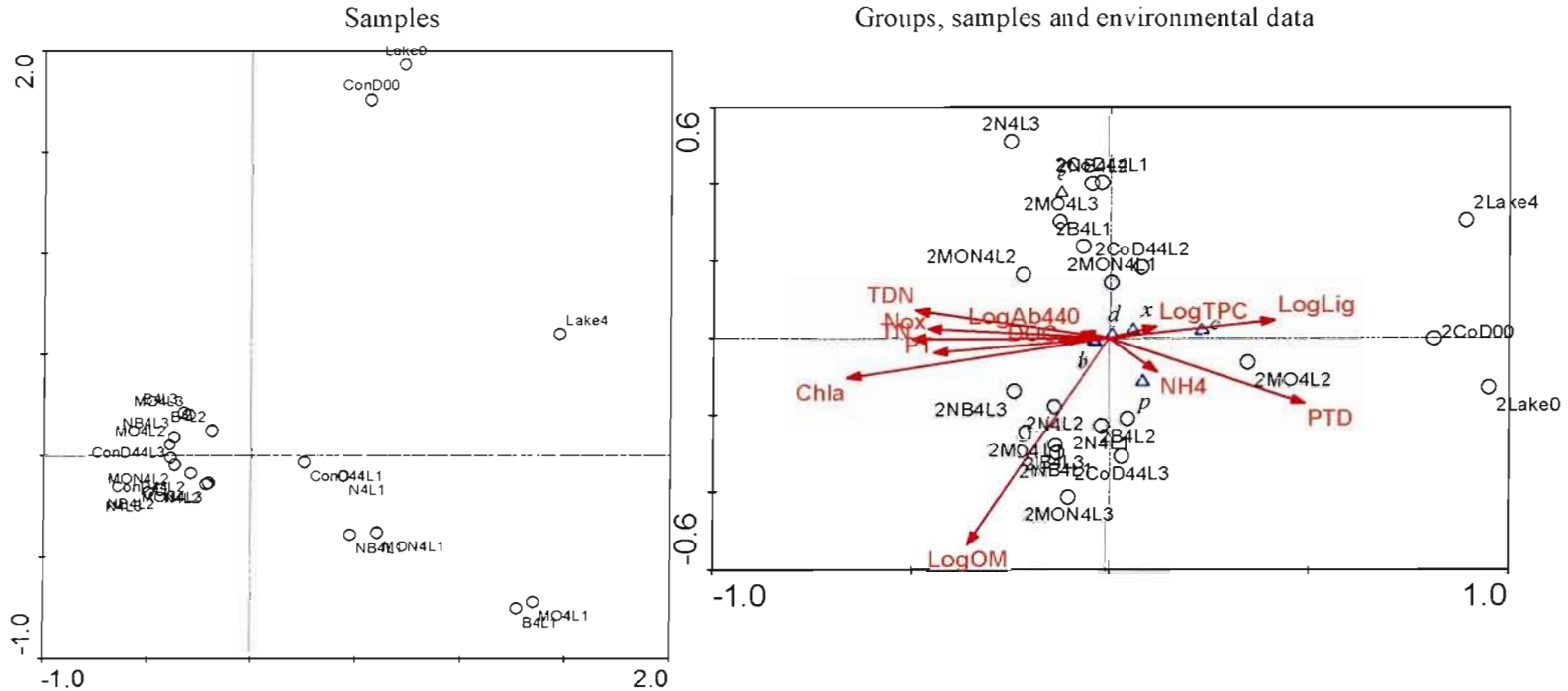


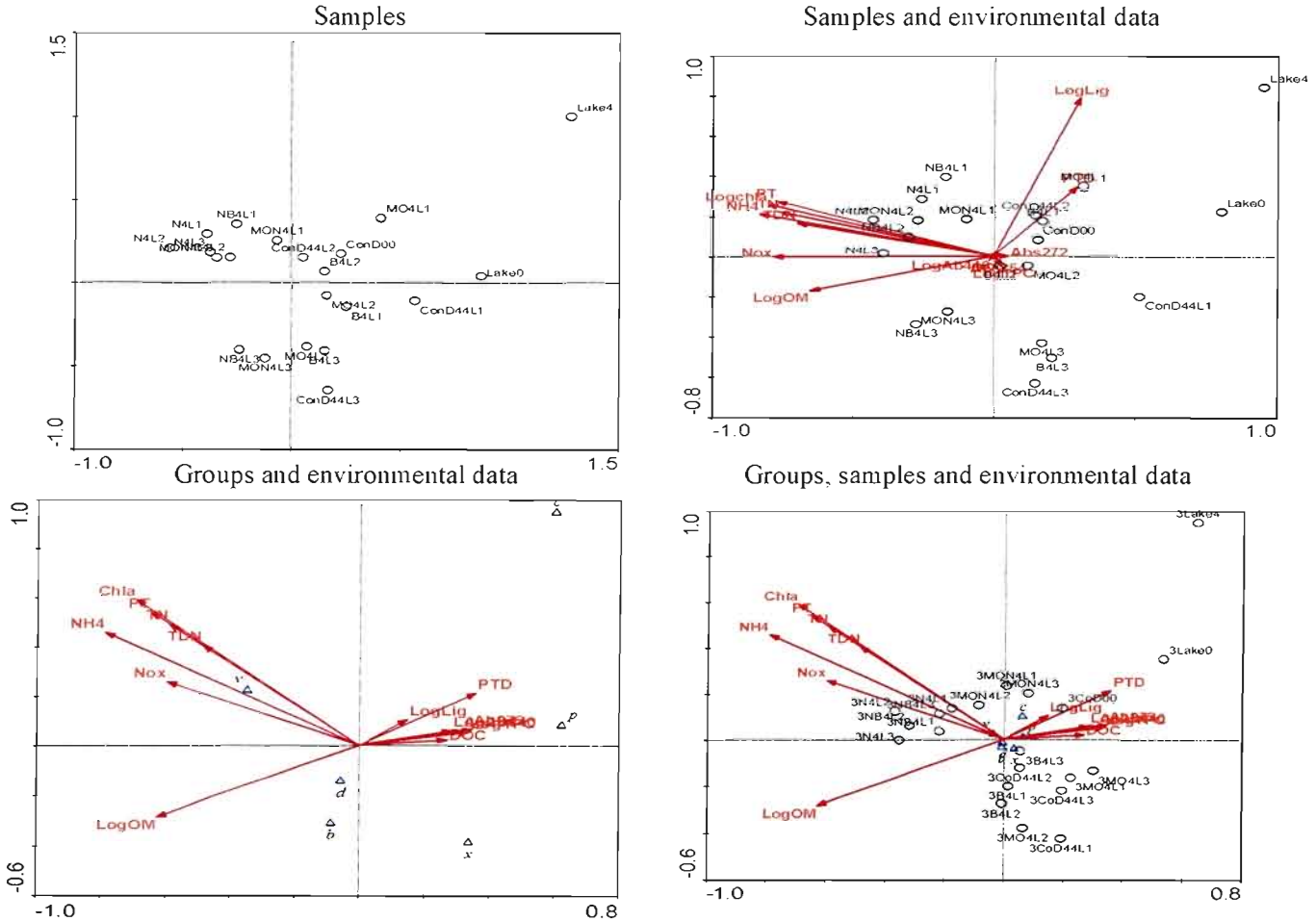
**Figure 21.** Correspondence analysis for all species and groups in experiment 2 – July 2007. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C-Co: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level. Between groups c: Cryptophyceae, x: Chrysophyceae, d: Bacillariophyceae, p: Dinophyceae, v: Chlorophyceae, b: Cyanophyceae.



Species composition in experiment 3 (September) was similar for the nutrient addition depicting the level of exposition to the light. Samples with barley, organic matter and nutrient addition presented a different taxonomical composition at the lowest light level (Figure 22). Taxonomical composition of samples with barley and organic matter addition plus nutrients was different from barley and organic matter addition alone and similar to nutrient composition alone, which suggests that in presence of nutrient there is no effect of barley and organic matter addition on the taxonomical composition. Taxonomical composition of samples with barley and organic matter addition was positively related to total dissolved phosphorus concentration at the highest light intensity (the identity of the species involved is an arbitrary function that was not investigated). At the intermediate light intensity, taxonomical composition of samples with barley and organic matter addition was positively related to total phenolic compounds and DOC concentration (Figure 22).

Chlorophyceae was positively related to nutrient concentration. Dinophyceae was positively related to DOC and total phenolic compounds concentration. Chrysophyceae was positively related to samples with barley and organic matter addition at all light levels (Figure 22).

**Figure 22.** Correspondence analysis for all species and groups in experiment 3 – September 2007. Between samples first number: Experiment number, N: Nutrient addition, B: Barley addition, MO: Organic matter addition, C-Co: Control, 4-D4: Day 4 of incubation experiment, L1: High light level, L2: Intermediate light level, L3: Low light level. Between groups c: Cryptophyceae, x: Chrysophyceae, d: Bacillariophyceae, p: Dinophyceae, v: Chlorophyceae, b: Cyanophyceae.



## CHAPTER IV: DISCUSSION - CONCLUSIONS

Although summer cyanobacterial blooms have been reported in the Missisquoi Bay from 1998 to 2006, in 2007 this was unfortunately not the case. The only advisory warning announcement of a cyanobacterial bloom was given at the beginning of September 2007, due to a slight increase in the abundance of Cyanobacteria. Yet on the basis of data from our general limnological characterization of the lake, there was no clear change in limnological parameters for the lake in 2007 that we could use to explain the absence of a cyanobacterial bloom (Figure 3, Table 1).

Our experiments tried to assess the effect of dissolved organic matter on the growth of Cyanobacterial species. Despite the unfortunate absence of a bloom in the summer of 2007, this work could evaluate the effect of dissolved organic matter addition under different factors (light, nutrient status, form of DOM added) on lake phytoplankton communities and in increases on emerging communities of Cyanobacteria in late summer (September 2007 - 3rd experiment).

Nutrient and light influence were evaluated as important factors controlling the effect of DOM on phytoplankton growth (Table 9). It was difficult to attribute changes in the concentrations of nutrients to fluctuations in populations of one particular group. In general, there was decrease in the concentration of all nutrients for all the treatments (especially for ammonium, nitrate and total dissolved phosphorus) as result of a high consumption rate that indicates strong nutrient limitation of the phytoplankton in the incubation experiments, especially for experiments 2 and 3 (July and September 2007). Strong light limitation for experiments was demonstrated by significant increase of chlorophyll a for the treatments at the highest light level (Figures 6, 7 and 8). High nutrient consumption at low light levels suggested predominant heterotrophic uptake on experiments by increased bacterial development or direct heterotrophic uptake from algae, especially when Dinophyceae was predominant as a group (Stoecker 1999; Tuchman et al. 2006).

Nutrient limitation for algal species was important for the experiments. As suggested by Klug and coworkers (Klug et al. 2001; Klug 2005) when algae and bacteria are competing directly for availability of nutrients, there is a strong influence on the community structure of original algae populations and light intensity for the determination of the group of organisms that predominate.

Chlorophyceae, Bacillariophyceae and Dinophyceae constitute the biggest part of the photosynthetically active biomass for the experiments. There was an increase in Chlorophyceae, Bacillariophyceae and Cyanophyceae with addition of nutrients, and an increase in the proportion of Dinophyceae with the addition of barley and organic matter particularly from the experiment made in September at high light intensities. Proportions of Bacillariophyceae were strongly related with low concentrations of organic matter. It appears that organic matter supplements allowed the dinoflagellates to more strongly outcompete the diatoms.

For experiments 1 and 2 small populations of Chlorophyceae originally present in the samples took advantage at high light levels and dominated, followed in proportion by Bacillariophyceae (Figure 9, Figure 10). This is consistent with the idea that the chlorophytes are superior competitors under eutrophic conditions in high light (shallow) environments, by virtue of their superior growth rates. In experiment 2, there was an important increase in the proportions of Dinophyceae when we added barley, organic matter and organic matter and nutrients for the lowest light intensity (Figure 10); probably higher heterotrophic consumption of increased nutrients by bacteria, provides them an additional food source as mixotrophs (Stoecker 1999) (Figure 10). Communities in experiment 3 were not strongly light limited. The increase of diatoms populations with the addition of nutrients suggested pure nutrient limitation of phytoplankton community biomass.

The use of resin XAD-8 was adequate to increase significantly the concentration of DOM, DOC and total phenolic compounds for the corresponding treatments in the incubations experiment (Figure 4). The natural concentration of total phenolic compounds for the bay

were already quite high, relative to a group of lakes in other countries (Box 1983; Hilt et al. 2006).

Concentrated dissolved organic matter was taken in June for the experiment made in July (experiment 2) and in August for the experiment made in September (experiment 3), from natural incoming water to Lake Champlain from Pike River. There was an important difference between results concerning dissolved organic matter characteristics and responses in treatments for experiment 2 and experiment 3. As suggested by Geller (1985) and Granéli et al. (1999), responses to such concentrated dissolved matter can be related with seasonal changes in watershed input and process, indicating differences in the quality of dissolved organic matter entering to the lake. The presence of easily photodegradable compounds in the concentration of dissolved organic matter was confirmed in all the experiments (Figure 4). For the second experiment in the early summer, dissolved organic matter had a biggest proportion of easily photodegradable low molecular weight compounds, perhaps from vegetal material rich in lignins, reflected in absorbance parameters (Annex 2). For the third experiment in the late summer the largest proportion of dissolved organic matter concentrated from Pike River was heavier compounds that might have come from runoff of agricultural activities developed in summer (Figure 14, Figure 15; Annex 2).

Regarding effect of DOM on phytoplankton communities (Table 9), increased light intensity could promote the release of low molecular compounds that can be related with the decrease of autotrophic algal populations predominant in experiment 2 and cyanobacterial populations in experiment 3. Also photodegradation of nutrients can improve bacterial growth that can directly compete with algae and reduce it abundance; as we didn't measure bacterial production we couldn't make an affirmation from the last statement. We recommend taking measures of bacterial growth that might clarify whether changes in the predominance of species are due to heterotrophic or mixotrophic ability of some algal groups and competition for resources among species, or direct interaction of compounds released by degradation of dissolved organic matter or barley in the growth of some algal species.

Cyanophyceae was an important group only in the third experiment, in September 2007 (Figure 11). There was a significant effect on the decrease of final chlorophyll *a* concentration with the addition of barley and organic matter for experiments 2 and 3, but the effect was only present in the interaction with nutrients (Table 3, 4). Proportions of Cyanophyceae were positively related to low concentrations of total phenolic compounds and dissolved organic matter. The effect was strongest for barley at the highest light levels for experiment 3, and for organic matter at low light level for experiment 2.

Growth of *Microcystis* was strongly nutrient limited (Figure 12). With the addition of barley and organic matter there was a decrease in the final biomass of *Microcystis* for the experiments especially for the intermediate light level (Figure 13) and high light level (Table 7, Figure 12). There is evidence than at highest light levels, the release of phenolic compounds that can inhibit growth of phytoplankton is strongest (Pillinger et al. 1994), and it can be one of the reasons for the significant effect of the addition of organic matter at high light intensities. Organic matter and barley both decreased the positive effect of nutrients in the growth of cyanobacterial species (Table 7). Negative effect on growth was statistically significant with barley addition (Table 8).

In conclusion, phytoplankton growth was strongly dependent on the availability of nutrients in Missisquoi Bay in 2007. The results of these experiments suggest that total biomass was limited in the lake in summer 2007 by a lack of nutrient recycling in the lake. Significant variation in taxonomical composition of the samples based in capacity of groups as Cyanophyceae and Chlorophyceae to increase growth with nutrients at high light intensities was presented for incubation experiments. The heterotrophic and mixotrophic ability of groups such as Dinophyceae and Bacillariophyceae allowed them to increase growth at low light levels, improved by the probable augmentation of bacteria (used as nutrition source) with the addition of dissolved organic matter. Probable release of photochemical degradation products (as total phenolic compounds) after the addition of barley and dissolved organic matter in the experiments (as demonstrated by spectrophotometric changes (Annex 2) inhibited the growth of phytoplankton, particularly Cyanobacterial species as *Microcystis*. The decreased growth was not sufficient to eliminate these toxic species, however. In

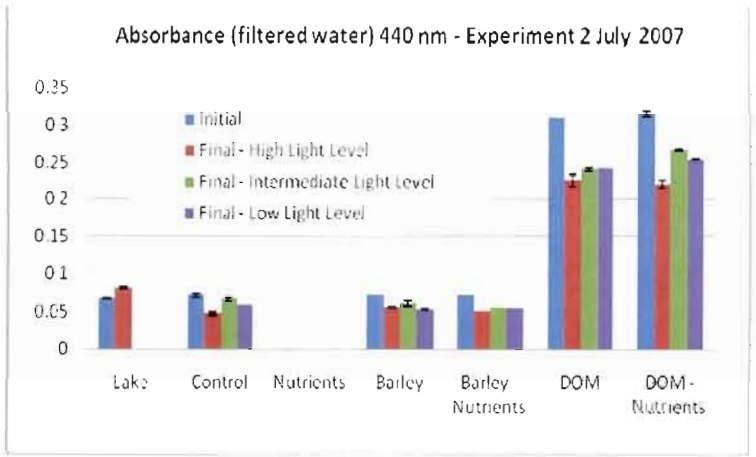
conclusion, it has been shown that dissolved organic carbon compounds both naturally ccurring and those added with barley extract, have the capacity to strongly negatively affect cyanobacterial growth rates, and to positively affect certain competing groups. The inhibitory effect is intimately related to light levels, and therefore the effect will be useful in control situations only when the right light conditions can be assured.

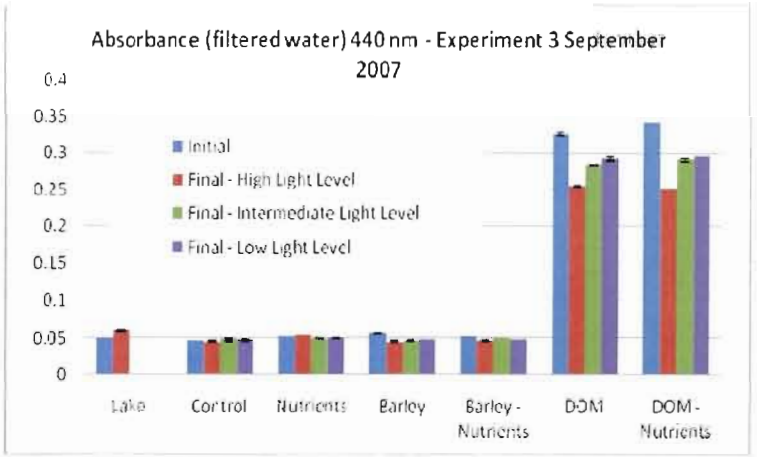
## ANNEXES

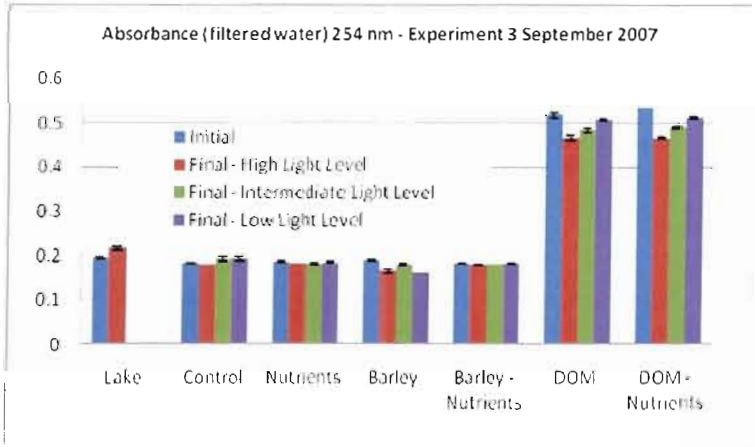
**Annex 1.** ANOVA of parameters related with characteristics and composition of DOM for the incubations.

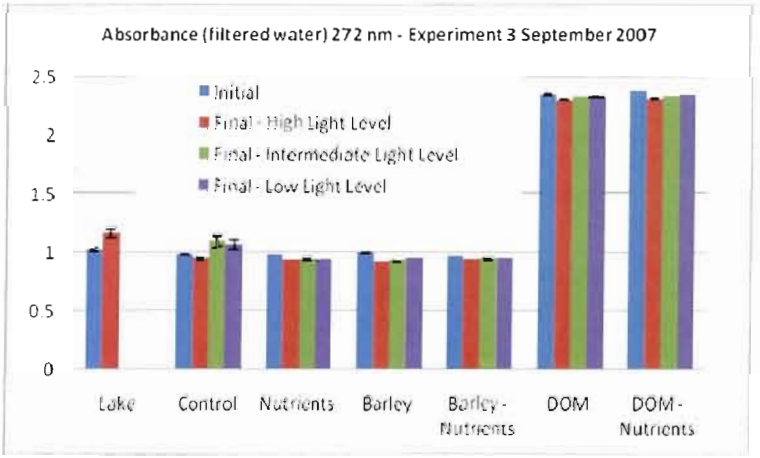
| | TN (mg/L) | | | TDN (mg/L) | | $NO_3$ (mg/L) | | | Ammonium (mg/L) | | | TP (mg/L) | | | TPD (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 |
| RSquare | 1.00 | 1.00 | 1.00 | 0.97 | 0.99 | 0.91 | 1.00 | 0.58 | 0.42 | 0.93 | 0.99 | 0.93 | 0.99 | 1.00 | 0.82 | 0.88 | 0.63 |
| RSquare Adj | 0.99 | 0.99 | 1.00 | 0.94 | 0.97 | 0.81 | 0.99 | 0.19 | -0.21 | 0.86 | 0.98 | 0.85 | 0.98 | 0.99 | 0.60 | 0.77 | 0.27 |
| Root Mean Square Error | 0.06 | 0.06 | 0.02 | 0.11 | 0.03 | 0.26 | 0.05 | 0.00 | 0.05 | 0.03 | 0.02 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Mean of Response | 1.98 | 1.49 | 0.93 | 1.05 | 0.55 | 1.07 | 0.66 | 0.00 | 0.13 | 0.09 | 0.15 | 0.09 | 0.07 | 0.08 | 0.03 | 0.01 | 0.02 |
| Observations (or Sum Wgts) | 22.00 | 36.00 | 36.00 | 36.00 | 34.00 | 22.00 | 36.00 | 36.00 | 22.00 | 35.00 | 36.00 | 22.00 | 36.00 | 36.00 | 21.00 | 36.00 | 35.00 |
| | | | | | | | | | | | | | | | | | |
| Light Level | | 0.01 | 0.05 | 0.00 | | | <.0001 | | | 0.00 | | | | 0.04 | | 0.00 | |
| Nutrients | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.02 | | 0.00 | <.0001 | <.0001 | <.0001 | <.0001 | 0.00 | 0.00 | |
| Light Level*Nutrients | | | | 0.01 | | | <.0001 | | | 0.02 | | | | | | 0.00 | |
| Barley | | | | | | | 0.02 | | | <.0001 | | | | | | | |
| Light Level*Barley | | | | | | | 0.04 | | | 0.00 | | | | 0.05 | | 0.01 | |
| Nutrients*Barley | | | | | | | 0.05 | | | | | | | 0.04 | | | |
| Light Level*Nutrients *Barley | | | | 0.03 | | | 0.01 | | | | | | | | | | |
| OM | | <.0001 | <.0001 | 0.02 | <.0001 | | | | | 0.01 | 0.00 | | 0.00 | <.0001 | | | 0.03 |
| Light Level*OM | | | 0.00 | | | | 0.00 | | | <.0001 | 0.04 | | | 0.00 | | 0.01 | |
| Nutrients*OM | | | 0.06 | | | | | | | 0.01 | 0.00 | | 0.04 | | | | |
| Light Level*Nutrients*OM | | | | 0.04 | | | 0.01 | | | 0.01 | 0.04 | | | | | | |

**Annex 2.** Parameters related to dissolved organic matter characterization

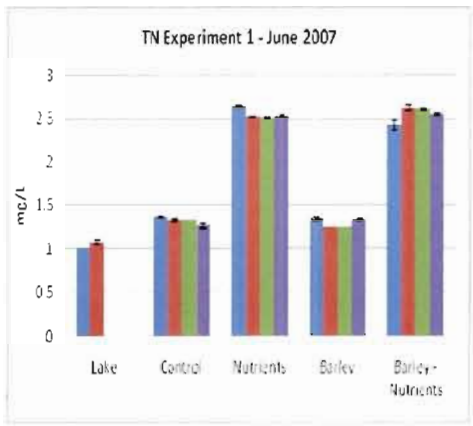


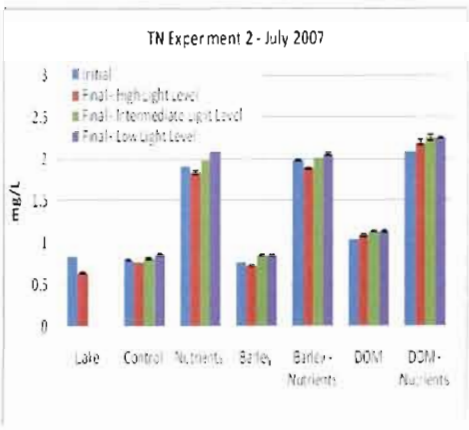



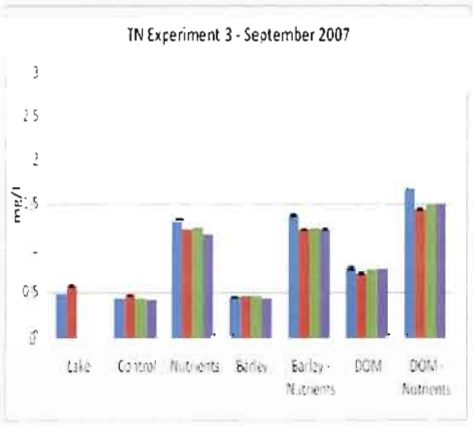



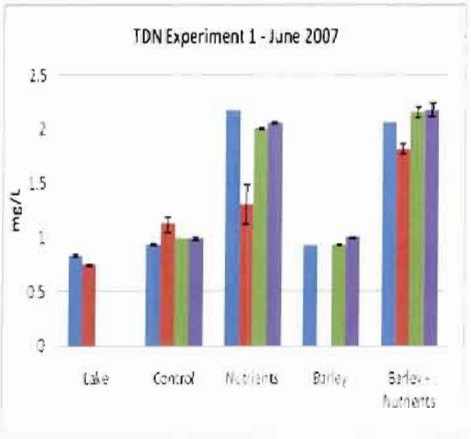



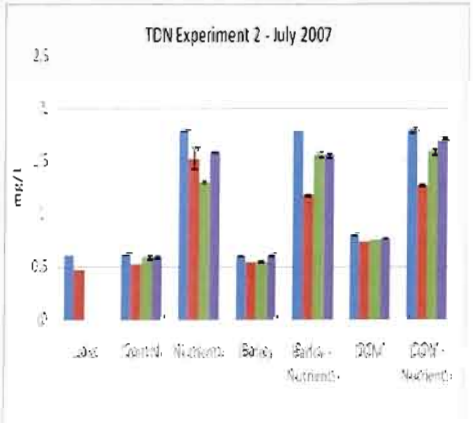
**Annex 3.** Concentration of nutrients for the incubation experiments

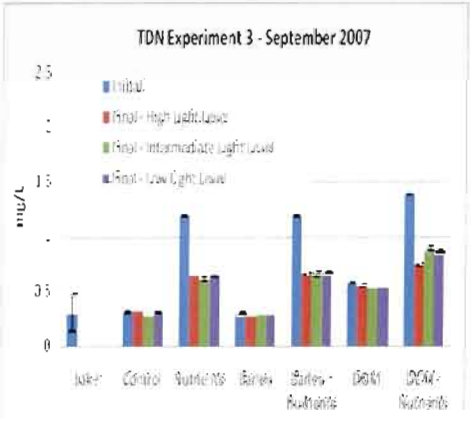


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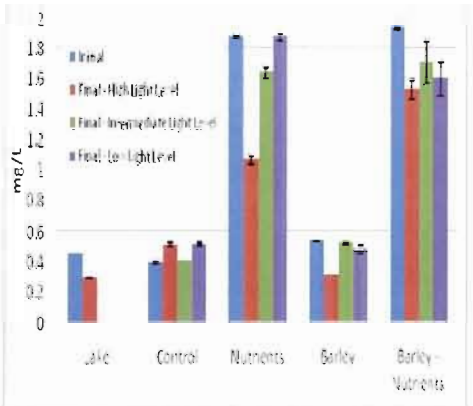


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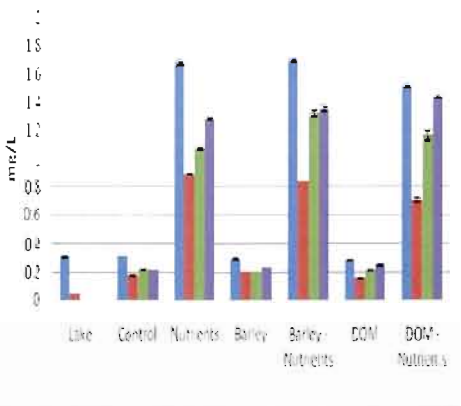


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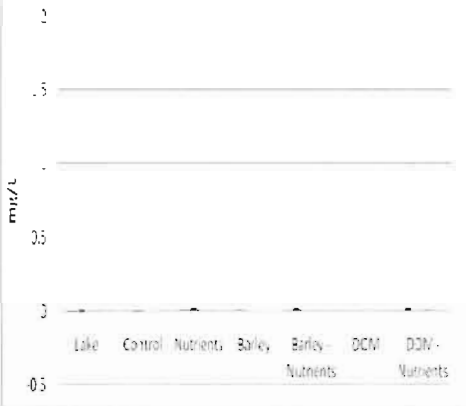


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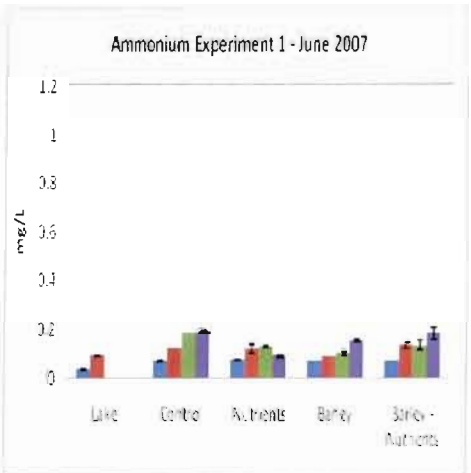


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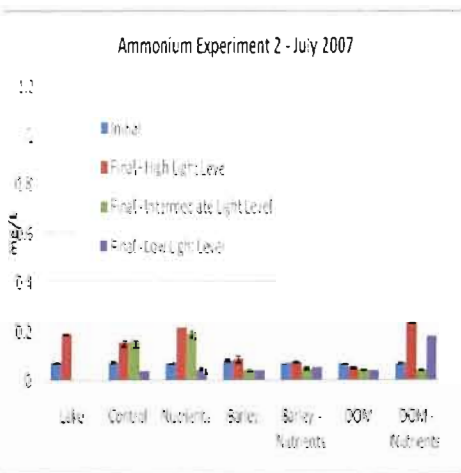


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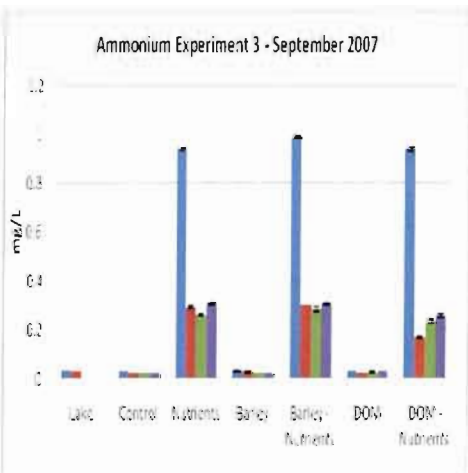
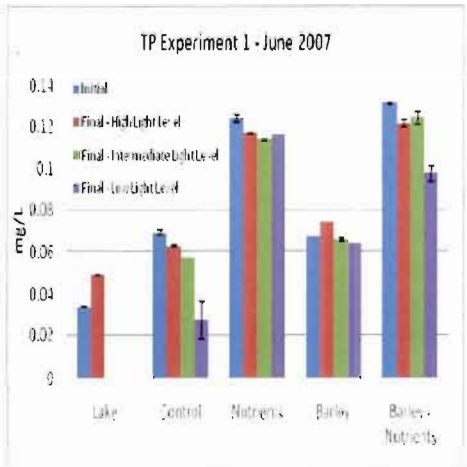
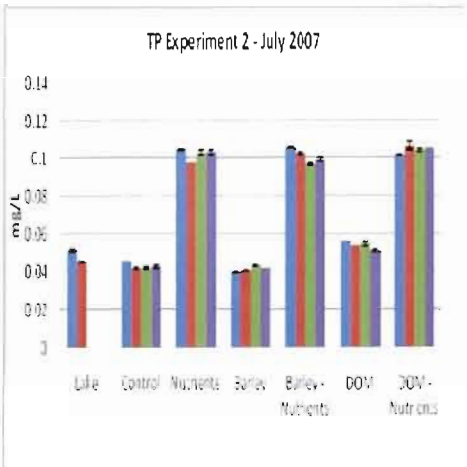
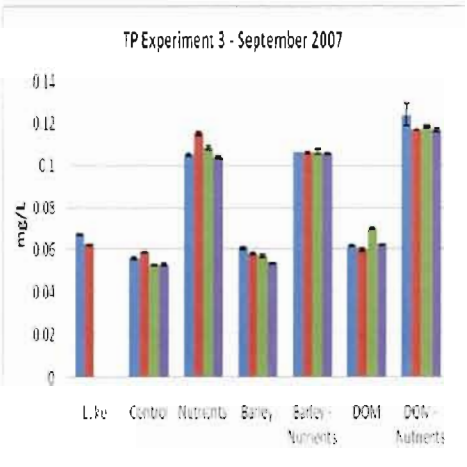
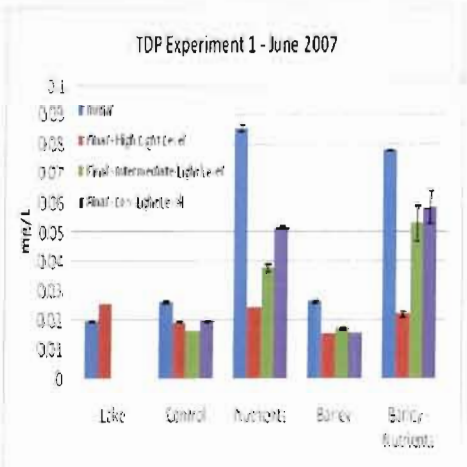
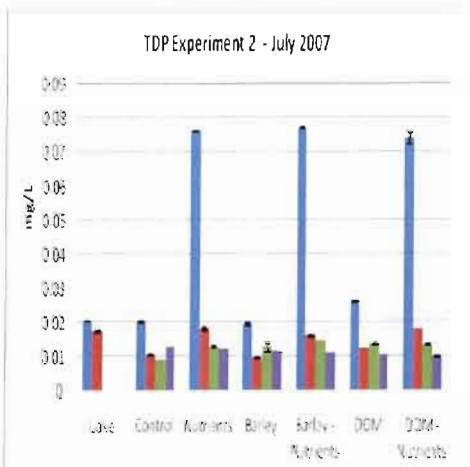


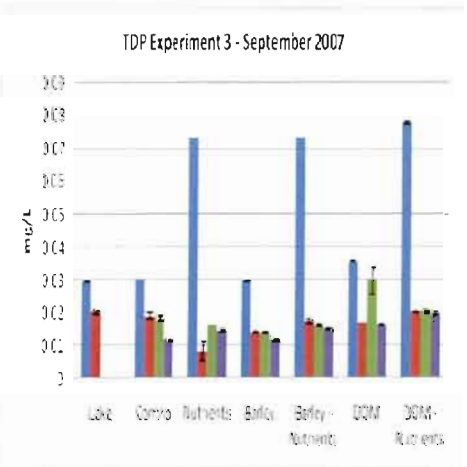

TP Experiment 1 - June 2007
mg/L
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Initial
Final - High Light Level
Final - Intermediate Light Level
Final - Low Light Level
Lake
Control
Nutrients
Barley
Barley - Nutrients


TP Experiment 2 - July 2007
mg/L
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Lake
Control
Nutrients
Barley
Barley - Nutrients
DOM
DOM - Nutrients


TP Experiment 3 - September 2007
mg/L
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Lake
Control
Nutrients
Barley
Barley - Nutrients
DOM
DOM - Nutrients


TDP Experiment 1 - June 2007
mg/L
Initial
Final - High Light Level
Final - Intermediate Light Level
Final - Low Light Level
Lake
Control
Nutrients
Barley
Barley - Nutrients


TDP Experiment 2 - July 2007
mg/L
Lake
Control
Nutrients
Barley
Barley - Nutrients
DOM
DOM - Nutrients


TDP Experiment 3 - September 2007
mg/L
Lake
Control
Nutrients
Barley
Barley - Nutrients
DOM
DOM - Nutrients

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