DEPENDENCIES OF FOOD WEB AND NUTRIENT CYCLING DYNAMICS ON DISSOLVED ORGANIC MATTER (DOM) AND INORGANIC NUTRIENT CONCENTRATIONS IN LAKE ENCLOSURES

by

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Abstract

An autotrophic–allotrophic gradient was established in 12 lake enclosures across a natural DOM concentration gradient. Phytoplankton were co-regulated by solar irradiance and inorganic nutrient concentrations, whereas bacterioplankton were strongly dependent on DOM in the reference enclosures. Nutrient scavenging in the reference enclosures was limited by efficient biotic incorporation and recycling, across the full DOM gradient. Nutrient enrichment stimulated a strong autotrophic response across the autotrophic-allotrophic gradient due to increased phytoplankton productivity. Bacterioplankton productivity was still strongly dependent on DOM, but bacterioplankton productivity also increased either as a direct or indirect result of nutrient enrichment. Carbon, nitrogen, and phosphorus were effectively scavenged from the water column by incorporation into biomass at high rates and then deposited in the sediments in the nutrient enriched enclosures, producing nutrient-rich sediments. The data further suggest that at DOM concentrations greater than 14 mg L^{-1}, allotrophy would dominate regardless of inorganic nutrient enrichment.

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1. Literature Review

1.1. General Introduction

Food web and nutrient cycling dynamics are an integral part of lake ecosystem function (Elser et. al. 1998; Elser and Foster 1998; Pace and Cole 2000; Elser et. al. 2000c). The concept of energy and nutrient transfers between biotic and abiotic components in lakes is important in understanding lake ecology (Sterner et. al. 1992; DeAngelis 1992; Elser et. al. 1995). Energy and nutrients are supplied to a lake ecosystem from the terrestrial environment, atmospheric deposition, and internally through transformation processes. The major energy and nutrient elements involved in biogeochemical cycling in lakes are carbon, nitrogen, and phosphorus. Carbon, nitrogen, and phosphorus are important primarily because of their importance in cell growth and metabolism (Redfield 1958; Elser et. al. 2000a; Sterner and Elser 2002). Therefore, food web dynamics influence nutrient cycling dynamics in lakes by affecting the proportion of energy and nutrients in each pool as well as the flux of energy and nutrients among pools (Stumm and Morgen 1995; Elser et. al. 1998; Elser et. al. 2000a; Elser et. al. 2000c; Hessen et. al. 2003). These pools include the dissolved and particulate phases of nutrients and nutrients deposited in the sediments.

The flux of carbon, nitrogen, and phosphorus between the dissolved and particulate phases and between inorganic and organic pools is dependent on the element ratios of the organisms and their environment as well as metabolic processes (Elser et. al. 1995; Elser et. al. 1998; Elser et. al. 2000c; Hessen et. al. 2003). Element ratios of an organism and its environment can help address the fate and flux of carbon, nitrogen, and phosphorus among pools in lake

ecosystems (Elser et. al. 1995; Elser et. al. 1998). The fate of nutrients includes reincorporation into biomass, remineralization into the dissolved inorganic pool, or settling out of the water column to form sediments (Santschi 1988).

Element ratio requirements of trophic levels as well as the primary metabolism driving lake productivity can affect biogeochemical cycling of energy and nutrients in lake ecosystems (Sterner et. al. 1998; Elser et. al. 1998; Elser et. al. 2000c; Elser et. al. 2003). Phytoplankton have flexible stoichiometric requirements, which often reflect the element ratios of its surrounding environment (Sterner et. al. 1997; Elser et. al. 2000b). Bacterioplankton are less flexible than phytoplankton and have higher nutrient requirements (Vadstein et. al. 1988; Makino et. al. 2003). Higher trophic levels, such as zooplankton, have rigid stoichiometric requirements and high nutrient requirements (Elser et. al. 2000b), but graze on phytoplankton with variable, and sometimes nutrient-poor, stoichiometry (Sterner et. al. 1992; Sterner et. al. 1998; Elser et. al. 2000c).

The majority of research on lake ecosystem function has been conducted on autotrophic lakes (Rich and Wetzel 1978; Currie 1990; Kirchman 1994). However, a trophic discontinuity appears to exist between autotrophic and allotrophic lakes. The differences in food web drivers (e.g. solar radiation and dissolved organic matter) between these lakes can result in different nutrient cycling dynamics that reflect the primary metabolism of the lake (Jones 1992; del Giorgio and Peters 1994; Jansson 1998; Jones 1998).

Autotrophic lakes are categorized primarily based on increasing inorganic nutrient concentrations and increasing primary productivity, and they are classified as either oligotrophic, mesotrophic, or eutrophic lakes (Fig. 1). Oligotrophic lakes generally have low inorganic nutrient concentrations and low primary productivity, whereas eutrophic lakes have high inorganic nutrient concentrations and high primary productivity. Autotrophic systems rely on phytoplankton production to mobilize energy to higher trophic levels (Birge and Juday 1927; Vadstein et. al. 1989; Currie 1990; Hessen 1992; Cole et. al. 2000).

Allotrophic or dystrophic lakes do not fit within this continuum as they are characterized by a high proportion of dissolved organic matter (DOM) derived from terrestrial sources (allochthonous), moderate to high nutrients concentrations, and low primary productivity relative to the total inorganic nutrient concentrations (Birge and Juday 1927; Jackson and Hecky 1980; Meili 1992; del Giorgio and Peters 1994; Jansson et. al. 1996). Bacterioplankton are the primary drivers of allotrophic food webs, and they are important in mobilizing organically bound carbon to higher trophic levels (Tranvik 1989; Jones 1992; Hessen 1998; Tranvik 1998). Therefore, the differences between autotrophic and allotrophic lakes are the way nutrients are utilized and the primary microbial metabolism that drives the system (Fig. 1.2) (Jansson 1998; Jansson et. al. 2000; Hakanson and Jansson 2002).

Current research indicates that the majority of lakes worldwide are allotrophic, driven by heterotrophic metabolism based on allochthonous organic matter (Kortelainen 1993; Cole et. al. 1994). It has also been proposed that only extremely oligotrophic and extremely eutrophic lakes are truly autotrophic systems (Baron et. al. 1991; del Giorgio and Peters

1993; del Giorgio and Peters 1994; Schindler et. al. 1997; del Giorgio et. al. 1999; Cole et. al. 2000; Jansson et. al. 2000). While allotrophic lakes may dominate our landscape the biogeochemical processes driving these systems are far less understood than autotrophic lakes.

The primary factors that appear to regulate lake metabolism and separate autotrophic lakes from allotrophic lakes are natural dissolved organic matter loading, the availability of solar irradiance, and inorganic nutrient concentrations (Jones 1992; Tranvik 1998). Allochthonous DOM provides bacterioplankton with a source of carbon independent of phytoplankton and a source of nutrients in allotrophic systems (Currie 1990; Jones 1992; Hessen 1992; Arvola et. al. 1996; Arvola and Tulonen 1998). Therefore, natural allochthonous DOM can stimulate bacterioplankton growth resulting in high bacterioplankton abundance and productivity in allotrophic systems (Tranvik 1989; Hessen et. al. 1994; Jansson et. al. 1999). Furthermore, allochthonous DOM has the ability to attenuate solar irradiance and to complex nutrients (Francko and Heath 1982; de Haan et. al. 1990; Shaw 1994; Scully and Lean 1994; Morris et. al. 1995). These properties of DOM can create light and nutrient limiting conditions for phytoplankton (Jackson and Hecky 1980; Jones 1998; Carpenter et. al. 1998). Thus, allotrophic and autotrophic lakes may respond differently to allochthonous DOM and nutrient loading and the availability of solar irradiance.

1.1.1. Objectives

This research investigated the food web and nutrient cycling dynamics in systems with variable natural DOM concentrations. These food web and nutrient cycling dynamic dependences were investigated using a 2 x 6 enclosure design set up in the Experimental

Lakes Area (ELA) in northwestern Ontario. A gradient of natural DOM concentration (measured analytically as dissolved organic carbon, DOC), potentially representing an autotrophic-allotrophic gradient, was established in two replicate sets of six enclosures. One set acted as a reference, and the other set was enriched with inorganic nutrients to stimulate an autotrophic response across the autotrophic-allotrophic gradient.

This research is presented as two experimental chapters. Chapter 2 investigated the dependencies of phytoplankton and bacterioplankton on DOC and inorganic nutrient concentration. The hypotheses tested in Chapter 2 were: 1. increasing DOC concentration will shift the pelagic microbial community from autotrophic to allotrophic; and 2. nutrient enrichment will prevent or dampen the shift of the pelagic microbial community from autotrophic to allotrophic.

Chapter 3 investigated the DOM dependence of nutrient scavenging from the water column and the DOM dependence of the element ratios of the sediments formed in the lake enclosures. The null hypotheses tested in Chapter 3 were: 1. scavenging of carbon, nitrogen, and phosphorus from the water column is independent of DOC concentration; 2. scavenging of carbon, nitrogen, and phosphorus from the water column is independent of nutrient enrichment; 3. element sediment ratios are independent of DOC concentration; and 4. element sediment ratios are independent of nutrient enrichment.

1.2. DOM

1.2.1. DOM Sources

Natural dissolved organic matter (DOM) originates from two sources: autochthonous and allochthonous (McKnight and Aiken 1998; Wetzel 2001). Autochthonous organic matter is derived within lake systems from the by-products of photosynthesis, microbial activity, leaching from macrophytes, and microbial and plant cell lysis (Lampert 1978; Meili 1992; Wetzel 1995). Autochthonous organic matter is less coloured than allochthonous organic matter and typically exists in smaller concentrations in lake systems (Meili 1992; Münster and de Haan 1998; Curtis 1998). This DOM is usually less absorbent and fluorescent, and it is also considered biologically labile, or readily usable by microbes (McKnight et. al. 1994; Tranvik 1998). Autochthonous DOM has a C:N element ratio of approximately 14:1 and, therefore, it is a nutrient-rich substrate for bacterioplankton (Wetzel 2001).

Allochthonous DOM is derived from decomposed plant material in the terrestrial environment (Fukushima et. al. 1996; McKnight and Aiken 1998), and it is transported to lake ecosystems via hydrologic pathways (Schindler et. al. 1997; Curtis 1998). Allochthonous DOM is transformed by soil microbes as it is transported through a watershed to a lake ecosystem, and as a result this DOM is generally considered recalcitrant, or resistant to microbial degradation (Singer and Munns 1987; Manahan 1994). Approximately 70 to 80% of allochthonous DOM consists of recalcitrant humic substances (Wetzel et. al. 1995), which strongly absorb light, reducing the depth of the photosynthetic zone as DOM concentration increases (McKnight and Aiken 1998). Allochthonous DOM is nutrient-poor

with a C:N element ratio of approximately 58:1 and, therefore, it is a poor organic substrate for bacterioplankton (Wetzel 1995; Wetzel 2001).

The recalcitrant allochthonous DOM results in lower assimilation rates, reduced incorporation efficiency, and greater respiration rates by bacterioplankton (Hope et. al. 1996; del Giorgio and Cole 1998). However, allochthonous DOM is a stable source of carbon and nutrients for bacterioplankton. Allochthonous DOM can support up to 90% of bacterioplankton productivity in allotrophic lakes, and it can result in 2 to 4 times greater bacterioplankton biomass and productivity in allotrophic lakes than autotrophic lakes (Tranvik 1989; Jansson et. al. 1996; Jansson et. al. 1999).

Climate, hydrology of the watershed, catchment characteristics, and vegetation types can influence the quantity and quality of allochthonous DOM entering a lake (Rasmussen et. al. 1989; Curtis 1998). Significant alterations to the hydrology of the watershed due to forest harvesting, fires, flooding or other factors may influence the quantity and quality of DOM and the amount of nutrients reaching lake systems (Jackson and Hecky 1980; Meyer and Tate 1983; Guildford et. al. 1987; Christensen et. al. 1996).

Global climate change may seriously affect allochthonous DOM loading. Currently there is a concern that precipitation and natural DOM concentrations are decreasing in temperate North American lakes (Schindler et. al. 1996; Schindler et. al. 1997; Schindler and Curtis 1997). The opposite trend is occurring in Scandinavia (Forsberg 1992). In addition to the increasing

DOM loading, nitrogen and phosphorus loading are also increasing in Scandinavia (Hessen et. al. 1994). Changes in DOM and nutrient loading to lake systems will affect the underwater light regime, nutrient availability, and ultimately the food web drivers and nutrient cycling dynamics of both allotrophic and autotrophic lakes.

Most temperate North American lakes have DOM concentrations ranging from 2 to 8 mg L^{-1}, and they are primarily composed of allochthonous DOM (Meili 1992; Kortelainen 1993; Lean 1998). Autochthonous DOM often dominates lakes associated with watersheds with low terrestrial biomass, such as alpine lakes. These lakes can have DOM concentrations < 3 mg L^{-1} (Baron et. al. 1991; Sommaruga et. al. 1999). In contrast, allotrophic lakes have DOM concentrations ranging from 5 to 30 mg L^{-1} and are associated with high absorbance and low transparency (Hessen 1998; Lean 1998). Regardless of the source, no more than 20% of the total natural DOM pool in lakes is ever biologically labile, but this fraction is continually replenished from the refractory portion by photolysis and enzymatic hydrolysis (Tranvik 1988; Münster et. al. 1992; Kroer 1993; Bushaw et. al. 1996; Tranvik 1998).

1.2.2. DOM Properties

Natural allochthonous DOM has the ability to attenuate solar irradiance and to complex nutrients, which creates an environment in allotrophic lakes that is structurally and functionally different from autotrophic lakes (Salonen et. al. 1992; Jones 1992; Hessen 1998; Jansson 1998; Jones 1998). The ability to attenuate solar irradiance can result in lower phytoplankton photosynthesis, but photolytic by-products can stimulate bacterioplankton productivity. Furthermore, DOM bound nutrients can result in nutrient limiting conditions

for phytoplankton, but can act as a source of nutrients to bacterioplankton through decomposition of DOM by bacterioplankton (Blomqvist et. al. 2001; Klug 2002).

1.2.2.1. Light Attenuation

Natural allochthonous DOM attenuates solar irradiance in surface waters, and in moderate concentrations DOM can protect organisms from harmful UV radiation (Scully and Lean 1994). Absorbance, a measure of solar irradiance attenuation, increases as a function of increasing DOM concentration (Morris et. al. 1995; Bukaveckas and Robbins-Forbes 2000). Researchers have shown that natural DOM concentrations can explain 85 to 92% of the among-lake variation in UV absorbance values, indicating a close relationship between DOM concentration, lake water absorbance, and transparency (Morris et. al. 1995; Fee et. al. 1996). Suspended particles are minor factors in solar irradiance attenuation in natural lake systems as compared to oceans, but suspended particles become increasingly more important in eutrified lake systems that typically support higher phytoplankton biomass (Fee et. al. 1996).

Availability of solar irradiance directly affects photosynthetic processes by phytoplankton. Photosynthesis increases as a function of increasing irradiances at low levels, but at moderate to high levels phytoplankton become light inhibited and primary productivity decreases (Long et. al. 1994; Lampert and Sommer 1997). High irradiances in the water column of a lake often correspond to low allochthonous DOM loading and to high autochthonous DOM loading, where autochthonous DOM is usually transparent in the photosynthetically active wavelengths (Morris et. al. 1995).

The effective light attenuating properties of DOM can suppress phytoplankton productivity (Arvola et. al. 1996; Carpenter et. al. 1998; Jones 1998). Attenuation of visible light and UV radiation by high loading of natural allochthonous DOM concentrations occurs within the first few centimeters of the water column (Lean 1998). More specifically, DOM can successfully attenuate photosynthetically active radiation (PAR: 400-750nm), which is extremely important for photosynthesis. DOM concentrations can account for 85% of the variation observed in PAR and 90% in UV attenuation (Bukaveckas and Robbins-Forbes 2000). Therefore, primary productivity is often light limited in allotrophic lakes (Arvola et. al. 1996; Carpenter et. al. 1998).

The attenuating properties of natural allochthonous DOM affect bacterioplankton in lake systems. DOM is susceptible to photolysis, or the breakdown by UV radiation, which occurs concomitantly with solar irradiance attenuation (Strome and Miller 1978; Stewart and Wetzel 1981). Photolysis can increase the labile fraction of allochthonous DOM resulting in higher bacterioplankton biomass and productivity than observed in autotrophic lakes (Lindell et. al. 1995; Wetzel et. al. 1995; Münster and de Haan 1998; Tranvik et. al. 2000). As a result, bacterioplankton are often relieved of their dependence on phytoplankton for their carbon source due to the high allochthonous DOM concentrations present (Hessen 1998; Grover and Chrzanowski 2000; Blomqvist et. al. 2001).

1.2.2.2. Nutrient Complexation

Natural allochthonous DOM complexes inorganic nutrients, therefore, reducing the inorganic nutrient concentrations available to the pelagic microbial community (Jackson and Hecky 1980; de Haan et. al. 1990; Jones 1992; Shaw 1994). Nitrogen and phosphorus are often

transported from watersheds to lakes bound to allochthonous DOM, therefore, systems dominated by allochthonous DOM loading may have lower biologically available inorganic nutrient concentrations than autotrophic lakes (Jansson 1998; Wetzel 2001). DOM can also complex nutrients in the water column, removing nitrogen and phosphorus from the available inorganic nutrient pool (de Haan et. al. 1990; Shaw 1994).

Phytoplankton productivity in high DOM systems is lower than that predicted from the high nutrient concentrations (Jackson and Hecky 1980; Meili 1992). Complexation by allochthonous DOM removes nitrogen and phosphorus from the available inorganic nutrient pool creating nutrient limiting conditions for phytoplankton (Jackson and Hecky 1980). Some phytoplankton, such as phagotrophic phytoflagellates, may assimilate DOM directly or consume bacterioplankton to obtain the necessary nutrients for productivity in allotrophic lakes as an adaptive strategy and, therefore, may dominate the phytoplankton community (Sanders and Porter 1988; Porter 1988; Isaksson et. al. 1999).

Bacterioplankton productivity is potentially limited by nutrients bound to DOM because of the recalcitrant nature of allochthonous DOM. However, DOM degradation by photolysis and bacterioplankton enzymatic hydrolysis are the two processes that can release nutrients from their complexed state. Photolytic processes are shown to release labile nitrogen- and phosphorus-rich organic substances from DOM (Francko and Heath 1982; Bushaw et. al. 1996; Kieber et. al. 1999; Tranvik et. al. 2000). These nutrient-rich substrates are rapidly assimilated by bacterioplankton to drive productivity. Bacterioplankton decomposition of allochthonous DOM by enzymatic hydrolysis is slow, but it can still help support

bacterioplankton nutritional requirements (Münster et. al. 1992; Wetzel 1995). Therefore, high allochthonous DOM concentrations are an important nutrient source for bacterioplankton in allotrophic lakes (de Haan et. al. 1990; Hessen et. al. 1994; Arvola and Tulonen 1998).

1.3. Pelagic Microbial Community

The pelagic microbial community includes but is not restricted to phytoplankton and bacterioplankton that exist within the water column of lakes, and they comprise a portion of the seston. Pelagic microbial productivity is defined by two trophic gradients driven by separate energy sources. Microbial productivity along the autotrophic gradient is driven by solar energy, whereas microbial productivity along the allotrophic gradient is driven by allochthonous organic matter (Jones 1992). As a result of these differences in microbial drivers and productivity, a trophic discontinuity appears to exists between autotrophic and allotrophic lakes.

The trophic discontinuity is the result of differences in food web drivers and nutrient cycling dynamics in autotrophic and allotrophic lakes (Jones 1992; del Giorgio and Peters 1994; Jansson et. al. 2000). Increasing inorganic nutrient concentrations and increasing phytoplankton biomass and productivity characterize the autotrophic gradient. In contrast, increasing allochthonous organic matter and increasing bacterioplankton biomass and productivity characterize the allotrophic gradient (Cole et. al. 2000). Therefore, autotrophic and allotrophic lakes differ in nutrient utilization, the primary microbial metabolism that

drives food web dynamics, and in the flux of energy and nutrients (Jansson 1998; Hakanson and Jansson 2002).

1.3.1. Phytoplankton

The microbial community of autotrophic lakes is dominated by phytoplankton that mobilize carbon to higher trophic levels. As primary producers in these systems, phytoplankton utilize light energy to convert inorganic carbon to biomass through photosynthesis. Inorganic carbon sources used by phytoplankton include carbon dioxide and bicarbonate. High rates of photosynthesis can result in the sequestering of carbon dioxide directly from the atmosphere, making the lake system a net sink for carbon dioxide (Schindler 1977; Stumm and Morgen 1995; Cole 1999; Hanson et. al. 2003).

In autotrophic lakes, dissolved inorganic nitrogen and phosphorus are used directly by phytoplankton to aid metabolism. High concentrations of inorganic nutrients can facilitate high photosynthetic rates, which can deplete the inorganic carbon pool in an aquatic system further increasing the need to sequester carbon from the atmosphere (Schindler 1977; Stumm and Morgen 1995; Wetzel 2001). However, phytoplankton are typically light or nutrient limited rather than carbon limited because of the large atmospheric source of inorganic carbon (Schindler 1977; Smith 1986; Sterner et. al. 1997).

Phytoplankton depend strongly on their environment for nutrients and can tolerate a wide range of solar irradiance and inorganic nutrient concentrations, which results in their flexible element ratios that reflect their environment (Sterner et. al. 1997; Frost and Elser 2002). For

example, phytoplankton C:N, C:P, and N:P ratios can range widely from 6 – 15, 8 – 1340, and 16 – 44, respectively (Downing and McCauley 1992; Coveney and Wetzel 1995; Hochstadter 2000; Mari et. al. 2001). The Redfield ratio (C:N = 6.6, C:P = 106, N:P = 16) represents the optimal element ratio for marine phytoplankton (Redfield 1958; Buffle and De Vitre 1994). Although freshwater phytoplankton ratios are typically more variable than marine phytoplankton (Hecky et. al. 1993; Elser and Hassett 1994; Sterner et. al. 1997; Elser et. al. 2000b), the Redfield ratio can serve as a useful comparison to lake phytoplankton or to seston ratios (Uehlinger and Bloesch 1987; Buffle and De Vitre 1994).

Phytoplankton productivity is strongly suppressed in allotrophic lakes (Jackson and Hecky 1980; Blomqvist et. al. 2001; Klug 2002; Drakare et. al. 2002), which is likely due to the strong light attenuating and nutrient complexation properties of allochthonous DOM (de Haan et. al. 1990; Jones 1992; Shaw 1994; Scully and Lean 1994; Morris et. al. 1995). The suppression of phytoplankton results in productivity levels that are insufficient to support the other trophic levels and, therefore, phytoplankton are not the base of the aquatic food web in allotrophic lakes (Scavia and Laird 1987; Jansson et. al. 1996).

Adaptive strategies by phytoplankton have been identified by researchers to minimize the effects of high allochthonous DOM loading in allotrophic lakes. Mobile phytoplankton can regulate their position within the water column to maximize solar irradiance availability for photosynthesis and, therefore, they dominate the phytoplankton community in allotrophic systems (Jones 1998). Furthermore, phagotrophic phytoflagellates can consume bacterioplankton and DOM directly and picophytoplankton, with high surface area to volume

ratios, can successfully compete with bacterioplankton for nutrients (Sanders and Porter 1988; Porter 1988; Nygaard and Tobiesen 1993; Jansson et. al. 1996; Isaksson et. al. 1999; Drakare et. al. 2003).

1.3.2. Bacterioplankton

The primary role of bacterioplankton in autotrophic lakes is decomposition of organic matter. As decomposers, bacterioplankton break down organic material and recycle nutrients back into the system. Dissolved and particulate organic matter are decomposed primarily by bacterioplankton in the water column making the nutrients bound to organic matter available to phytoplankton for primary production (Wetzel 1995). As a result, phytoplankton depend on bacterioplankton to remineralize organically bound nutrients (Azam et. al. 1983; Currie and Kalff 1984c).

Bacterioplankton are consumers of organic matter, and they are an important component of the microbial loop in autotrophic lakes (Azam et. al. 1983). Bacterioplankton are typically carbon limited and, therefore, they rely on phytoplankton for their organic carbon energy source (Chrzanowski and Hubbard 1989; Coveney and Wetzel 1995; Stumm and Morgen 1995). Bacterioplankton rapidly incorporate labile autochthonous organic matter into biomass, which is transferred to higher trophic levels via the microbial loop (Azam et. al. 1983; Wetzel 1995; del Giorgio and Cole 1998). Therefore, a close coupling between phytoplankton and bacterioplankton is often observed in autotrophic lakes (Søndergaard et. al. 1988; Chrzanowski and Hubbard 1989; Coveney and Wetzel 1995).

In contrast to autotrophic systems, bacterioplankton in allotrophic lakes are not coupled to phytoplankton (Jones and Salonen 1985; Hessen et. al. 1994; Jansson et. al. 1996; Jansson et. al. 1999). The large pool of allochthonous DOM in allotrophic lakes is a stable supply of carbon and nutrients for bacterioplankton metabolism, despite the recalcitrant state of this carbon source (Salonen et. al. 1983; de Haan et. al. 1990; Hessen 1992; Hessen et. al. 1994; Wetzel 1995; Arvola and Tulonen 1998). Therefore, bacterioplankton are the base of the aquatic food web in allotrophic lakes by utilizing allochthonous organic carbon and nutrients for biomass production (Tranvik 1989; Salonen et. al. 1992; Tranvik 1998; Grover and Chrzanowski 2000).

Bacterioplankton in allotrophic lakes serve as a major pathway for carbon and nutrients to higher trophic levels through grazing (Salonen and Jokinen 1988; Hessen 1992; Salonen et. al. 1992; Arvola et. al. 1996; Kankaala et. al. 1996; Jansson et. al. 1999; Blomqvist et. al. 2001). Carbon flows through heterotrophic nanoflagellates (protozoans) and phagotrophic phytoflagellates, the primary bacterivores, to higher trophic levels, such as zooplankton (Porter 1988; Salonen and Jokinen 1988; Sherr et. al. 1992; Isaksson et. al. 1999). Typically bacterioplankton are considered carbon limited in autotrophic systems, but this is unlikely in allotrophic systems with high rates of allochthonous DOM loading (Cotner and Wetzel 1992; Grover 2000). Furthermore, nutrient enrichment studies of allotrophic systems indicate that bacterioplankton are nutrient limited rather than carbon limited (Vadstein et. al. 1988; Morris and Lewis 1992; Morris and Lewis 1992; Hessen et. al. 1994; Chrzanowski et. al. 1995; Jansson et. al. 1996).

Bacterioplankton can outcompete phytoplankton for the low available nutrient concentrations in allotrophic systems (Currie and Kalff 1984a; Currie and Kalff 1984b; Currie and Kalff 1984c; Chrzanowski et. al. 1995; Vadstein 2000). Bacterioplankton have high metabolic demands for inorganic nutrient concentrations, and they can assimilate low nutrient concentrations more efficiently than phytoplankton when they are not carbon limited (Currie and Kalff 1984b; Vadstein et. al. 1988). These characteristics of bacterioplankton can contribute to the suppression of phytoplankton growth in allotrophic systems (Jansson et. al. 1996; Grover 2000; Blomqvist et. al. 2001; Joint et. al. 2002; Drakare et. al. 2002). Therefore, bacterioplankton abundance and productivity are generally high in allotrophic lakes (Arvola et. al. 1996; Arvola and Tulonen 1998).

Decomposition of allochthonous DOM by bacterioplankton enzymatic hydrolysis is slow, but is an important nutrient recycling process in allotrophic systems (Wetzel 1995; Münster and de Haan 1998). Bacterioplankton can immobilize nutrients during decomposition of DOM to meet their high metabolic demands rather than recycling nutrients back to the inorganic nutrient pool (Vadstein et. al. 1988; Tezuka 1990; Elser et. al. 1995). In contrast, nutrient mobilization by bacterioplankton for phytoplankton productivity is important in autotrophic systems (Sterner et. al. 1995). The immobilization of nutrients by bacterioplankton can further enhance the nutrient limited state of phytoplankton in allotrophic lakes (Vadstein et. al. 1988; Jansson 1998; Blomqvist et. al. 2001).

Nutrient immobilization by bacterioplankton results from the need to maintain elemental stoichiometry (Vadstein et. al. 1989; Tezuka 1990; Hessen 1992; Vadstein et. al. 1993; Elser

et. al. 1995). Bacterioplankton have high metabolic nutrient requirements and less flexible element ratios than phytoplankton (Vadstein et. al. 1988; Makino et. al. 2003). The C:N, C:P, and N:P ratios for bacterioplankton reported in the literature ranges from 6 – 15, 8 – 173, and 5 – 22, respectively (Coveney and Wetzel 1995; Chrzanowski et. al. 1996; Jansson et. al. 2001).

Bacterioplankton incorporate nutrients and respire carbon during decomposition of nutrient-poor substrates to meet their metabolic demands (Vadstein et. al. 1988; Hessen 1992). Therefore, lakes that depend on bacterioplankton productivity generated from allochthonous organic matter are typically net sources of carbon dioxide (Salonen et. al. 1983; Hessen et. al. 1994). In contrast, autochthonous DOM generated by phytoplankton productivity is assimilated more efficiently by bacterioplankton, resulting in lower respiration rates than the primary productivity (del Giorgio et. al. 1997; del Giorgio and Cole 1998; del Giorgio and Cole 1998; Hanson et. al. 2003). Therefore, autotrophic systems are generally net sinks for carbon dioxide due to CO_2 sequestering from the atmosphere by phytoplankton for photosynthesis (Schindler et. al. 1997; Cole 1999; Kelly et. al. 2001).

1.4. Elemental Stoichiometry

Food web and nutrient cycling dynamics in autotrophic and allotrophic lakes are driven by phytoplankton and bacterioplankton productivity, respectively. Elemental stoichiometry is a method used to study the relationships between internal cycling processes and food web dynamics within lakes (Elser et. al. 2000a; Elser et. al. 2000c; Sterner and Elser 2002). The stoichiometric or element ratios of an organism and its environment can help address the fate

and flux of carbon, nitrogen, and phosphorus in lake systems among pools (Elser et. al. 1998), which can help elucidate the different biogeochemical mechanisms that are occurring in autotrophic and allotrophic systems (Elser et. al. 1995).

The element ratios of the different biotic and abiotic pools represent nutrient availability, metabolic processes, metabolic requirements of organisms, and the nutritional quality of food sources (Elser et. al. 1998; Sterner and Elser 2002). The element ratios of the dissolved and particulate phases in the water column represent the balance of available nutrients as well as the nutrients scavenged from the water column (Stumm and Morgen 1995; Elser et. al. 2000a; Elser et. al. 2000c; Sterner and Elser 2002; Hessen et. al. 2003). Element ratios of recent sediments reflect the metabolic and nutrient scavenging processes occurring within the water column of a lake (Buffle and De Vitre 1994; Elser and Foster 1998; Elser et. al. 2000c; Hakanson and Jansson 2002).

Nutrients are scavenged from the water column via three processes: incorporation, adsorption, and coagulation (Santschi 1988). Incorporation of carbon, nitrogen, and phosphorus by organisms involves the assimilation of these nutrients to form biomass. Nutrients can also adsorb onto particles, and coagulation occurs between small particles that combine to form larger particles. Coagulation is a less important scavenging process in soft water lakes because dissolved organic matter can stabilize nutrients in solution, thereby limiting coagulation (Weilenmann et. al. 1989). The fates of scavenged nutrients include remineralization into the dissolved inorganic pool, reincorporation into biomass, or settling out of the water column to form sediments.

The element ratios of seston (i.e. suspended living and non-living organic matter) represent the balance between carbon, nitrogen, and phosphorus bound in particulates. The ratios reported in the literature for seston vary widely (e.g. C:P ranges from 100 – 1632) (Sterner et. al. 1997; Elser et. al. 1998; Elser and Foster 1998; Elser et. al. 2002). Element ratios of seston may represent the dominant organism comprising the pelagic microbial community, the food quality of the system, and or the importance of nutrient-poor detrital particles (Elser et. al. 2000a; Elser et. al. 2000c; Hessen et. al. 2003). Typically seston ratios are nutrient deficient relative to the individual pelagic organisms, suggesting that detritus is a major component of seston (Uehlinger and Bloesch 1987; Wetzel 1995).

1.5. Literature Cited

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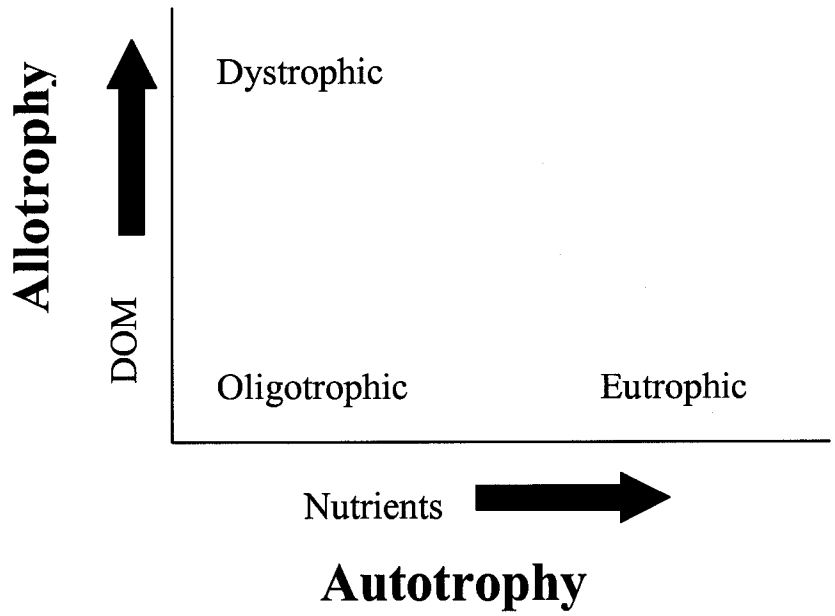

Figure 1.1. Autotrophy-allotrophy continuum graph (Hakanson and Jansson 2002).

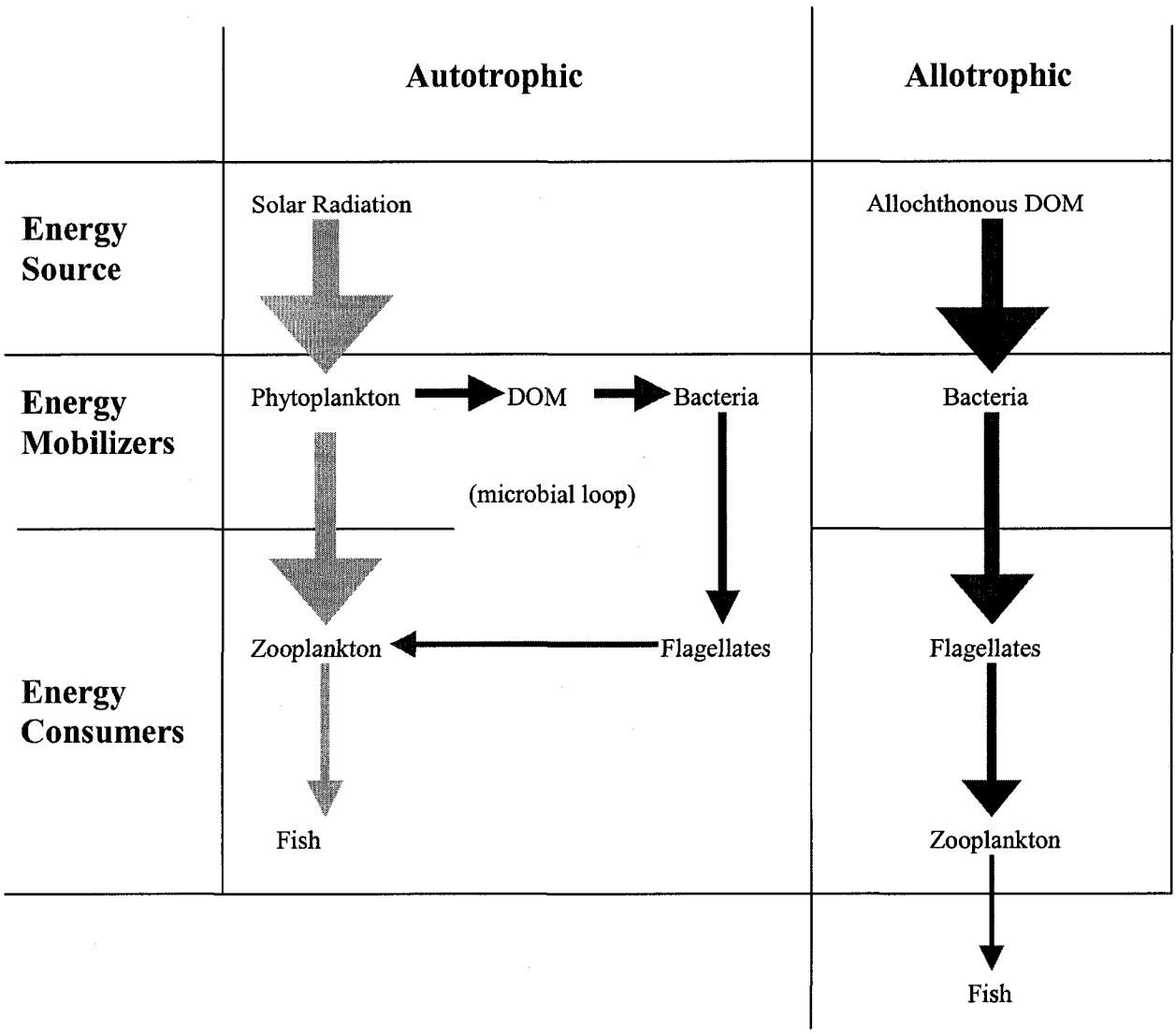

Figure 1.2. Comparison of the autotrophic and allotrophic food webs (Jansson et. al. 2000).

2. Dependencies of Phytoplankton and Bacterioplankton Biomass and Productivity on Dissolved Organic Matter (DOM) and Inorganic Nutrients

2.1. Introduction

A trophic discontinuity appears to exist between autotrophic and allotrophic lakes due to differences in food web drivers and nutrient cycling dynamics (Jones 1992; del Giorgio and Peters 1994; Jansson et. al. 2000). Most researchers have assumed that lakes are primarily autotrophic (Rich and Wetzel 1978; Currie 1990), however, recent research indicates that the majority of temperate North American lakes are allotrophic and that the primary food web drivers are allochthonous dissolved organic matter (DOM) and bacterioplankton productivity (Hesslein et. al. 1980; Cole et. al. 1994; del Giorgio and Peters 1994).

Solar radiation and allochthonous DOM are the two energy sources that drive the autotrophic and allotrophic gradients and determine pelagic microbial productivity. Solar energy drives microbial productivity in autotrophic lakes, whereas allochthonous DOM drives microbial productivity in allotrophic lakes (Jones 1992).

The autotrophic gradient is characterized by increasing inorganic nutrient concentrations and increasing phytoplankton productivity. Autotrophic lakes are clearwater lakes that are typically undersaturated in carbon dioxide and, therefore, they are net sinks for CO_2 (Jones 1992; Schindler et. al. 1997). In contrast, the allotrophic gradient is characterized by increasing allochthonous organic matter and increasing heterotrophic productivity.

Allotrophic systems are generally oversaturated in carbon dioxide due to decomposition of allochthonous DOM and, therefore, they are net sources of CO_2 to the atmosphere (Salonen et. al. 1983; Hessen et. al. 1994; Cole et. al. 1994).

Allotrophic or dystrophic lakes are characterized by high proportions of allochthonous DOM, low primary productivity relative to the apparent nutrient availability, and high bacterioplankton biomass and productivity (Jackson and Hecky 1980; Tranvik 1989; Jones 1992; Meili 1992; Tranvik 1998). Mechanisms hypothesized to cause dystrophy include attenuation of photosynthetically active radiation by DOM and direct or indirect control of nutrient availability by dissolved organic matter (Jones 1992; Jansson 1998; Jones 1998).

In allotrophic lakes, DOM probably competes with phytoplankton for available solar irradiance resulting in light limiting conditions and low phytoplankton productivity (Jones 1992; Morris et. al. 1995; Arvola et. al. 1996; Carpenter et. al. 1998; Jones 1998). DOM also has the ability to complex nutrients, which likely makes the nutrients unavailable to phytoplankton for photosynthesis (Jackson and Hecky 1980; de Haan et. al. 1990; Shaw 1994; Jones 1998). Furthermore, bacterioplankton are not likely carbon limited in allotrophic lakes because of high allochthonous DOM concentrations (Cotner and Wetzel 1992; Grover 2000). Photolysis of DOM produces nutrient-rich organic substrates that stimulate bacterioplankton growth (Francko and Heath 1982; Wetzel et. al. 1995; Lindell et. al. 1995; Bushaw et. al. 1996), and bacterioplankton can hydrolyse DOM releasing complexed nutrients (Münster et. al. 1992; Münster and de Haan 1998). In addition, bacterioplankton may outcompete phytoplankton for available nutrient concentrations, further suppressing

phytoplankton growth in allotrophic lakes (Currie and Kalff 1984a; Currie and Kalff 1984b; Chrzanowski et. al. 1995; Joint et. al. 2002).

The objective of this study was to investigate microbial food web dynamics as a function of DOM and inorganic nutrient concentrations. To meet this objective, two hypotheses were tested: 1. increasing DOM concentration will shift the pelagic microbial community from autotrophic to allotrophic; and 2. nutrient enrichment will prevent or dampen the shift of the pelagic microbial community from autotrophic to allotrophic. Support for these hypotheses will suggest that dystrophy is not a discrete trophic state as previously thought (Wetzel 2001), but is part of a continuous distribution of lake trophic states. The effect of natural dissolved organic matter (DOM) and inorganic nutrient concentrations on food web dynamics were tested by determining the dependencies of phytoplankton, bacterioplankton, and inorganic carbon on DOM.

2.2. Methods

2.2.1. Site Description

This study was conducted at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (93°30'-94°00'W, 49°30'-49°45'N)(Fig. 2.1). The area ranges in elevation from 360 m to 380 m above sea level and is located on the south western region of the Precambrian Shield. The soils are poorly developed and underlain by granite, glacial sands, and gravels. The vegetation is primarily boreal subclimax forest consisting of jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), trembling aspen (*Populus tremuloides*), and white birch (*Betula papyrifera*). The ELA mean annual temperatures range from 0.5 °C to 2.2 °C,

and the annual precipitation ranges from 500 mm to 750 mm (Brunskill and Schindler 1971; Curtis and Schindler 1997). The lakes involved in this study were Lake 224 and Lake 225 and were chosen primarily because Lake 224 and Lake 225 represent the most extreme DOC concentrations of all active ELA lakes and, therefore, would provide an adequate DOC concentration gradient. Furthermore, Lake 225 flows directly into Lake 224 making these lakes hydrologically linked. Lake 224 has a surface area of 25.9 ha, a drainage area of 97.5 ha, and a mean depth of 11.59 m. Lake 225 has a surface area of 5.6 ha and a drainage area of 30.5 ha (Curtis and Schindler 1997).

2.2.2. Enclosures

Twelve, closed-bottom lake enclosures were randomized along a floating linear wooden frame, and they were set up and remained in Lake 224 from July to September 2002. The surface area and depths of each enclosure was 0.84 m^2 and 1.5 m, respectively. DOM concentration gradients were established in two sets of six enclosures by pumping water from Lake 224 and Lake 225 into the enclosures in approximately 0%, 20%, 40%, 60%, 80%, and 100% proportions (relative to Lake 225)(Fig 2.2). These proportions potentially represent an autotrophic-allotrophic gradient. Plankton were readily transferred into the lake enclosures during pumping, but it is unlikely fish were transferred and none were observed in the lake enclosures.

One set of six lake enclosures acted as a reference (referred hereafter as reference enclosures), and the other set was enriched with inorganic nutrients (referred hereafter as nutrient enriched enclosures) to test the effects of autotrophic stimulation on microbial biomass and productivity along an autotrophic-allotrophic gradient. The nutrient enriched

enclosures were enriched with $NaNO_3$ and KH_2PO_4 at the beginning of the experiment and again 4 weeks later. The total amount of nitrogen and phosphorus added to the enclosures was 4500 mg N and 300 mg P, which elevated the concentrations by factors of 10 and 7, respectively.

2.2.3. Sample Collection and Preparation

Depth integrated water samples were collected weekly from each of the enclosures using a 1.5 m tube sampler and a bucket. Following each sample collection day, the enclosures were mixed with an oar. Subsamples were taken from the integrated sample for analyses of water chemistry and bacterioplankton abundance and productivity. Samples collected for water chemistry analyses were stored in 500 mL polyethylene bottles. From these samples, 100 mL aliquots were filtered through pre-ashed Whatman GF/C glass fibre filters and frozen. One frozen filter from each sample was analysed for chlorophyll a. The filtrate was kept for dissolved organic carbon (DOC; an analytical measure of DOM), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) analyses. Bacterioplankton abundance and productivity samples were stored in 60 mL tissue bottles and preserved with a final concentration of 3% glutaraldehyde buffered to pH 7 with 5N NaOH (Kepner and Pratt 1994). All water samples collected were stored and shipped at 4 °C.

Surface samples for dissolved inorganic carbon (DIC) and partial pressure of carbon dioxide (pCO_2) were collected biweekly using headspace vials presalted with KCl to maintain pH and to inhibit microbial activity between collection and analysis (Hesslein et. al. 1990; Kelly et. al. 2001). These samples were analysed immediately following collection. In addition, depth integrated samples for phytoplankton productivity and community respiration were

collected four times during the experiment. Analyses of these samples were performed immediately following collection and subsequent incubation.

2.2.4. Sample Analyses

2.2.4.1. Water Chemistry

2.2.4.1.1. Dissolved Organic Carbon

Filtered sample water was analysed for dissolved organic carbon (DOC) using a Shimadzu TOC-5000A Total Organic Carbon Analyzer with a detection limit of 0.05 mg L^{-1}. A 40 mL sample was acidified with 200 µL of select grade HCl and sparged for 7 mins with oxygen to volatilize and strip inorganic carbon from the sample. The sample was combusted to carbon dioxide and measured with an infrared detector. This instrument was operated with a 2% coefficient of variation according to the instruction manual.

2.2.4.1.2. Total Dissolved Nitrogen

Total dissolved nitrogen (TDN) concentrations were determined on filtered samples using a Shimadzu TNM-1 Total Nitrogen Analyzer with a detection limit of 0.06 mg L^{-1}. Samples were combusted to nitrogen monoxide and nitrogen dioxide and then reacted with ozone to create an excited state of nitrogen dioxide. A chemiluminescence detector measured the light emitted when the nitrogen dioxide returned to ground state.

2.2.4.1.3. Total Dissolved Phosphorus

Total dissolved phosphorus (TDP) concentrations were determined on filtered samples using the persulphate digestion and ascorbic acid methods (Greenberg, Clesceri, and Eaton 1992). The samples were digested with persulphate for 1 hr in an autoclave and then analysed on a

Milton Roy Spectrophotometer using a 5 cm pathlength. Sample absorbance was measured at 885 nm. The detection limit of this method is 0.01 mg L^{-1}.

2.2.4.1.4. Dissolved Inorganic Carbon

Surface water samples for analysis of dissolved inorganic carbon (DIC) were measured to determine the amount of inorganic carbon available in the system. DIC samples were acidified and then equilibrated with the headspace and analysed using a Varian Chrompack CP-3800 Gas Chromatograph. A criteria of ±5% was used for reproducibility of multiple injections.

$$DIC = CO_{2(aq)} + HCO_3^- + CO_3^{2-} \qquad \text{Eq 2.1}$$

where, DIC concentration is the sum of aqueous carbon dioxide (CO_2) and is expressed as the partial pressure of CO_2 (pCO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}). At pH < 9, the contribution of carbonate is negligible, therefore, the equation becomes:

$$DIC = pCO_2 + HCO_3^- \qquad \text{Eq 2.2}$$

The DIC concentration at atmospheric equilibrium (DIC_{eq}) was used as a reference point to determine the utilization of DIC in the enclosures, and it was calculated using the following equations:

$$DIC_{eq} = pCO_{2\,eq} + HCO_{3\,eq}^- \qquad \text{Eq. 2.3}$$

$$DIC_{xs} = pCO_{2\,xs} + HCO_{3\,xs}^{-} \qquad \text{Eq 2.4}$$

where, the subscript *xs* stands for in excess, or oversaturation. The assumption that $HCO_3^-{}_{xs} = HCO_3^-{}_{eq}$ is made here because oversaturation of CO_2 will affect the concentration of DIC, but will not significantly affect the concentration of HCO_3^-. This assumption is not valid when CO_2 is undersaturated because the equilibrium shifts to produce CO_2 and OH^- in Eq 2.5. The assumption becomes $HCO_3^-{}_{eq} = HCO_3^-{}_{unsat} + OH^-$ and OH^- was not measured.

$$HCO_3^- \leftrightarrow CO_2 + OH^- \qquad \text{Eq 2.5}$$

Therefore, solving Eq 2.4 for $HCO_3^-{}_{xs}$ and inserting it into Eq 2.3, we get Eq 2.6:

$$DIC_{eq} = DIC_{xs} - pCO_{2xs} + pCO_{2eq} \qquad \text{Eq 2.6}$$

where, DIC_{xs} is measured DIC concentration in the reference enclosures in μM; pCO_{2xs} is the measured partial pressure of CO_2 in the reference enclosures in μM, which was determined to be close to or above equilibrium (see results); and pCO_{2eq} is the measured partial pressure of CO_2 at atmospheric equilibrium. Then δDIC was calculated using the following equation to determine if bicarbonate (HCO_3^-) concentrations were changed in the nutrient enriched enclosures as a result of CO_2 drawdown.

$$\delta DIC = DIC_m - DIC_{eq} \qquad \text{Eq 2.7}$$

where, DIC_m is the measured DIC concentration in either the reference or nutrient enriched enclosures, and DIC_{eq} is the value calculated in Eq 2.6 for each reference enclosure value.

2.2.4.1.5. pH

pH was measured on each of the enclosure samples using a Beckman glass double junction Ag-Ag/Cl pH electrode coupled with a Beckman 320 pH meter. The pH meter was standardized using pH 4 and 7 buffers prior to each use.

2.2.4.2. Microbial Biomass

2.2.4.2.1. Chlorophyll a

Chlorophyll a, a surrogate for phytoplankton biomass (PB), was analysed by the technique described by (Wetzel and Likens 2000). Filters were left to extract for 24 hours in 10 mL of 85% denatured alcohol. Analysis of fluorescence at 660 nm with an excitation wavelength of 436 nm was performed using a Shimadzu RF-1501 Spectrofluorophotometer. A stock solution for the calibration standards was standardized using a Milton Roy Spectrophotometer following the method of (Wetzel and Likens 2000). All samples were above the 4 $\mu g\ L^{-1}$ detection limit for this technique. Chlorophyll a was also converted to units of carbon using a conversion factor of 50 μg C $\mu g\ Chl^{-1}$ (Coveney and Wetzel 1995). However, it is important to recognize that the proportion of carbon in a plankton cell can vary widely, especially under nutrient deficiencies and light limiting conditions (Hecky et. al. 1993).

2.2.4.2.2. Bacterioplankton Abundance

Bacterioplankton abundance, a surrogate for bacterioplankton biomass (BB), was determined by epifluorescence direct counts (Hobbie et. al. 1977; Kepner and Pratt 1994). Samples were stained with acridine orange to a final concentration of 110 μg L^{-1}, filtered onto 0.2 μm black polycarbonate filters (Millipore ™), and counted at 1000x magnification on an Olympus BH-2 microscope. Minimums of 200 cells were counted per filter. To prevent photobleaching, the coverslips were mounted onto slides using non-drying immersion oil and 1,4-diazabicyclo (DABCO, Triethylenediamine). Prepared filters were counted within 2 hours of staining. Bacterioplankton abundance was also converted to units of carbon using a conversion factor of 0.121 pg C μm^{-1} and an average cell biovolume of 0.06 μm^3 (Hagström et. al. 1979; Riemann et. al. 1984). The carbon per cell biovolume conversion factor is variable (Newell and Christian 1981), but only the relative values were of interest here. Furthermore, applying an average cell biovolume is acceptable because bacteria exhibit a small range in biovolume.

2.2.4.3. Microbial Productivity and Respiration

2.2.4.3.1. Phytoplankton Productivity and Community Respiration

Gross phytoplankton productivity (GPP) and community respiration (R) were measured as changes in dissolved inorganic carbon (DIC) concentration during 4 to 7 hour incubations (Hesslein et. al. 1990; Kelly et. al. 2001). An integrated water sample from each enclosure was transferred to three (initial, light, dark) 50 mL headspace vials making sure to fill from the bottom and allowing the bottle to overflow. The bottles were capped to ensure no bubbles. The initial CO_2 sample bottles were immediately fixed with 300 μL of concentrated phosphoric acid. The light and dark bottles were covered with a solar mesh to reduce light intensity and incubated in Lake 239. Following the incubation, a 5 mL aliquot of sample was

simultaneously removed from the bottles and replaced with N_2 gas, and then the sample was fixed with phosphoric acid. DIC was analysed for CO_2 concentration in the headspace for all bottles based on a precision of ±5% using a Varian Chrompack CP-3800 Gas Chromatograph. The following equations were used to determine community metabolism:

Dark – Light = Gross primary production Eq 2.8

Dark – Initial = Community respiration Eq 2.9

2.2.4.3.2. Specific Photosynthetic Rate

The specific photosynthetic rate (SPR) was calculated to normalize gross primary productivity per unit of phytoplankton biomass using the following equation:

$$\frac{\mu g\ C\ L^{-1}\ h^{-1}}{\mu g\ Chl\ L^{-1}} = \mu g\ C\ \mu g\ Chl^{-1}\ h^{-1} \qquad \text{Eq 2.10}$$

where, the numerator is gross primary productivity, and the denominator is chlorophyll a concentration.

2.2.4.3.3. Bacterioplankton Productivity

Bacterioplankton productivity (BP) was determined using the frequency of dividing cells (FDC) method (Hagström et. al. 1979). During bacterioplankton enumeration, dividing cells (those cells showing invaginations but not separated) were also counted. This study used the relationship between %FDC and specific growth rate established by (Riemann et. al. 1984). An average bacterial cell biovolume of 0.06 μm^3 was used based on literature values

(Hagström et. al. 1979; Riemann et. al. 1984). Bacteria exhibit a small range in cell biovolume, therefore, it is acceptable to use an averaged literature biovolume when absolute numbers are not required. The carbon per cell biovolume conversion factor of 0.121 pg C μm^{-3} is frequently reported in the literature and was used in this study (Riemann et. al. 1984; Tranvik 1988). The carbon per cell biovolume conversion factor can range widely from 0.087 pg C μm^{-3} to 0.380 pg C μm^{-3} (Newell and Christian 1981; Salonen et. al. 1992; Friedrich et. al. 1999; Drakare et. al. 2002), but only the relative values are of interest in this study, therefore, the conversion factor will not affect the relative differences observed.

2.2.4.4. Net Metabolism

Surface water samples for analysis of dissolved inorganic carbon (DIC) and the partial pressure of CO_2 (pCO_2) were measured to determine the amount of inorganic carbon available in the system and the net metabolism of the enclosures, respectively. The pCO_2 is equal to $CO_{2(aq)}$ in Eq 2.1, expressed as a partial pressure.

The pCO_2 samples were shaken to equilibrate the dissolved and gaseous phases with the headspace. Henry's Law (Eq 2.11) was used to directly relate $CO_{2\,(aq)}$ to pCO_2, where K_h is Henry's constant.

$$CO_{2(aq)} = K_H \times pCO_2 \qquad \text{Eq 2.11}$$

Changes to CO_2 solubility were accounted for by correcting for the water temperature and salt effects (Hesslein et. al. 1990; Kelly et. al. 2001). These samples were analysed using a

Varian Chrompack CP-3800 Gas Chromatograph and the data were assessed with a precision of ±5%.

The difference (δpCO_2) between the measured pCO_2 (pCO_{2m}) of the water sample and the atmospheric equilibrium value (pCO_{2eq}), ranging from 12.5 μM to 14.8 μM, was used to determine if the system was a net sink of CO_2 or a net source of CO_2.

$$\delta pCO_2 = pCO_{2m} - pCO_{2eq} \qquad \text{Eq 2.12}$$

A negative value indicated that the system was a net sink for CO_2 and CO_2 was sequestered from the atmosphere. A positive value indicated that the system was a net source and excess CO_2 was released from the water into the atmosphere (Kelly et. al. 2001).

2.2.5. Statistical Analyses

All dependent variable data were integrated over the course of the experiment and expressed as a time weight average using the following equation (Eq 2.13):

$$\left(\frac{\left(\left(\frac{(y_0+y_1)}{2}\right)\times(x_1-x_0)\right)+\left(\left(\frac{(y_1+y_2)}{2}\right)\times(x_2-x_1)\right)+\ldots+\left(\left(\frac{(y_{n-1}+y_n)}{2}\right)\times(x_n-x_{n-1})\right)}{x_n-x_0}\right)$$

where, y is the measured parameter from day 0 to day *n* in cumulative days, and x is the cumulative number of days since the beginning of the experiment from day 0 to day 61.

The initial DOC concentration for the enclosures was used as the independent variable, and the reference and nutrient enrichment treatments were fixed factors. Data were analysed using simple and stepwise multivariate regression analyses with SPSS 8.0 and assessed using an alpha value of 0.05. Simple regression analyses were used to determine the DOM dependence of the measured parameters and to compare between the reference and nutrient enriched enclosures. The regression coefficients (slope and y-intercepts) were compared between the reference and nutrient enriched enclosures to see if they were significantly different using a homogeneity of regression analysis. The homogeneity of regression analysis also determined whether the initial DOC concentration in the enclosures was a significant covariate. Equal slopes between the reference and nutrient enriched enclosures and DOC concentration as a covariate allowed an ANCOVA to be performed. ANCOVA removed the effects of the DOC covariate and assessed whether nutrient enrichment significantly affected the measured parameter. Stepwise multivariate analysis was used for exploratory purposes to better understand the relationships between parameters. MANOVA tested the effects of nutrient enrichment on the measured parameters that were independent of DOM concentration.

2.3. *Results*

2.3.1. Water Chemistry

The initial DOC concentrations in the both the reference and nutrient enriched enclosures ranged from 3.6 mg L^{-1} to 11.4 mg L^{-1}. The time weighted average TDN concentrations ranged from 0.18 mg L^{-1} to 0.34 mg L^{-1} and from 2.16 mg L^{-1} to 2.77 mg L^{-1} in the reference

and nutrient enriched enclosures, respectively. The time weighted average TDP concentrations were at or below the detection limit (0.01 mg L^{-1}) in the reference enclosures and ranged from 0.05 mg L^{-1} to 0.12 mg L^{-1} in the nutrient enriched enclosures. The time weighted average pH values ranged from 6.0 to 6.4 in the reference enclosures and ranged from 6.4 to 7.4 in the nutrient enriched enclosures.

2.3.2. Phytoplankton

Phytoplankton biomass (PB), measured as chlorophyll a concentration, increased as a function of increasing DOC concentration in the reference enclosures and ranged from 12 μg L^{-1} to 48 μg L^{-1} ($r^2 = 0.81$; $P < 0.01$) (Table 2.1)(Fig. 2.3A). PB was independent of DOC concentration, but increased by one order of magnitude as a function of nutrient enrichment in the nutrient enriched enclosures ($P < 0.001$). Chlorophyll a concentrations ranged from 239 μg L^{-1} to 745 μg L^{-1} in the nutrient enriched enclosures. Conversion of chlorophyll a concentrations to carbon units resulted in carbon biomass values ranging from 615 μg C L^{-1} to 2410 μg C L^{-1} in the reference enclosures and from 11900 μg C L^{-1} to 37200 μg C L^{-1} in the nutrient enriched enclosures (Fig. 2.3A).

Gross primary productivity (GPP) was independent of DOC concentration in the reference enclosures and ranged from 8.10 μg C L^{-1} h^{-1} to 15.31 μg C L^{-1} h^{-1} (Fig. 2.3B). GPP increased significantly in the nutrient enriched enclosures as compared to the reference enclosures and ranged from 25.02 μg C L^{-1} h^{-1} to 54.95 μg C L^{-1} h^{-1} ($P < 0.001$). GPP in the nutrient enriched enclosures peaked between approximately 5.7 mg L^{-1} and 6.2 mg L^{-1} of DOC, but GPP was suppressed in the low DOC and high DOC nutrient enriched enclosures. GPP did not correlate with chlorophyll a concentration in either of the two sets of enclosures.

The specific photosynthetic rate (SPR) decreased with increasing DOC concentration in the reference enclosures ranging from 0.42 μg C μg Chl^{-1} h^{-1} to 0.85 μg C μg Chl^{-1} h^{-1}, but the regression was non-significant ($P > 0.05$). SPR was independent of DOC concentration in the nutrient enriched enclosures and ranged from 0.20 μg C μg Chl^{-1} h^{-1} to 0.39 μg C μg Chl^{-1} h^{-1} (Fig. 2.3C). The SPR was significantly lower in the nutrient enriched enclosures than in the reference enclosures ($P < 0.001$).

2.3.3. Bacterioplankton

Bacterioplankton biomass (BB), measured as bacterioplankton abundance, increased as a function of increasing DOC concentration in the reference enclosures and ranged from 3.51 x 10^6 cells mL^{-1} to 9.34 x 10^6 cells mL^{-1} ($r^2 = 0.97$; $P < 0.001$)(Table 2.1). BB increased with increasing DOC concentration and ranged from 4.24 x 10^6 cells mL^{-1} to 6.56 x 10^6 cells mL^{-1} in the nutrient enriched enclosures, but the regression was non-significant ($P > 0.05$)(Fig. 2.4A). The BB dependence on DOC concentration changed significantly as a function of nutrient enrichment, where BB in the low DOC nutrient enriched enclosures was significantly higher than in the low DOC reference enclosures ($P < 0.01$). BB converted to carbon biomass units ranged from 26 μg C L^{-1} to 68 μg C L^{-1} in the reference enclosures and ranged from 31 μg C L^{-1} to 48 μg C L^{-1} in the nutrient enriched enclosures (Fig. 2.4A).

Using DOC, chlorophyll a, TDP, and TDN concentrations as independent variables, stepwise multivariate regression analyses showed that DOC concentration was the only variable that significantly predicted BB in the reference enclosures ($r^2 = 0.97$; $P < 0.001$). None of these

other independent variables predicted BB in the nutrient enriched enclosures, suggesting that some other variable, such as grazing, was regulating BB in these enclosures.

The dependence of bacterioplankton productivity (BP) on DOC concentration was similar in both sets of enclosures, regardless of nutrient enrichment, but BP was significantly higher in the nutrient enriched enclosures ($P < 0.05$). BP was marginally dependent on DOC concentration in the reference enclosures ($r^2 = 0.44$; $P = 0.09$)(Fig. 2.4B) and ranged from 1.32 μg C L^{-1} h^{-1} to 3.01 μg C L^{-1} h^{-1}. BP in the nutrient enriched enclosures was strongly dependent on DOC concentration ($r^2 = 0.74$; $P< 0.05$)(Table 2.1) and ranged from 3.13 μg C L^{-1} h^{-1} to 4.71 μg C L^{-1} h^{-1}. The results from ANCOVA showed that nutrient enrichment explained 84% of the variation in adjusted BP values between the two sets of enclosures ($P < 0.001$).

DOC, chlorophyll a, TDP, and TDN concentrations were used as predictors for BP in a stepwise multivariate regression analysis, but none of these variables predicted BP separately in the reference enclosures. Together, DOC, TDP, and TDN concentrations significantly predicted BP in the reference enclosures ($r^2 = 0.99$; $P < 0.01$), indicating that DOC and DOC bound nutrients were the primary factors regulating BP in the reference enclosures.

DOC, TDP, and TDN concentrations all predicted BP in the nutrient enclosures separately, but in a stepwise regression TDP was the only variable selected to predict BP ($r^2 = 0.89$; $P < 0.01$). Together, TDP and chlorophyll a concentrations also significantly predicted BP in the nutrient enriched enclosures, but chlorophyll a did not significantly increase the r^2-value

obtained with TDP ($r^2 = 0.89$; $P < 0.05$). The added phosphorus was important to bacterioplankton growth and the bacterioplankton were likely P-limited. Phytoplankton derived DOC may have also contributed to the increase in BP.

2.3.4. Net Enclosure Metabolism

The partial pressure of carbon dioxide (pCO_2) was marginally dependent on DOC concentration and ranged from 12.1 μM to 21.4 μM in the reference enclosures ($r^2 = 0.55$; $P = 0.056$)(Table 2.1). The pCO_2 was strongly dependent on DOC concentration in the nutrient enriched enclosures and ranged from 3.3 μM to 5.8 μM ($r^2 = 0.72$; $P < 0.05$)(Fig 2.5A). Nutrient enrichment did not change the dependence of pCO_2 on increasing DOC concentration, but pCO_2 was significantly lower in the nutrient enriched enclosures than in the reference enclosures ($P < 0.05$). ANCOVA showed that the nutrient enrichment treatment explained 92% of the variance in the adjusted pCO_2 values between the two sets of enclosures ($P < 0.001$).

The δpCO_2 values for the reference enclosures were close to atmospheric equilibrium at low DOC concentrations and then increased from equilibrium as DOC concentration increased, indicating allotrophy at high DOC concentrations in the reference enclosures ($r^2 = 0.55$; $P = 0.056$)(Table 2.1)(Fig 2.5B). The δpCO_2 values ranged from –1.4 μM to 7.8 μM in the reference enclosures. The δpCO_2 values decreased well below atmospheric equilibrium ranging from –10.3 μM to –7.8 μM, but the δpCO_2 increased towards equilibrium with increasing DOC concentration ($r^2 = 0.72$; $P < 0.05$). This suggests that the nutrient enriched enclosures were approaching allotrophy at higher DOC concentrations.

2.3.5. Dissolved Inorganic Carbon

Dissolved inorganic carbon (DIC) concentrations decreased as a function of increasing DOC concentration in both the reference and nutrient enriched enclosures (r^2 = 0.95, 0.88, respectively; $P < 0.01$)(Table 2.1)(Fig 2.6A). The DIC concentrations ranged from 34.4 μM to 100.8 μM in the reference enclosures and from 21.8 μM to 50.7 μM in the nutrient enriched enclosures. The DIC concentration regression line in the reference enclosures did not differ significantly from the DIC_{eq} line ($P > 0.05$)(Eq 2.2), but the DIC dependence on DOC concentration changed significantly as a function of nutrient enrichment. DIC concentration at low DOC concentration in the nutrient enriched enclosures was significantly lower than at low DOC concentration in the reference enclosures ($P < 0.01$). The reference and nutrient enriched regression lines would converge at a DOC concentration of approximately 13.8 mg L^{-1}, indicating that at this point DOC becomes the primary factor controlling the available DIC concentrations.

The δDIC concentrations were marginally dependent on DOC concentration in the reference enclosures (r^2 = 0.55; $P = 0.056$)(Table 2.1)(Fig 2.6B), and they were strongly dependent on DOC concentration in the nutrient enriched enclosures (r^2 = 0.83; $P = 0.01$). The δDIC values ranged from –1.9 μM to 7.5 μM and from –48.3 μM to –3.3 μM in the reference and nutrient enriched enclosures, respectively. The δDIC values were close to equilibrium at high DOC concentrations, indicating that the enclosures were close to allotrophy at high DOC concentrations in the nutrient enriched enclosures.

2.3.6. Community Respiration

Community respiration (R) was independent of DOC concentration in the reference enclosures ($P > 0.05$) and ranged from 7.12 μg C L^{-1} h^{-1} to 10.73 μg C L^{-1} h^{-1}. R followed a similar pattern as GPP in the nutrient enriched enclosures suggesting that respiration was dependant on GPP (Fig 2.7). Respiration ranged from 11.40 μg C L^{-1} h^{-1} to 28.25 μg C L^{-1} h^{-1} in the nutrient enriched enclosures. R was significantly higher in the nutrient enriched enclosures as compared to the reference enclosures ($P < 0.01$), but R was suppressed in the low DOC and high DOC nutrient enriched enclosures. R correlated with GPP in the reference enclosures at a P-value of 0.1 ($r^2 = 0.73$) and was correlated with GPP in the nutrient enriched enclosures ($r^2 = 0.88$; $P < 0.05$). Respiration did not correlate with BB or BP in either the reference of nutrient enriched enclosures.

2.3.7. GPP:R and GPP:BP Ratios

The phytoplankton productivity:community respiration ratios (GPP:R) were independent of DOC concentration in the reference and nutrient enriched enclosures and were not significantly different between the reference and nutrient enriched enclosures ($P > 0.05$)(Fig 2.8A). The GPP:R ratios ranged from 0.40 to 1.9 in the reference enclosures and ranged from 1.1 to 2.3 in the nutrient enriched enclosures.

The phytoplankton productivity:bacterioplankton productivity ratios (GPP:BP) were independent of DOC concentration in both the reference and nutrient enriched enclosures (Fig 2.8B). These ratios were not significantly different between the reference and nutrient enriched enclosures ($P > 0.05$), but the nutrient enriched enclosures had higher ratio values at

low DOC concentrations. The GPP:BP ratios ranged from 1.1 to 12 in the reference enclosures and ranged from 6.2 to 74 in the nutrient enriched enclosures.

2.4. Discussion

2.4.1. Autotrophy-Allotrophy Gradient

An autotrophic-allotrophic gradient was established across the natural DOM concentration gradient in the reference enclosures, which supports hypothesis one. The shift from autotrophy to allotrophy occurred at approximately 6 mg L^{-1} of DOC. The dependences of pCO_2 concentration and δpCO_2 on DOC concentration suggested that CO_2 accumulated in the water column of the reference enclosures at DOC concentrations greater than 6 mg L^{-1}.

At DOC concentrations of at least 6 mg L^{-1}, the δpCO_2 data in the reference enclosures were consistently greater than atmospheric equilibrium indicating that the enclosures were net sources of CO_2 and, therefore, were likely allotrophic (Hope et. al. 1996; Dillon and Molot 1997; Duarte and Agustí 1998). These conclusions are consistent with findings from other researchers who reported CO_2 supersaturation and phytoplankton productivity suppression at DOC concentrations greater than 6 mg L^{-1} (del Giorgio and Peters 1994; Hope et. al. 1996; Prairie et. al. 2002). Other researchers measured the shift from autotrophic to allotrophic at 10 mg L^{-1} of DOC, but this higher DOC concentration is likely explained by higher total phosphorus concentrations measured in those study lakes (Jansson et. al. 2000; Hanson et. al. 2003).

Carbon dioxide accumulation in the water column of lakes is likely the result of respiration from the decomposition and subsequent assimilation of organic matter by bacterioplankton (Hessen 1992; del Giorgio and Peters 1994; Hope et. al. 1996; Prairie et. al. 2002), but may also be from photochemical reactions (Graneli et. al. 1996). Results from research on five Swedish oligotrophic lakes ranging from 4 mg L^{-1} to 19 mg L^{-1} of DOC concentration indicate that photooxidation of DOC produces CO_2 and may contribute significantly to supersaturation of lakes (Graneli et. al. 1996).

Phytoplankton appeared co-regulated by both solar irradiance and inorganic nutrient concentrations in the reference enclosures. The direct dependence of phytoplankton biomass (PB) on DOC concentration in the reference enclosures was likely due to increased chlorophyll a abundance by phytoplankton in response to increasing light limitation at higher DOC concentrations. This conclusion is consistent with the independence of phytoplankton productivity (GPP) on DOC concentration and specific photosynthetic rate (SPR) data in the reference enclosures. The SPR data shows that less carbon is actually produced per unit of chlorophyll a as DOC concentration increases. This conclusion is supported by researchers who suggest DOM is an important indirect regulator of phytoplankton growth by directly regulating solar irradiance in aquatic ecosystems (Jackson and Hecky 1980; Lean 1998; Carpenter et. al. 1998).

Phytoplankton growth in the reference enclosures was nutrient limited, which is supported by the relative increase in GPP observed in the nutrient enriched enclosures. Therefore, phytoplankton did not utilize organic nutrient sources, either directly or indirectly from

bacterioplankton decomposition to increase biomass at higher DOC concentrations in the reference enclosures as suggested by other researchers (Sanders and Porter 1988; Porter 1988; Isaksson et. al. 1999). Bacterioplankton are more efficient assimilators of low available nutrient concentrations than phytoplankton and may have contributed to phytoplankton nutrient limitation (Currie and Kalff 1984a; Currie and Kalff 1984b; Chrzanowski et. al. 1995; Joint et. al. 2002).

In addition to solar irradiance and inorganic nutrient concentrations, low DIC (< 100 μM) concentrations suggest that DIC may have also limited phytoplankton growth in the reference enclosures. However, the large atmospheric source of CO_2 and quick dissolution of CO_2 into the water likely prevented inorganic carbon from limiting GPP (Schindler 1977; Wetzel 2001). The DIC concentrations in the reference enclosures were the same as DIC atmospheric equilibrium concentrations, which suggests that biological activity within the reference enclosures was insufficient to change the DIC concentration from atmospheric equilibrium.

The direct dependence of bacterioplankton biomass (BB) on DOC concentration in the reference enclosures suggests the bacterioplankton were using the DOC for growth. This result was supported by the direct dependence of bacterioplankton productivity on DOC concentration also observed in the reference enclosures. Results from other research support the conclusion that bacterioplankton directly utilize allochthonous DOM for production of biomass (Tranvik 1988; McCauley et. al. 1989). Allochthonous DOM can act as a source of carbon independent of phytoplankton and as a source of nutrients for bacterioplankton,

especially at high DOC concentrations (Tranvik 1988; McCauley et. al. 1989; de Haan et. al. 1990; Hessen 1992; Shaw 1994; Wetzel 1995; Arvola et. al. 1996; Arvola and Tulonen 1998). Bacterioplankton typically rely on allochthonous DOM to support productivity regardless of the trophic state, however, the proportion assimilated will increase with allotrophy (Chrzanowski and Hubbard 1989; Arvola et. al. 1996; Jansson et. al. 1999).

2.4.2. Autotrophic Shift with Nutrient Enrichment

Autotrophy appeared to be the dominant trophic state in the nutrient enriched enclosures across the natural DOM concentration gradient and supports hypothesis two. Nutrient enrichment stimulated a strong autotrophic response across the autotrophic-allotrophic gradient established in the reference enclosures. The pCO_2 data suggested that the nutrient enriched enclosures were undersaturated in carbon dioxide and, therefore, were autotrophic systems (Schindler 1977; Schindler et. al. 1997; Duarte and Agustí 1998; Carpenter et. al. 2001). The δpCO_2 data in the nutrient enriched enclosures were less than atmospheric equilibrium for all DOC concentrations indicating that the enclosures were net sinks of CO_2. These conclusions are supported by results of other studies that suggest highly eutrophic systems are typically net sinks for carbon dioxide (del Giorgio and Peters 1993; Schindler et. al. 1997; Jansson et. al. 2000).

The δpCO_2 dependence on DOC concentration indicated that the nutrient enriched enclosures would probably become net sources of CO_2 at DOC concentration values higher than used in this study. This suggests that high nutrient availability cannot compete with significant decreases in solar irradiance and in DIC concentrations due to high DOC concentrations and, therefore, under these conditions the system would be allotrophic. This conclusion is

supported by results from research on systems with high allochthonous DOC loading, which have low phytoplankton growth relative to the high nutrient availability (Jansson et. al. 2000; Hanson et. al. 2003). Furthermore, these results provide support for the suggestion that dystrophy is not a discrete trophic status (Fig 1.1), but rather is part of a continuous distribution of trophic states, which are a function of DOC concentration.

The DIC concentration data indicated that at a DOC concentration of approximately 14 mg L^{-1}, DOC concentration was likely the primary regulator of DIC concentration. DOC concentration can inhibit or reduce the solubility of carbon dioxide in water indicating that, at high DOC concentrations and high nutrient availability, DIC concentration may limit phytoplankton productivity (Wetzel 2001).

The available pCO_2 concentrations were likely fully utilized in the nutrient enriched enclosures and additional carbon sources were required for photosynthesis, which is supported by the larger δDIC values than δpCO_2 values. Phytoplankton in the nutrient enriched enclosures likely consumed most of the available pCO_2 in the nutrient enriched enclosures and then relied on bicarbonate sources for photosynthesis. pCO_2 is preferentially consumed by phytoplankton before other inorganic carbon sources (Gavis and Ferguson 1975; Wetzel 2001), but under high nutrient concentrations in the nutrient enriched enclosures bicarbonate may also have been used. Alternatively, the low pCO_2 may have resulted in an indirect drawdown of bicarbonate by shifting the equilibrium towards CO_2 formation (Gavis and Ferguson 1975).

Phytoplankton in the nutrient enriched enclosures were no longer nutrient limited, which is supported by the significant increase in gross phytoplankton productivity (GPP) observed. GPP was suppressed in the nutrient enriched enclosures at low and high DOC concentrations, which was likely due to light inhibition and light limitation, respectively. Low DOC concentrations can offered little protection to phytoplankton from harmful UV damage (Scully and Lean 1994; Lean 1998), which was amplified by the relatively shallow depth of the enclosures used in this study. High DOC concentration can limit productivity because DOC competes with phytoplankton for available solar irradiance (Long et. al. 1994; Arvola et. al. 1996; Carpenter et. al. 1998). Results from other research further supports the conclusion that DOC concentration is an important indirect regulator of phytoplankton by regulating the availability of solar irradiance (Morris et. al. 1995; Fee et. al. 1996; Jones 1998; Bukaveckas and Robbins-Forbes 2000).

BB was probably regulated by predation in the nutrient enriched enclosures. This conclusion was supported by the direct dependence of bacterioplankton productivity on DOC concentration observed in the nutrient enriched enclosures. High bacterioplankton productivity and low bacterioplankton biomass suggested that a high loss rate of bacterioplankton biomass was occurring in the nutrient enriched enclosures. Predation is a strong regulator of bacterioplankton biomass and is enhanced in more productive ecosystems, whereas oligotrophic systems tend to have low biomass insufficient to sustain large predator populations (Currie 1990; Persson et. al. 1992; Pace and Cole 1996; Arvola and Tulonen 1998; Langenheder and Jurgens 2001).

The similar direct dependence of bacterioplankton productivity (BP) on DOC concentration in the reference and nutrient enriched enclosures suggested that allochthonous DOM was the primary carbon source for bacterioplankton. Results from other studies show that 15-90% of bacterioplankton productivity is dependent on allochthonous DOM in a variety of lake systems (Chrzanowski and Hubbard 1989; Arvola et. al. 1996; Jansson et. al. 1999). The added nutrients in the nutrient enriched enclosures also supported bacterioplankton growth either directly or indirectly through phytoplankton productivity, which is supported by results from other research (Currie 1990; Vadstein et. al. 1993; Hessen et. al. 1994; Coveney and Wetzel 1995; Jansson et. al. 1996).

The community respiration (R), GPP:R ratios, and GPP:BP ratios provided no clear demarcation between autotrophy and allotrophy in the reference and nutrient enriched enclosures. The community respiration (R) data were independent of DOC concentration in both the reference and nutrient enriched enclosures and did not support the pCO_2 data. pCO_2 is a direct measure of the amount of CO_2 in the lake enclosures, whereas community respiration is based on short term incubations and this technique may not have been sensitive enough to detect changes in DIC concentrations (Davies et. al. 2003).

The GPP:R and GPP:BP ratios did not show clear trends or relationships with DOC concentration or nutrient enrichment. Most natural systems with high DOM concentrations are not associated with high inorganic nutrient concentrations, which could result in high variability and masking of trends. In addition, the lack of trends may be the result of using very different techniques to measure GPP and BP.

2.5. Conclusion

The data from this paper show that an autotrophic-allotrophic gradient was established across the DOM concentration gradient in the reference enclosures. Phytoplankton were co-regulated by both solar irradiance and inorganic nutrient concentrations, whereas bacterioplankton were strongly dependent on DOM concentration.

Autotrophic stimulation on the autotrophic-allotrophic gradient was achieved with nutrient enrichment. However, GPP appeared limited by solar irradiance at high DOC concentrations and BP maintained a strong dependence on DOC concentration. Taken together this suggests that allotrophy dominates at DOC concentrations higher than investigated in this study, regardless of inorganic nutrient concentrations, which suggests that allotrophy is a continuous function of DOC resulting in a continuous distribution of trophic states.

2.6. Literature Cited

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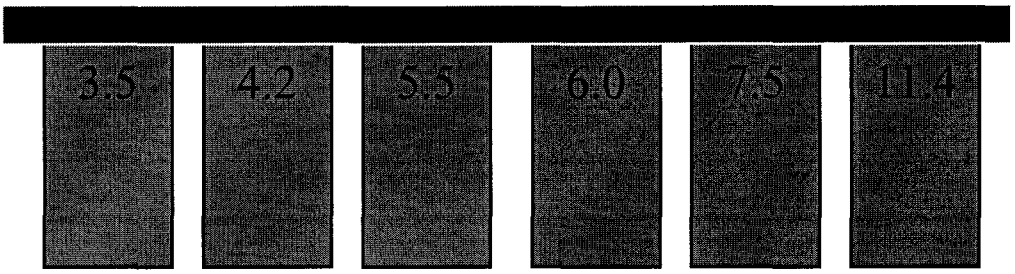
Table 2.1 Results of significant simple regression models. DOC is DOC concentration (mg L^{-1}), Chla is chlorophyll a concentration (mg L^{-1}), BB is bacterioplankton biomass (10^6 mL^{-1}), pCO_2 is partial pressure of CO2 (μM), δpCO_2 is the difference between the measured pCO_2 value and atmospheric equilibrium value (μM), DIC is DIC concentration (μM), δDIC is the difference between the measured DIC value and DIC at atmospheric equilibrium (μM), and BP is bacterioplankton productivity (μg C L^{-1} h^{-1}).

DV	Model	r^2	P-value	n
	Reference Enclosures			
Chla	4.60(DOC) + 9.58 x 10^{-2}	0.81	0.009	6
BB	0.755(DOC) + 0.956	0.97	0.000	6
pCO_2	0.998(DOC) + 9.695	0.55	0.056	6
δpCO_2	0.997(DOC) - 3.894	0.55	0.056	6
DIC	-8.03(DOC) + 123	0.95	0.001	6
δDIC	0.998(DOC) - 3.90	0.55	0.056	6
	Nutrient Enriched Enclosures			
BP	0.192(DOC) + 2.67	0.74	0.017	6
pCO_2	0.289(DOC) + 2.63	0.72	0.020	6
δpCO_2	0.288(DOC) – 11.0	0.72	0.021	6
DIC	-3.94(DOC) + 66.22	0.88	0.004	6
δDIC	5.06(DOC) - 58.6	0.83	0.008	6

Figure 2.1. Map of site location. Experimental Lakes Area, northwestern Ontario, Canada.

Reference Enclosures



Nutrient Enriched Enclosures

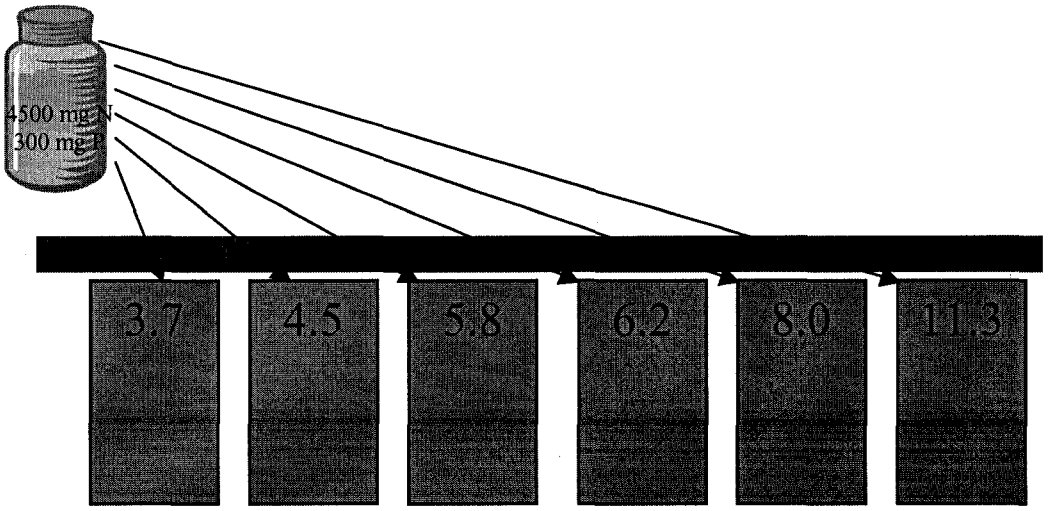


Figure 2.2. Schematic of the lake enclosures used in this study. The values in each of the enclosures indicates the initial DOC concentration in mg L^{-1}.

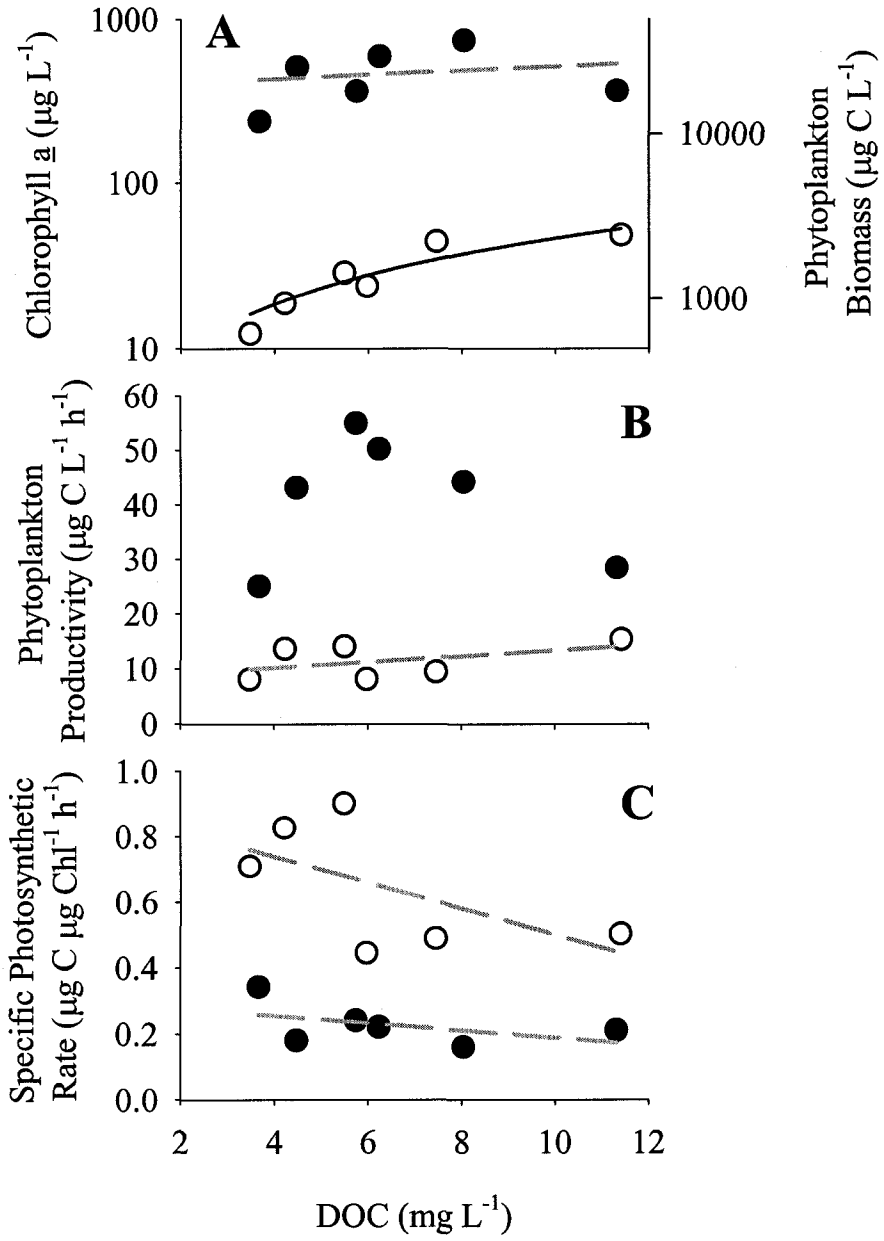


Figure 2.3. Dependencies of phytoplankton biomass, phytoplankton productivity, and specific photosynthetic rate on DOC concentration and inorganic nutrient enrichment. A. Chlorophyll a and phytoplankton biomass plotted on a log scale. B. Phytoplankton productivity measured as gross primary productivity. C. Specific photosynthetic rate. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black line represents a significant regression at $P < 0.05$. The grey dashed lines represent non-significant regressions.

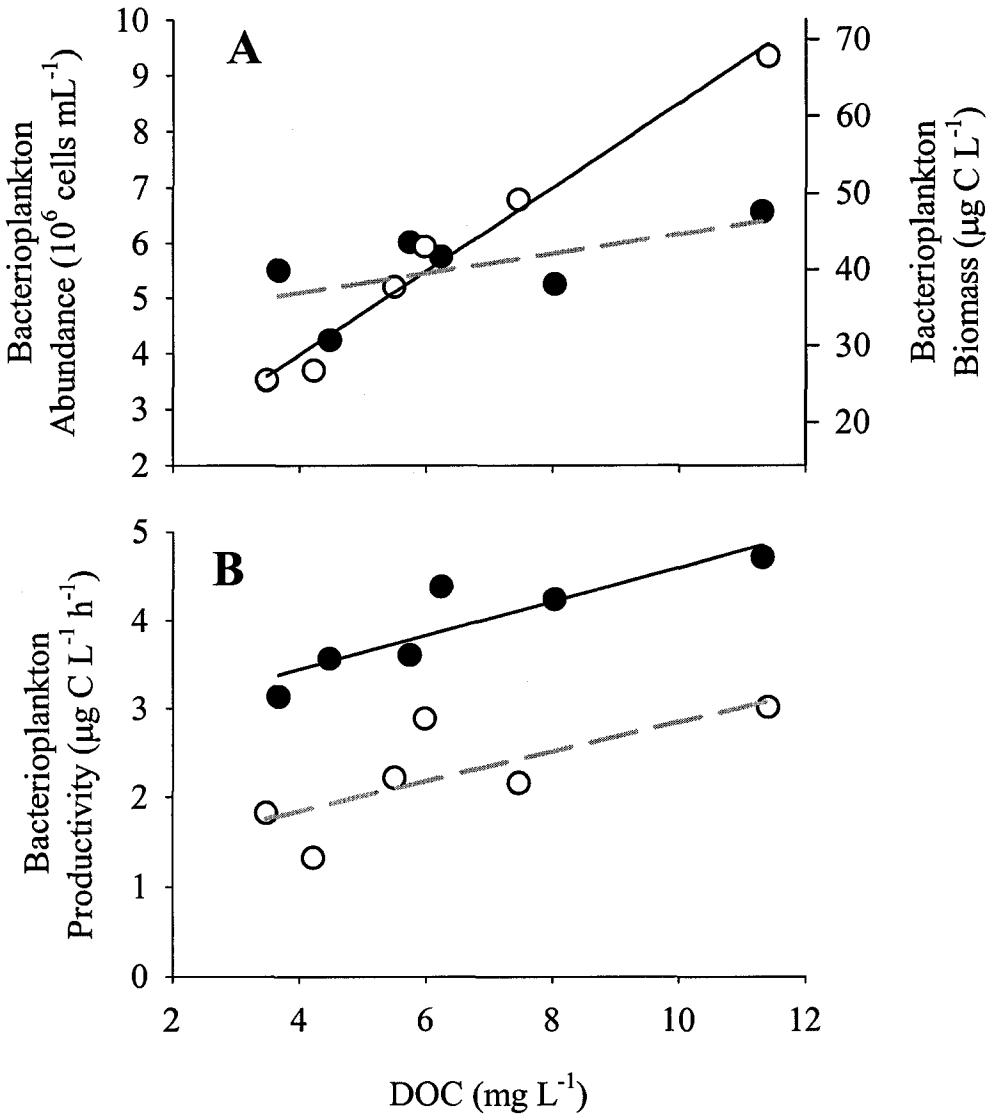


Figure 2.4. Dependencies of bacterioplankton biomass and productivity on DOC concentration and inorganic nutrient enrichment. A. Bacterioplankton abundance and biomass. B. Bacterioplankton productivity. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent a significant regression at $P < 0.05$. The grey dashed lines represent non-significant regressions.

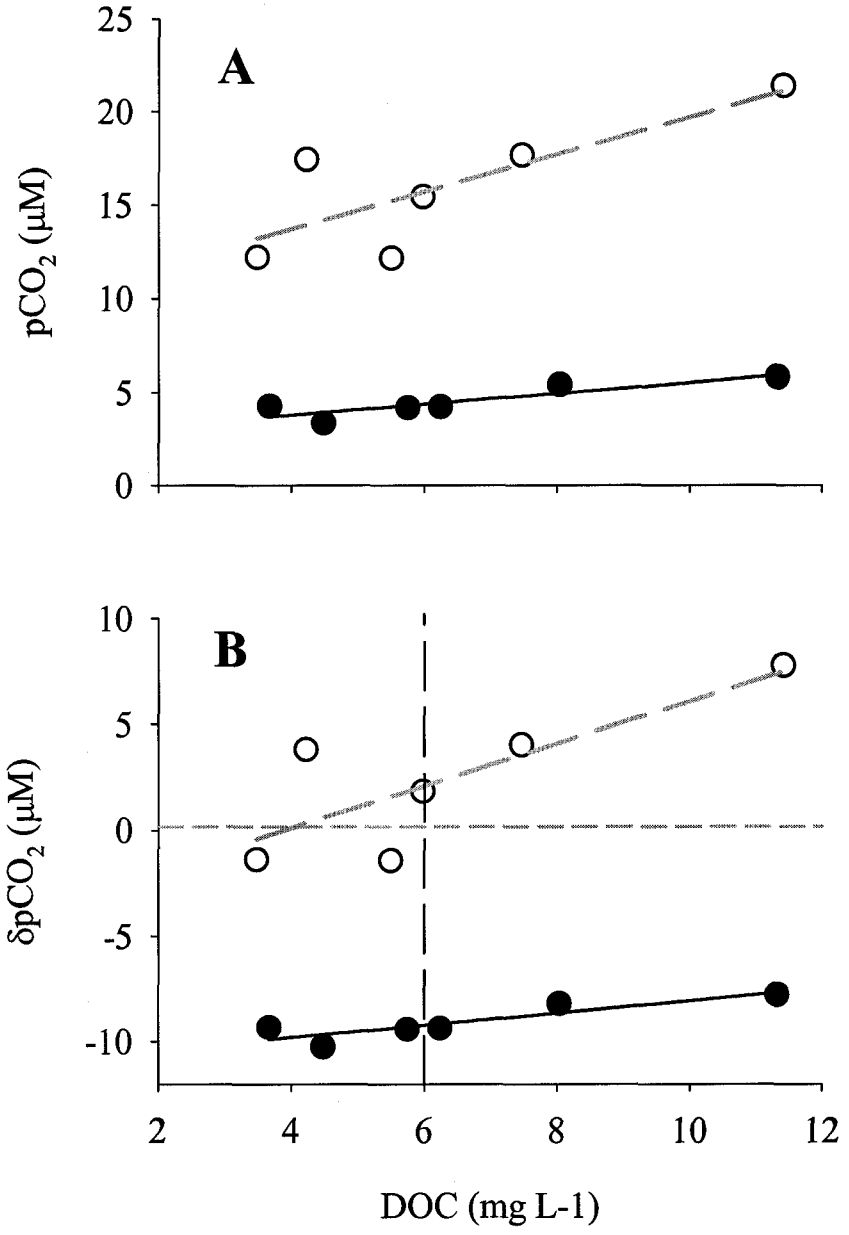


Figure 2.5. Dependencies of pCO_2 concentrations on DOC concentration and inorganic nutrient enrichment. A. Direct pCO_2 measures. B. The change in pCO_2 as calculated as the difference between the measured pCO_2 value and the atmospheric equilibrium value. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent significant regressions at $P < 0.05$. The long grey dashed lines represent non-significant regressions, the short grey dashed line represents atmospheric equilibrium, and the long black dashed line at 6 mg L^{-1} of DOC represents the shift from autotrophy to allotrophy.

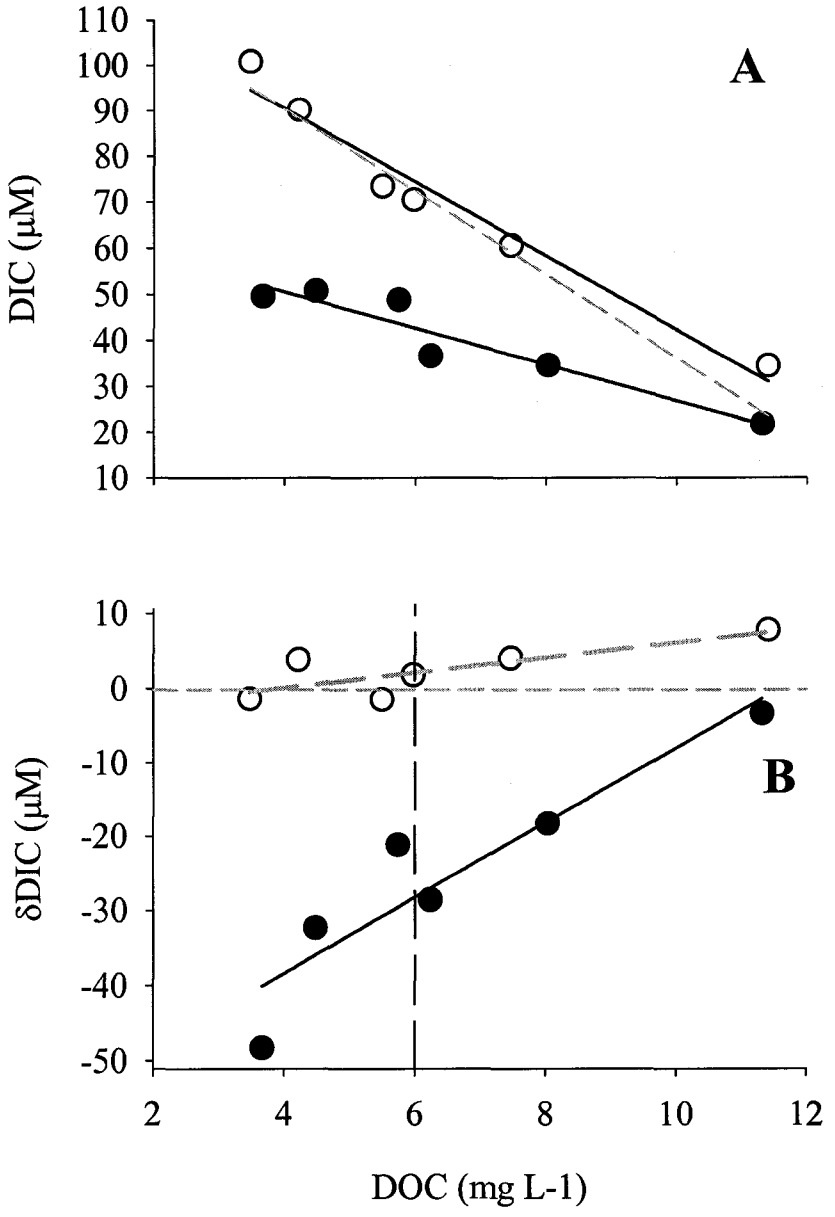


Figure 2.6. Dependence of DIC concentration on DOC concentration and inorganic nutrient enrichment. A. DIC concentration. B. The change in DIC as calculated as the difference between the measured DIC value and the atmospheric equilibrium value. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent significant regressions at $P < 0.05$. The long grey dashed lines represent non-significant regressions, the short grey dashed line represents atmospheric equilibrium, and the long black dashed line at 6 mg L^{-1} of DOC represents the shift from autotrophy to allotrophy.

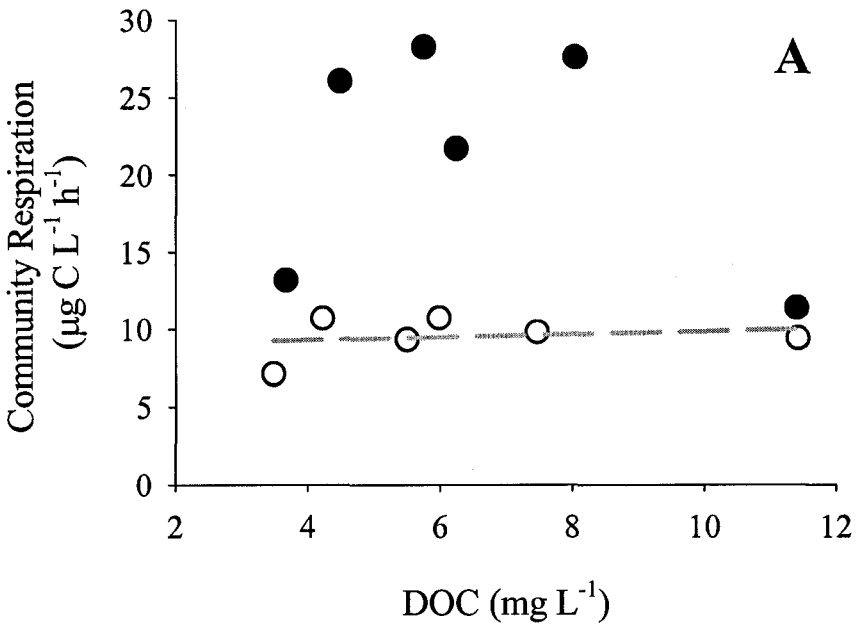


Figure 2.7. Dependence of community respiration on DOC concentration and inorganic nutrient enrichment. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The grey dashed lines represent non-significant regressions.

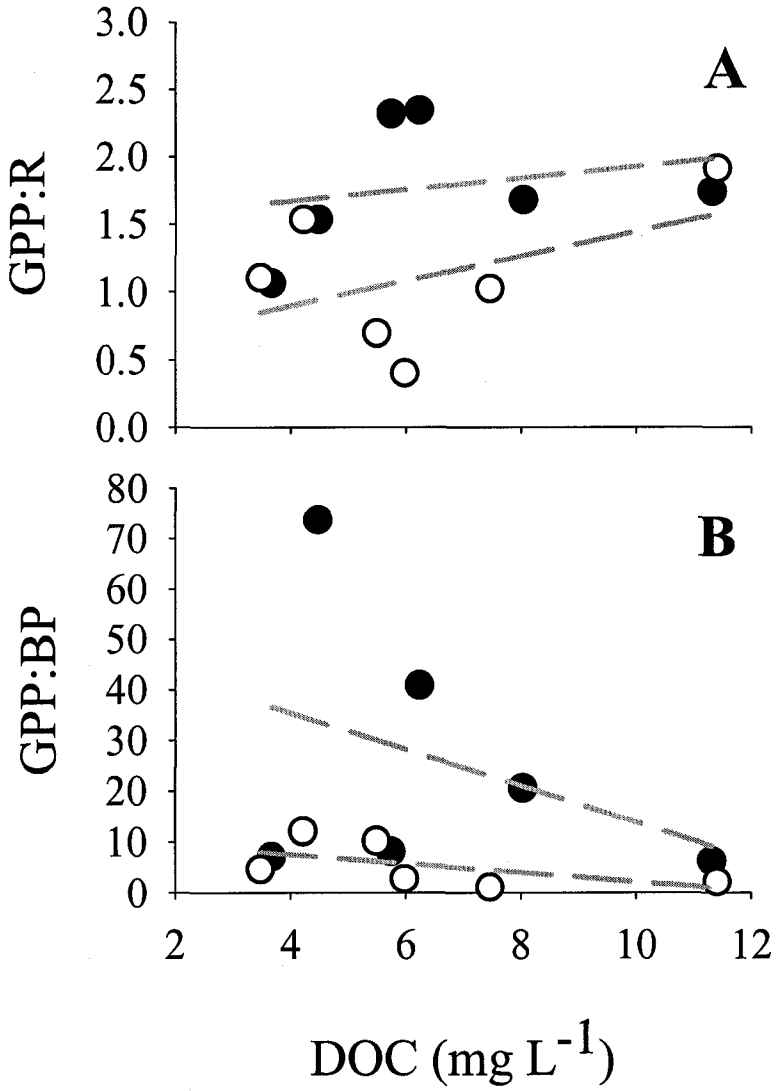


Figure 2.8. Dependencies of GPP:R and GPP:BP ratios on DOC concentration and inorganic nutrient enrichment. A. Gross phytoplankton productivity:community respiration ratios. B. Gross phytoplankton productivity:bacterioplankton productivity ratios. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The grey dashed lines represent non-significant regressions.

3. Element Ratios of Sediments Produced from DOM and Inorganic Nutrient Dependent Scavenging

3.1. Introduction

Autotrophic and allotrophic lakes differ based on the primary energy source, the microbial metabolism that drives the system, and nutrient utilization by the microbial community (Jones 1992; del Giorgio and Peters 1994; Jones 1998; Jansson 1998). Autotrophic lake microbial metabolism is driven by phytoplankton productivity based on solar irradiance, whereas allotrophic lake microbial metabolism is driven by bacterioplankton productivity based on allochthonous dissolved organic matter (DOM) (Currie 1990; Jansson et. al. 2000). These differences in energy source and metabolism result in differences in nutrient cycling (Sterner et. al. 1997; Sterner et. al. 1998).

Investigating biogeochemical processes may help to distinguish between autotrophic and allotrophic lakes (Sterner et. al. 1992; Elser et. al. 1995). Biogeochemical processes involve the transfer of energy and nutrients between living and non-living components. The primary components of a lake include dissolved nutrients, the seston, and the sediments. Elemental stoichiometry is a method that can provide a better understanding of food web and nutrient cycling dynamics in autotrophic and allotrophic lakes by relating the energy and nutrient requirements of organisms to the element ratios of their environment (Sterner et. al. 1998; Elser et. al. 1998; Elser et. al. 2000c).

The most common element ratios are C:N, C:P, and N:P because carbon, nitrogen, and phosphorus are important in cell growth and metabolism (Elser et. al. 2000a; Sterner and

Elser 2002). The distribution of C, N, and P among components and the flux between components will differ between autotrophic and allotrophic lakes and can be expressed by element ratios. Comparison of element ratios provides a measure of organic matter quality and nutrient availability to biota and can reflect the balance among C, N, and P scavenged from the water column (Redfield 1958; Elser et. al. 2000a; Elser et. al. 2000c; Sterner and Elser 2002; Hessen et. al. 2003). For example, the Redfield ratio (C:N = 6.6, C:P = 106, N:P = 16) represents the optimal element ratio for marine phytoplankton (Redfield 1958). Freshwater phytoplankton ratios are typically higher, and more variable than marine phytoplankton (Elser and Hassett 1994; Sterner et. al. 1997; Elser et. al. 2000b), but the Redfield ratio can serve as a useful comparison to lake seston ratios (Buffle and De Vitre 1994). Comparison of the seston and sediment ratios can provide insight into metabolic and scavenging processes occurring within the water column of a lake (Elser and Foster 1998; Elser et. al. 2000c; Hakanson and Jansson 2002).

Sediments are formed through scavenging of dissolved nutrients, such as dissolved organic carbon (DOC), nitrogen, and phosphorus, from the water column and the settling of particles. Nutrient scavenging can occur in three ways (Santschi 1988): 1. incorporation, 2. adsorption, and 3. coagulation. Incorporation involves the assimilation of nutrients by biota to generate biomass. Nutrients can also adsorb onto particles and coagulation occurs between small particles that combine to form larger particles. The fates of scavenged nutrients include remineralization into the dissolved inorganic pool, reincorporation into biomass, or settling out of the water column to form sediments.

The objective of this study was to investigate nutrient cycling dynamics as a function of DOM and inorganic nutrient concentrations. To meet this objective four null hypotheses were tested: 1. scavenging of carbon, nitrogen, and phosphorus from the water column is independent of DOC concentration; 2. scavenging of carbon, nitrogen, and phosphorus from the water column is independent of nutrient enrichment; 3. element sediment ratios are independent of DOC concentration; and 4. element sediment ratios are independent of nutrient enrichment. Rejection of these hypothesis will support the suggestion that autotrophic and allotrophic lakes exists along the same continuum as opposed to existing as discrete trophic states (Fig 1.1).

These hypotheses were tested by studying the DOM dependence of nutrient scavenging from the water column and the DOM dependence of the element ratios of the sediments formed during a lake enclosure experiment. Scavenging was calculated as the difference between initial and final concentrations in the water column, element ratios were calculated for the dissolved, seston, and sediments, and a mass balance was calculated relative to phosphorus because it is the conservative element among the three.

3.2. Methods

3.2.1. Site Description

This study was conducted at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (93°30'-94°00'W, 49°30'-49°45'N)(Fig. 3.1). The area ranges in elevation from 360 m to 380 m above sea level and is located on the south western region of the Precambrian Shield. The soils are poorly developed and underlain by granite, glacial sands, and gravels.

The vegetation is primarily boreal subclimax forest consisting of jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), trembling aspen (*Populus tremuloides*), and white birch (*Betula papyrifera*). The ELA mean annual temperatures range from 0.5 °C to 2.2 °C, and the annual precipitation ranges from 500 mm to 750 mm (Brunskill and Schindler 1971; Curtis and Schindler 1997). The lakes involved in this study were Lake 224 and Lake 225 and were chosen primarily because Lake 224 and Lake 225 represent the most extreme DOC concentrations of all active ELA lakes and, therefore, would provide an adequate DOC concentration gradient. Furthermore, Lake 225 flows directly into Lake 224 making these lakes hydrologically linked. Lake 224 has a surface area of 25.9 ha, a drainage area of 97.5 ha, and a mean depth of 11.59 m. Lake 225 has a surface area of 5.6 ha and a drainage area of 30.5 ha (Curtis and Schindler 1997).

3.2.2. Enclosures

Twelve, closed-bottom lake enclosures were randomized along a floating linear wooden frame, and they were set up in Lake 224 from July to September 2002. The surface area and depths of each enclosures was 0.84 m^2 and 1.5 m, respectively. DOM concentration gradients were established in 2 sets of 6 enclosures by pumping water from Lake 224 and Lake 225 into the enclosures in approximately 0%, 20%, 40%, 60%, 80%, and 100% proportions (relative to Lake 225)(Fig 3.2). These proportions represent an autotrophic-allotrophic gradient (Chapter 2). Plankton were readily transferred into the lake enclosures during pumping, but it is unlikely fish were transferred and none were observed in the lake enclosures.

One set of enclosures acted as a reference (referred hereafter as reference enclosures), and the other set was enriched with inorganic nutrients (referred hereafter as nutrient enriched enclosures) to test the effects of autotrophic stimulation on microbial biomass and productivity along an autotrophic-allotrophic gradient. The nutrient enriched enclosures were enriched with $NaNO_3$ and KH_2PO_4 at the beginning of the experiment and again 4 weeks later. The total amount of nitrogen and phosphorus added to the enclosures was 4500 mg N and 300 mg P, which elevated the concentrations by factors of 10 and 7, respectively (Table 3.1).

3.2.3. Sample Collection and Preparation

Depth integrated water samples were collected weekly from each of the enclosures using a 1.5 m tube sampler and a bucket. Following each sample collection day, the enclosures were mixed with an oar. Samples were taken from the integrated sample for water chemistry analyses in 500 mL polyethylene bottles. From these samples, 100 mL aliquots were filtered through pre-ashed Whatman GF/C glass fibre filters, and then the filters were frozen for seston carbon, seston nitrogen, and seston phosphorus. The filtrate was stored for dissolved organic carbon (DOC; an analytical measure of DOM), total dissolved nitrogen (TDN) analyses, and total dissolved phosphorus (TDP) analyses. All water samples collected were stored and shipped under refrigeration.

Non-quantitative sediment samples were collected from each of the enclosures at the end of the experiment and then frozen. The frozen sediment samples were freeze dried using a Labconco Freeze Dry System/Freezone 4.5 and then homogenized using a mortar and pestle.

3.2.4. Sample Analyses

3.2.4.1. Dissolved Organic Carbon

Filtered sample water was analysed for dissolved organic carbon (DOC) using a Shimadzu TOC-5000A Total Organic Carbon Analyzer with a detection limit of 0.05 mg L^{-1}. A 40 mL sample was acidified with 200 µL of select grade HCl and sparged for 7 mins with oxygen to volatilize and strip inorganic carbon from the sample. The sample was combusted to carbon dioxide and measured with an infrared detector. This instrument was operated with a 2% coefficient of variation according to the instruction manual.

3.2.4.2. Total Dissolved Nitrogen

Total dissolved nitrogen (TDN) concentrations were measured on filtered samples using a Shimadzu TNM-1 Total Nitrogen Analyzer with a detection limit of 0.06 mg L^{-1}. Samples were combusted to nitrogen monoxide and nitrogen dioxide and then reacted with ozone to create an excited state of nitrogen dioxide. A chemiluminescence detector measured the light emitted when the nitrogen dioxide returned to ground state.

3.2.4.3. Total Dissolved Phosphorus

Total dissolved phosphorus (TDP) concentrations were measured on filtered samples using the persulphate digestion and ascorbic acid methods (Greenberg, Clesceri, and Eaton 1992). The samples were digested with persulphate for 1 hr in an autoclave and then analysed on a Milton Roy Spectrophotometer using a 5 cm pathlength. Sample absorbance was measured at 885 nm. The detection limit of this method is 0.01 mg L^{-1}.

3.2.4.4. Seston and Sediment C, N, and P

Carbon (C) and nitrogen (N) concentrations of the seston and sediment were analysed by a PerkinElmer Series II 2400 CHNS/O Elemental Analyzer following the instruction manual. The detection limit of this method is 0.01 mg L^{-1} for nitrogen and 0.11 mg L^{-1} for carbon. Seston and sediment samples analyzed for phosphorus (P) were pre-ashed and then digested in 0.01 N HCl for 2 hrs at 85 °C. A subsample of the digested sample was diluted and analyzed for phosphorus following the ascorbic acid method (Greenberg, Clesceri, and Eaton 1992). The sample was analysed on a Milton Roy Spectrophotometer using a 5 cm pathlength and absorbance was measured at 885 nm. The detection limit of this method is 0.01 mg L^{-1}.

3.2.5. C, N, and P Scavenging

Scavenging of C, N, and P from the water column was determined as the difference between the initial and final measures. The initial nitrogen and phosphorus values included dissolved, seston, and precipitation data, and the final values included the dissolved and seston data. The initial and final organic carbon data included the dissolved and seston data. The concentrations of C, N, and P were converted to a total mass for the entire water column of the enclosures.

3.2.6. Mass Balance

Conservative estimates of sediment generated in the enclosures were calculated from phosphorus. Phosphorus is a non-volatile substance, therefore, it was assumed that the amount of phosphorus lost from the water column was gained in the sediments. Total carbon and nitrogen concentrations in the sediments were calculated from this conservative estimate of sediment formed in the enclosures. Concentrations of nitrogen and phosphorus in the

precipitation that fell during the experiment were also incorporated into the mass balance (Table 3.1). Gains and losses of gaseous carbon and nitrogen were not measured directly, but based on the water column and sediment data, the amount of C and N lost or gained to the system could be determined. The mass balance was calculated using the following equation:

$$\Delta M = \left|\left(X_{di} + X_{si} + X_a\right) - \left(X_{df} + X_{sf} + X_{sed}\right)\right| \qquad \text{Eq. 3.1}$$

where, X_{di} is the initial mass of dissolved C, N, or P; X_{si} is the initial mass of seston C, N, or P; X_a is the mass of N or P from precipitation and or from added nutrients; X_{df} is the final mass of dissolved C, N, or P; X_{sf} is the final mass of seston C, N, or P; X_{sed} is the mass of C, N, or P in the sediments.

3.2.7. Mass Transfer Coefficient and % Retention

The mass transfer coefficients were calculated to represent the rate at which the C, N, and P were removed from the water column.

$$\frac{X_{sed}}{\frac{A \times t}{[X_i]}}$$

where, X_{sed} is the total mass of C, N, or P in the sediments (mg), A is the area of the enclosures (0.84 m^2), t is the length of time of the experiment (61 days), and $[X_i]$ is the initial concentration of C, N, or P in the water column of the enclosures (mg m^{-3}).

Percent retention was calculated to represent the proportion of C, N, or P retained in the sediments relative to the initial mass in the water column. However, C and N have volatile

states and, therefore, this retention does not account for exchanges with the atmosphere. The % retention of C, N, and P were calculated using the following equation:

$$\frac{X_{sed}}{X_i} \times 100\% \qquad \text{Eq. 3.2}$$

where, X_{sed} is the total mass of C, N, or P in the sediments (mg) and X_i is the initial mass of C, N, or P in the water column of the enclosures (mg).

3.2.8. Element Ratios

Element ratios of C:N, C:P, and N:P were calculated for dissolved, seston, and sediment carbon, nitrogen, and phosphorus. The element ratio is calculated by dividing mass by the appropriate molecular weight.

3.2.9. Statistical Analyses

All dependent variable data were integrated over the course of the experiment and expressed as a time weight average using the following equation (Eq 3.3):

$$\left(\frac{\left(\left(\frac{(y_0 + y_1)}{2}\right) \times (x_1 - x_0)\right) + \left(\left(\frac{(y_1 + y_2)}{2}\right) \times (x_2 - x_1)\right) + \ldots + \left(\left(\frac{(y_{n-1} + y_n)}{2}\right) \times (x_n - x_{n-1})\right)}{x_n - x_0} \right)$$

where, y is the measured parameter from day 0 to day *n* in cumulative days, and x is the cumulative number of days since the beginning of the experiment from day 0 to day 61.

The initial DOC concentration for the enclosures was used as the independent variable and the reference and nutrient enrichment treatments were fixed factors. Data were analysed using simple regression analyses with SPSS 8.0 and assessed using an alpha value of 0.05. Simple regression analyses were used to determine the DOM dependence of the measured parameters and to compare between the reference and nutrient enriched enclosures. The regression coefficients (slope and y-intercepts) were compared between the reference and nutrient enriched enclosures to see if they were significantly different using a homogeneity of regression analysis. The homogeneity of regression analysis also determined whether the initial DOC concentration in the enclosures was a significant covariate. Equal slopes between the reference and nutrient enriched enclosures and DOC concentration as a covariate allowed an ANCOVA to be performed. ANCOVA removed the effects of the DOC covariate and assessed whether nutrient enrichment significantly affected the measured parameter. MANOVA tested the effects of nutrient enrichment on the measured parameters that were independent of DOM concentration.

3.3. Results

3.3.1. C, N, and P Enclosure Concentrations

3.3.1.1. Dissolved

The initial DOC concentrations in both the reference and nutrient enriched enclosures ranged from 3.6 mg L^{-1} to 11.4 mg L^{-1}. The time weighted average TDN concentrations ranged from 0.18 mg L^{-1} to 0.34 mg L^{-1} in the reference enclosures and ranged from 2.16 mg L^{-1} to 2.77 mg L^{-1} in the nutrient enriched enclosures. The time weighted average TDP concentrations were at or below the detection limit of 0.01 mg L^{-1} in the reference enclosures

and ranged from 0.05 mg L^{-1} to 0.12 mg L^{-1} in the nutrient enriched enclosures (Table 3.2). The total initial mass of the dissolved C, N, and P in the enclosures is shown in Table 3.1.

3.3.1.2. Seston

The time weighted average seston carbon concentrations ranged from 0.17 mg L^{-1} to 0.75 mg L^{-1} in the reference enclosures and ranged from 0.93 mg L^{-1} to 1.48 mg L^{-1} in the nutrient enriched enclosures. The time weighted average seston nitrogen concentrations ranged from 0.02 mg L^{-1} to 0.10 mg L^{-1} in the reference enclosures and ranged from 0.14 mg L^{-1} to 0.24 mg L^{-1} in the nutrient enriched enclosures. The time weighted average seston phosphorus concentrations were at or below the detection limit of 0.01 mg L^{-1} in the reference enclosures and ranged from 0.05 mg L^{-1} to 0.08 mg L^{-1} in the nutrient enriched enclosures (Table 3.2). The total initial mass of the seston C, N, and P in the enclosures is shown in Table 3.1.

3.3.1.3. Sediments

The sediment carbon concentrations ranged from 340 mg g^{-1} to 370 mg g^{-1} in the reference enclosures and ranged from 430 mg g^{-1} to 490 mg g^{-1} in the nutrient enriched enclosures. The sediment nitrogen concentrations ranged from 26 mg g^{-1} to 30 mg g^{-1} in the reference enclosures and ranged from 43 mg g^{-1} to 62 mg g^{-1} in the nutrient enriched enclosures. The sediment phosphorus concentrations ranged from 0.34 mg g^{-1} to 0.89 mg g^{-1} in the reference enclosures and ranged from 1.5 mg g^{-1} to 6.1 mg g^{-1} in the nutrient enriched enclosures (Table 3.2).

3.3.2. C, N, and P Scavenging

3.3.2.1. Carbon

The change in total carbon (δTC) in the water column of the lake enclosures showed that carbon was gained in all enclosures except at high DOC concentrations in the reference enclosures (Fig 3.3A). The δTC increased with increasing DOC concentration in the reference enclosures, but this regression was non-significant ($P > 0.05$), which supports hypothesis one. The δTC ranged from -1900 mg to 820 mg in the reference enclosures. The δTC depended directly on DOC concentration in the nutrient enriched enclosures ($r^2 = 0.82$; $P < 0.01$)(Table 3.3) and ranged from -5300 mg to -1100 mg, which were significantly greater increases in carbon than in the reference enclosures ($P < 0.001$) and does not support hypothesis two. Nutrient enrichment explained 78% of the variation in δTC values adjusted for covariate effects of DOC concentration between the two sets of enclosures ($P < 0.001$). The two regression lines would converge at approximately 14 mg L^{-1} of DOC (Fig 3.3A).

The total mass of carbon sedimentation in the reference enclosures was independent of DOC concentration and ranged from 1300 mg to 6300 mg (Fig 3.4A). The mass of carbon sedimentation in the nutrient enriched enclosures was directly dependent on DOC concentration ($r^2 = 0.70$; $P < 0.05$)(Table 3.3) and ranged from 26000 mg to 79000 mg. The mass transfer coefficients of carbon ranged from 4.2 mm d^{-1} to 27 mm d^{-1} in the reference enclosures and ranged from 87 mm d^{-1} to 190 mm d^{-1} in the nutrient enriched enclosures (Table 3.4). The proportion of carbon retained in the sediments ranged from 17% to 110% in the reference enclosures and ranged from 350% to 790% in the nutrient enriched enclosures (Table 3.4).

3.3.2.2. Nitrogen

The change in total nitrogen (δTN) in the water column of the lake enclosures showed that nitrogen was scavenged detectably from all of the reference and nutrient enriched enclosures except one (Fig 3.3B). The δTN was independent of DOC concentration in the reference enclosures and ranged from –5.0 mg to 250 mg, which supports hypothesis one. The δTN was inversely dependent on DOC concentration in the nutrient enriched enclosures ($r^2 = 0.77$; $P < 0.05$)(Table 3.3) and ranged from 3200 mg to 4700 mg. More nitrogen was scavenged from the water columns in the nutrient enriched enclosures than in the reference enclosures ($P < 0.001$), which does not support hypothesis two.

The total mass of nitrogen sedimentation in the reference enclosures was independent of DOC concentration and ranged from 110 mg to 520 mg. The mass of nitrogen sedimentation in the nutrient enriched enclosures increased with increasing DOC concentration in the nutrient enriched enclosures, but this regression was non-significant ($P > 0.05$)(Fig 3.4B). The total nitrogen sedimentation ranged from 3300 mg to 7900 mg in the nutrient enriched enclosures and was greater than sedimentation in the reference enclosures ($P < 0.001$). The mass transfer coefficients of nitrogen ranged from 0.63 mm d^{-1} to 2.8 mm d^{-1} in the reference enclosures and from 14 mm d^{-1} to 31 mm d^{-1} in the nutrient enriched enclosures (Table 3.4). The proportion of nitrogen retained in the sediments ranged from 2.6% to 12% in the reference enclosures and ranged from 56% to 120% in the nutrient enriched enclosures (Table 3.4).

3.3.2.3. Phosphorus

The change in total phosphorus (δTP) in the water column of the lake enclosures showed that phosphorus was scavenged from all of the reference and nutrient enriched enclosures. The δTP depended directly on DOC concentration in the reference enclosures ($r^2 = 0.59$; $P < 0.05$)(Table 3.3) and ranged from 2.3 mg to 11 mg, which does not support hypothesis one. The δTP decreased with increasing DOC concentration in the nutrient enriched enclosures, but this regression was non-significant ($P > 0.05$)(Fig 3.3C). The δTP ranged from 230 mg to 370 mg in the nutrient enriched enclosures and more phosphorus was scavenged from the water columns in the nutrient enriched enclosures than in the reference enclosures ($P < 0.001$), which does not support hypothesis two.

The total mass of phosphorus sedimentation in the reference enclosures depended on DOC concentration in the reference enclosures ($r^2 = 0.59$; $P < 0.05$)(Table 3.3) and ranged from 2.3 mg to 11 mg. The mass of phosphorus sedimentation decreased with increasing DOC concentration in the nutrient enriched enclosures, but this regression was non-significant ($P > 0.05$)(Fig 3.4C). The total phosphorus sedimentation in the nutrient enriched enclosures ranged from 230 mg to 370 mg, which was greater than the phosphorus sedimentation in the reference enclosures ($P < 0.001$). The mass transfer coefficients of phosphorus ranged from 0.20 mm d^{-1} to 0.94 mm d^{-1} in the reference enclosures and from 16 mm d^{-1} to 23 mm d^{-1} in the nutrient enriched enclosures (Table 3.4). The proportion of phosphorus retained in the sediments ranged from 0.83% to 3.8% in the reference enclosures and ranged from 54% to 84% in the nutrient enriched enclosures (Table 3.4).

3.3.2.4. Mass Balance

Phosphorus is a conservative substance with no volatile state, therefore, phosphorus was neither lost nor gained from the lake enclosures. Thus, the phosphorus scavenged from the water column was sedimented, and it was used to calculate the carbon and nitrogen mass balances.

The carbon mass balance showed that carbon was gained in all of the lake enclosures (Fig 3.5A). The carbon mass balance was independent of DOC concentration in the reference enclosures ranging from 2300 mg ± 3900 mg to 6800 mg ± 28000 mg. The error associated with the reference enclosures was high and suggests that carbon could have been either gained or lost from these enclosures. The carbon mass balance in the nutrient enriched enclosures depended on DOC concentration ($r^2 = 0.62$; $P < 0.05$)(Table 3.3) and ranged from 29000 mg ± 5400 mg to 79000 mg ± 15000 mg. The amount of carbon gained in the nutrient enriched enclosures was one order of magnitude greater that the amount of carbon gained in the reference enclosures.

The nitrogen mass balance showed that nitrogen was gained in all of the enclosures except three (Fig 3.5B). The nitrogen mass balance was independent of DOC concentration in the reference enclosures, but dependent on DOC concentration in the nutrient enriched enclosures ($r^2 = 0.71$; $P < 0.05$)(Table 3.3). The total gain in nitrogen ranged from –220 mg ± 380 mg to 290 mg ± 320 mg in the reference enclosures and ranged from –1100 mg ± 240 mg to 4700 mg ± 1100 mg in the nutrient enriched enclosures. The amount of nitrogen gained at high DOC concentrations in the nutrient enriched enclosures was one order of

magnitude greater that the amount of nitrogen gained at high DOC concentrations in the reference enclosures.

3.3.3. C:N, C:P, N:P Element Ratios

3.3.3.1. Dissolved Element Ratios

The dissolved C:N element ratios of the water column in the reference and nutrient enriched enclosures both depended on DOC concentration and ranged from 26 to 38 in the reference enclosures and from 2.7 to 5.2 in the nutrient enriched enclosures ($r^2 = 0.91, 0.94$, respectively; $P < 0.01$)(Fig 3.6A)(Table 3.3). The C:N ratios of the water column of the nutrient enriched enclosures were significantly lower than the C:N ratios in the reference enclosures ($P < 0.001$).

The dissolved C:P element ratios of the water column in the reference enclosures directly depended on DOC concentration and ranged from 950 to 2600 ($r^2 = 0.99$; $P < 0.001$)(Table 3.3). The C:P ratios were independent of DOC concentration in the nutrient enriched enclosures and ranged from 240 to 450 (Fig 3.6B)

The dissolved N:P element ratios of the water column in the reference enclosures depended on DOC concentration and ranged from 36 to 69 ($r^2 = 0.99$; $P < 0.001$)(Table 3.3). The N:P ratios decreased with increasing DOC concentration in the nutrient enriched enclosures, but the regression was non-significant ($P > 0.05$)(Fig 3.6C). The dissolved N:P ratios in the nutrient enriched enclosures ranged from 62 to 160.

3.3.3.2. Seston Element Ratios

The seston C:N element ratios were independent of DOC concentration in both the reference and nutrient enriched enclosures (Fig 3.7A). Seston C:N ratios ranged from 7.9 to 11 in the reference enclosures and from 6.8 to 7.9 in the nutrient enriched enclosures.

The seston C:P and N:P element ratios depended on DOC concentration ($r^2 = 0.74, 0.69$, respectively; $P < 0.05$)(Table 3.3) in the reference enclosures and ranged from 43 to 164 and from 5.6 to 19, respectively. The C:P and N:P ratios were independent of DOC concentration in the nutrient enriched enclosures and ranged from 42 to 56 and from 5.3 to 8.1, respectively (Fig 3.7).

3.3.3.3. Sediment Element Ratios

The sediment C:N element ratios decreased with increasing DOC concentration in the reference enclosures (Fig 3.8A), but the regression was non-significant ($P > 0.05$), which supports hypothesis one. The C:N ratios of the sediments ranged from 14 to 16 in the reference enclosures. The C:N ratios directly depended on DOC concentration in the nutrient enriched enclosures ($r^2 = 0.63$; $P < 0.05$) (Table 3.3) and ranged from 8.9 to 12, which were significantly lower than the reference enclosure ratios ($P < 0.001$) and does not support hypothesis two.

The sediment C:P and N:P element ratios decreased with increasing DOC concentration in the reference enclosures (Fig 3.8), but the regressions were non-significant ($P > 0.05$), which supports hypothesis one. The sediment C:P and N:P ratios in the reference enclosures ranged from 980 to 2800 and from 64 to 180, respectively. The C:P and N:P ratios were directly

dependent on DOC concentration in the nutrient enriched enclosures (r^2 = 0.78, 0.77, respectively; $P < 0.05$)(Table 3.3) and ranged from 190 to 730 and from 22 to 63, respectively, which were significantly lower than the reference ratios ($P < 0.01$) and does not support hypothesis two.

The regression models of the sediment C:N, C:P, and N:P element ratios in the reference and nutrient enriched enclosures would all converge at a DOC concentration range between 14 mg L^{-1} and 16 mg L^{-1} (Fig 3.8).

3.4. Discussion

3.4.1. C, N, and P Scavenging

Carbon (C) and nitrogen (N) scavenging under low nutrient conditions in the reference enclosures were independent of DOC concentration, which supports hypothesis one. Phosphorus (P) scavenging was dependent on DOC concentration and does not support hypothesis one. This suggests that either more P was scavenged from the water column at higher DOC concentrations or different scavenging mechanisms are affecting the different elements.

Nutrient recycling within the water column by biota (biota includes zooplankton, phytoplankton, and bacterioplankton) was an important mechanism preventing scavenging of nutrients to sediments in the reference enclosures. Results from another study showed that recycled nutrients were an important source of nutrients to both phytoplankton and bacterioplankton (Sterner et. al. 1995). Scavenging of C, N, and P from the water columns

of the reference enclosures was low compared to the nutrient enriched enclosures, which was consistent with the low mass transfer coefficients and proportions retained in the sediments as well as the low concentrations of C, N, and P deposited in the sediments of the reference enclosures.

Scavenging of C, N, and P in the nutrient enriched enclosures was greater than the reference enclosures. The higher mass transfer coefficients in the nutrient enriched enclosures suggest that C, N, and P were rapidly scavenged from the water columns and deposited in the sediments of the nutrient enriched enclosures resulting in a higher proportion retained than in the reference enclosures. These results suggest that nutrient scavenging and nutrient sedimentation were greater with eutrophication and autotrophy in the nutrient enriched enclosures. Results from another study reported greater annual sedimentation of organic carbon, nitrogen, and phosphorus from a eutrophic lake than from a mesotrophic lake in Switzerland (Bloesch et. al. 1977).

The carbon and nitrogen mass balances in the reference enclosures indicate net positive gains in C and N, but were independent of the autotrophic-allotrophic gradient established in the reference enclosures (Chapter 2). The excess carbon and nitrogen were likely from atmospheric sources and sequestered from the atmosphere to drive photosynthesis (Schindler 1977; Carpenter et. al. 2001). Data presented previously (Chapter 2) suggested that the reference enclosures at high DOC concentrations were net CO_2 producers, which does not directly support the result that C and N were sequestered for photosynthesis. However, phytoplankton productivity in the reference enclosures was limited by inorganic nutrients and

DIC concentrations were low (<100 μM), therefore, it is possible that phytoplankton needed atmospheric C and N for photosynthesis. An additional explanation for the observed C and N sequestering in the reference enclosures is that we could not detect the mass balance changes at these levels due to the large errors associated with the mass balance. Phosphorus occurred in lower concentrations than carbon or nitrogen in the reference enclosures, and resulted in large errors when the mass balance was calculated (see Fig 3.5).

Net positive gains in carbon and nitrogen were observed in the mass balance of the nutrient enriched enclosures and indicate that carbon and nitrogen were sequestered from the atmosphere. C and N scavenging to the sediments likely resulted in sequestering from the atmosphere for photosynthesis by phytoplankton (Schindler 1977; Carpenter et. al. 2001).

3.4.2. Element Ratios as Indicators of Processes

The element ratios of DOM in the reference enclosures indicate that the organic matter was nutrient-poor. The element ratios of the water column in the reference enclosures were extremely nutrient-deficient relative to the seston suggesting that biota rapidly incorporated and recycled nitrogen and phosphorus into biomass in the water column, thereby removing most of the nutrients from the dissolved pool (Tezuka 1990; Currie 1990; Elser et. al. 1995). The element ratios in the nutrient enriched enclosures showed that the dissolved organic matter in the water columns was nutrient-rich. The dissolved organic matter element ratios indicate that the water column was likely a stable source of nutrients to biota (Elser and George 1993).

The nutrient-rich seston ratios in both the reference and nutrient enriched enclosures suggest that incorporation was the primary scavenging mechanism of C, N, and P. This conclusion is supported by results from another study that looked at fifteen lakes ranging in trophic status, which found seston C:N ratios that ranged from 6 to 13 to represent phytoplankton (Baines and Pace 1994). Seston element ratios in the enclosures were similar to the Redfield ratio (C:N = 6.6, C:P = 106, N:P = 16) and to ratios representing biota as reported in the literature, therefore, these seston ratios likely reflected biotic ratios (Uehlinger and Bloesch 1987; Hochstadter 2000). The seston ratios deviated from the Redfield ratio at high DOC concentrations in the reference enclosures and were probably the result of the high element ratios of the dissolved organic matter in the water column. Other researchers have also found no relationship between seston ratios and trophic state (Uehlinger and Bloesch 1987) because similar seston ratios may actually be a product of different growth rates under different environmental conditions. For example, phytoplankton grow slowly under high solar irradiance, and they exhibit low C:P ratios (Xenopoulos et. al. 2002). Phytoplankton can also have low C:P ratios when growth rate and P concentrations are high (Hochstadter 2000).

The sediment element ratios typically became more enriched in nitrogen and phosphorus at high DOC concentrations in the reference enclosures, which does not support hypothesis three. This result suggests that more nitrogen and phosphorus were scavenged from the water column at higher DOC concentrations, which was supported by the phosphorus scavenging data. The nitrogen scavenging data did not support this conclusion directly, but the mass balance indicated that atmospheric sequestering occurred and the scavenging calculation did not account for atmospheric fluxes. Results from other studies using lake

enclosures at the ELA showed that DOM can either decrease or increase scavenging of trace metals from aquatic systems (Santschi 1988; Curtis 1993).

Although scavenging likely occurred in the reference enclosures by incorporation, the high element ratios of the sediments suggested that the nutrients were not readily scavenged into sediments. Therefore, a nutrient-poor water column produced nutrient-rich seston and nutrient-deficient sediments, which indicates that nutrient recycling in the water column of the reference enclosures was more efficient than in the nutrient enriched enclosures (Currie 1990; Kroer 1993; del Giorgio and Peters 1993; Sterner et. al. 1995; Cotner and Biddanda 2002). This result is in contrast to results from another study of 20 temperate lakes, representing a wide trophic gradient, that found that lower P concentrations did not result in higher nutrient cycling efficiency (Hudson et. al. 1999).

Scavenging in the nutrient enriched enclosures was likely dominated by incorporation by biota based on the nutrient-rich seston ratios. Less nutrient recycling occurred in the water column of the nutrient enriched enclosures producing nutrient-rich sediments. Furthermore, the higher mass transfer coefficients and nutrient retention in the sediments suggests that biotic sedimentation rates were higher under eutrophic conditions in the nutrient enriched enclosures, resulting in nutrient-enriched sediments, which is supported by results from other studies (Bloesch et. al. 1977; del Giorgio and Peters 1993). Another study has shown that sedimentation of primary productivity is less in eutrophic systems, but the relative amounts lost to the sediments from eutrophic systems as compared to oligotrophic systems were 5 times higher, producing low C:N ratios in the sediments (Baines and Pace 1994).

3.4.3. Autotrophy-Allotrophy Convergence

DOC concentrations greater than 14 mg L^{-1} would likely result in allotrophy, regardless of inorganic nutrient concentrations. Autotrophy was observed in the nutrient enriched enclosures across the DOC concentration gradient (Chapter 2), but a common convergence, at approximately 14 mg L^{-1}, was observed in the change in total carbon of the water column (δTC) and the sediment element ratios. Furthermore, these convergences are consistent with the interpretation of the DIC concentration convergence observed at approximately 14 mg L^{-1} of DOC (Chapter 2). At the convergence point, inorganic nutrient concentrations had no effect on δTC or on the element ratios of the sediments. Furthermore, the δTC and sediment element ratio values resemble the values predicted under low nutrient conditions in the reference enclosures. Therefore, these results suggest that DOC concentration can be a primary driver of nutrient cycling dynamics (Currie 1990; Sterner et. al. 1992; Chrzanowski et. al. 1996; Sterner et. al. 1997; Chrzanowski and Grover 2001). More importantly, these transitions to allotrophy further indicate that dystrophy is a continuous function of DOC concentration rather than a discrete trophic state (Fig 1.1).

3.5. Conclusions

Seston element ratios indicated that incorporation by biota was likely an important C, N, and P scavenging mechanism in the reference enclosures, but the sediment element ratios indicate that the nutrients were retained within the water columns under low nutrient conditions. Therefore, nutrient scavenging and subsequent sedimentation in the reference enclosures was limited by efficient biotic incorporation and recycling along the autotrophic-allotrophic gradient.

The autotrophic stimulation of the autotrophic-allotrophic gradient resulted in greater scavenging than observed in the reference enclosures and nutrient-enriched sediments. Carbon, nitrogen, and phosphorus were effectively scavenged from the water column by incorporation into biomass followed by rapid sedimentation. However, nutrient enrichment of water having DOC concentrations higher than used in this experiment would likely not stimulate an autotrophic response. Thus, allotrophy is likely a continuous function of DOC concentration and, therefore, dystrophy is not a discrete trophic state.

3.6. Literature Cited

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Table 3.1 Initial total mass of each component contributing carbon, nitrogen, and phosphorus. N_{add} and P_{add} are the total mass of N and P added to the nutrient enriched enclosures. Half of the total mass was added July 13, 2002 and the other half was added August 22, 2002. N_{ppt} and P_{ppt} are the total mass of N and P contributed to the enclosures from precipitation during the experiment.

DOC (mg)	Seston C (mg)	TDN (mg)	Seston N (mg)	N_{add} (mg)	N_{ppt} (mg)	TDP (mg)	Seston P (mg)	P_{add} (mg)	P_{ppt} (mg)
Reference Enclosures									
4600	29	2500	51	n/a	180	110	45	n/a	5.5
5600	77	2200	72	n/a	180	86	63	n/a	5.5
7200	31	2000	20	n/a	180	66	76	n/a	5.5
7800	510	2400	140	n/a	180	94	94	n/a	5.5
10000	440	2500	130	n/a	180	78	100	n/a	5.5
14000	990	2200	210	n/a	180	41	130	n/a	5.5
Nutrient Enriched Enclosures									
4400	31	220	39	4500	180	13	13	300	5.5
5300	150	210	72	4500	180	13	13	300	5.5
6900	410	380	89	4500	180	13	13	300	5.5
7500	220	270	76	4500	180	13	13	300	5.5
9400	570	310	110	4500	180	13	15	300	5.5
14000	1000	420	190	4500	180	13	21	300	5.5

Table 3.2 Concentrations of carbon, nitrogen, and phosphorus in the dissolved water column, in the seston, and in the sediments. Values of TDN, TDP, and Seston C, N, and P are time weighted averages.

Initial DOC ($mg\ L^{-1}$)	TDN ($mg\ L^{-1}$)	TDP ($mg\ L^{-1}$)	Seston C ($mg\ L^{-1}$)	Seston N ($mg\ L^{-1}$)	Seston P ($mg\ L^{-1}$)	Sediment C ($mg\ g^{-1}$)	Sediment N ($mg\ g^{-1}$)	Sediment P ($mg\ g^{-1}$)
Reference Enclosures								
3.48	0.18	0.01	0.06	0.03	<0.01	370	29	0.40
4.22	0.19	0.01	0.19	0.04	<0.01	370	27	0.34
5.50	0.21	0.01	0.27	0.05	<0.01	340	26	0.89
5.98	0.24	0.01	0.27	0.06	<0.01	370	30	0.64
7.47	0.26	0.01	0.17	0.04	<0.01	350	27	0.49
11.41	0.34	0.01	0.68	0.11	0.01	360	30	0.63
Nutrient Enriched Enclosures								
3.67	2.34	0.05	0.88	0.16	0.05	440	57	4.5
4.48	2.25	0.07	1.25	0.24	0.05	480	62	4.8
5.75	2.17	0.06	1.06	0.19	0.06	460	59	6.1
6.24	2.50	0.08	1.38	0.26	0.08	470	61	4.4
8.04	2.64	0.09	1.47	0.27	0.07	490	61	3.4
11.32	2.77	0.12	0.87	0.17	0.06	430	43	1.5

Table 3.3 Results of significant simple regression models. DOC is DOC concentration (mg L-1); δTC, δTN, and δTP are the change in total concentration in the water column (mg); Dissolved C:N, C:P, N:P are element ratios from dissolved C, N, and P in the water column; Sediment C, N, and P are the total amount deposited in the sediments (mg).

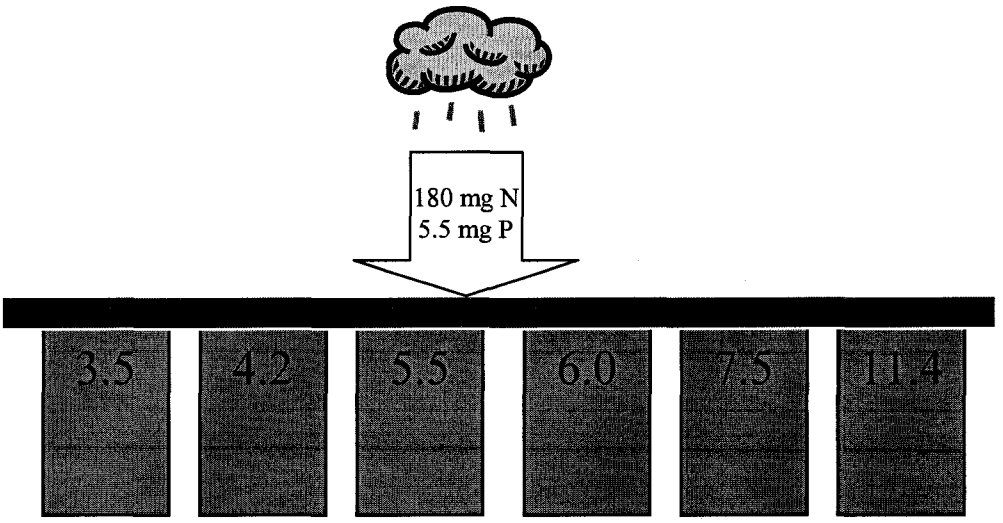
DV	Model	r^2	P-value	n
	Reference Enclosures			
δTP	0.889(DOC) + 0.511	0.59	0.045	6
Sediment P	0.889(DOC) + 0.511	0.59	0.045	6
Dissolved C:N	1.58(DOC) + 21.3	0.91	0.002	6
Dissolved C:P	214(DOC) + 192	0.99	0.000	6
Dissolved N:P	4.12(DOC) + 22.3	0.99	0.000	6
Seston C:P	14.1(DOC) - 24.9	0.74	0.018	6
Seston N:P	1.61(DOC) + 1.46	0.68	0.026	6
	Nutrient Enriched Enclosures			
δTC	608(DOC) - 7460	0.82	0.008	6
δTN	-184(DOC) + 4790	0.77	0.013	6
Sediment C	4980(DOC) + 6870	0.70	0.024	6
C Mass Balance	4370(DOC) + 14300	0.62	0.038	6
N Mass Balance	588(DOC) - 2650	0.71	0.023	6
Dissolved C:N	0.303(DOC) + 1.72	0.94	0.001	6
Sediment C:N	0.371(DOC) + 7.06	0.63	0.037	6
Sediment C:P	64.0(DOC) - 75.5	0.78	0.013	6
Sediment N:P	4.87(DOC) + 3.14	0.77	0.014	6

Table 3.4 Carbon, nitrogen, and phosphorus mass transfer coefficients (MTC) (mm d^{-1}) and percent sediment retention.

Initial DOC (mg L^{-1})	C MTC	% C Retention	N MTC	% N Retention	P MTC	% P Retention
			Reference Enclosures			
3.48	22.3	90.8	1.8	7.3	0.37	1.5
4.22	27.5	111.8	2.6	10.5	0.48	2.0
5.50	7.0	28.6	0.89	3.6	0.48	2.0
5.98	4.2	17.3	0.63	2.6	0.20	0.8
7.47	14.5	59.2	2.6	10.4	0.70	2.9
11.41	10.1	41.1	2.8	11.5	0.94	3.8
			Nutrient Enriched Enclosures			
3.67	202	718.9	19	67.7	23	83.2
4.48	156	546.2	18	64.0	22	76.4
5.75	88	307.2	14	47.6	21	73.2
6.24	115	407.3	19	68.0	21	72.7
8.04	139	500.0	28	101.2	23	83.7
11.32	127	426.0	31	102.6	16	54.0

Figure 3.1. Map of site location. Experimental Lakes Area, northwestern Ontario, Canada.

Reference Enclosures



Nutrient Enriched Enclosures

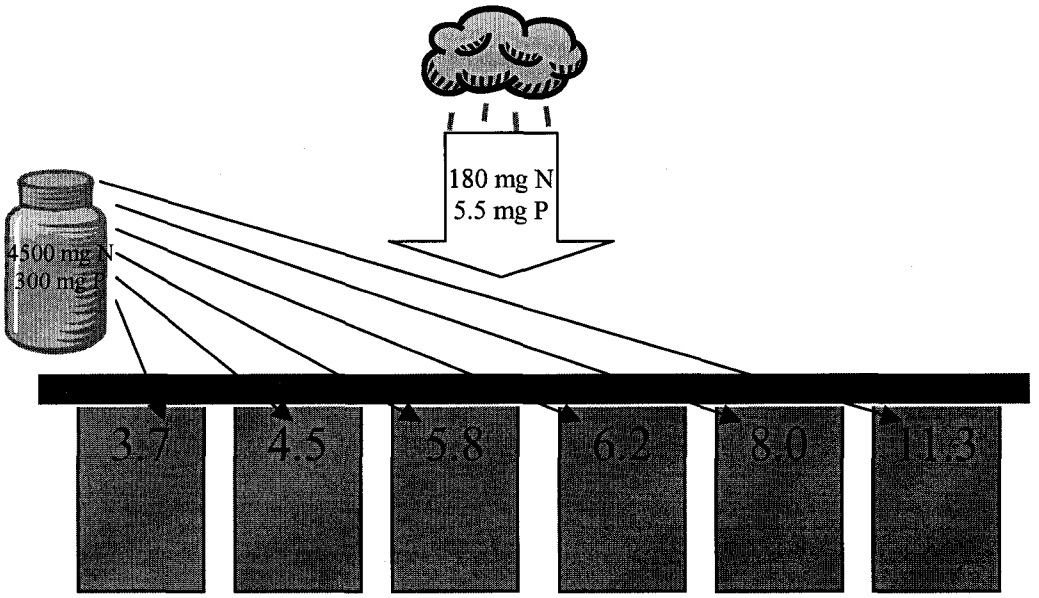


Figure 3.2. Schematic of the lake enclosures used in this study. The values in each of the enclosures indicates the initial DOC concentration in mg L^{-1}. The contribution of N and P from precipitation and the amount of added N and P are also shown.

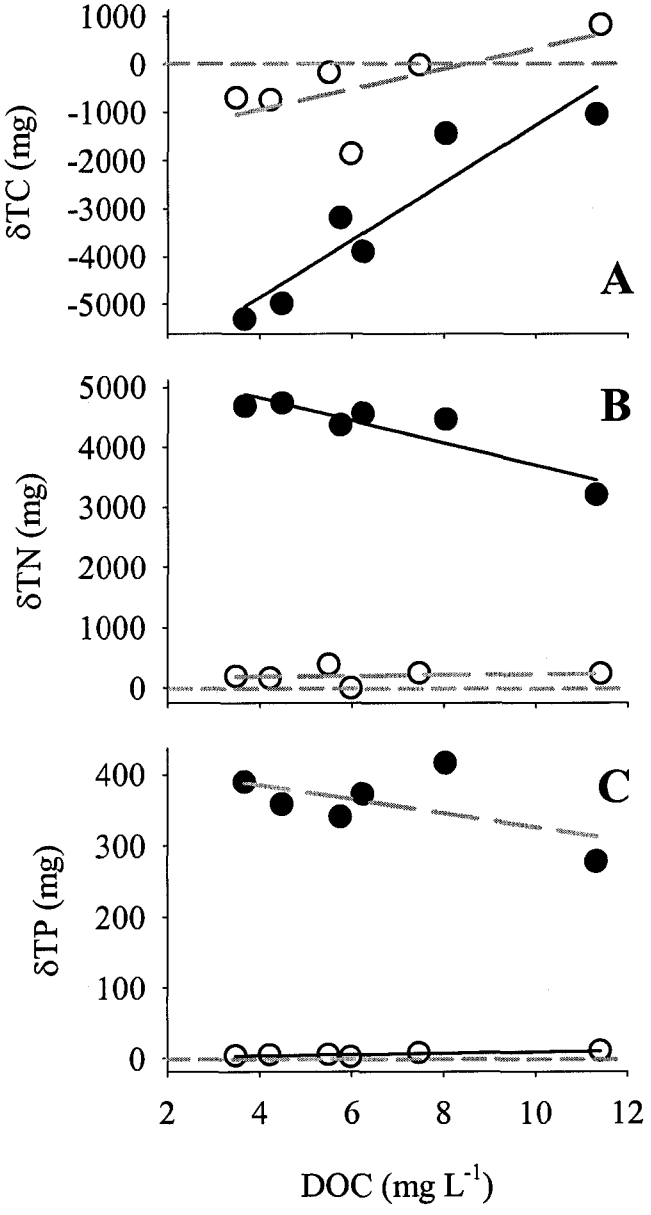


Figure 3.3. Carbon, nitrogen, and phosphorus scavenging (initial – final) dependence on DOC concentration. A. The change in total carbon in the water column (δTC). B. The change in total nitrogen in the water column (δTN). C. The change in total phosphorus in the water column (δTP). The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent a significant regression at $P < 0.05$. The long grey dashed lines represent non-significant regressions. The short grey dashed lines represent the zero or no change value.

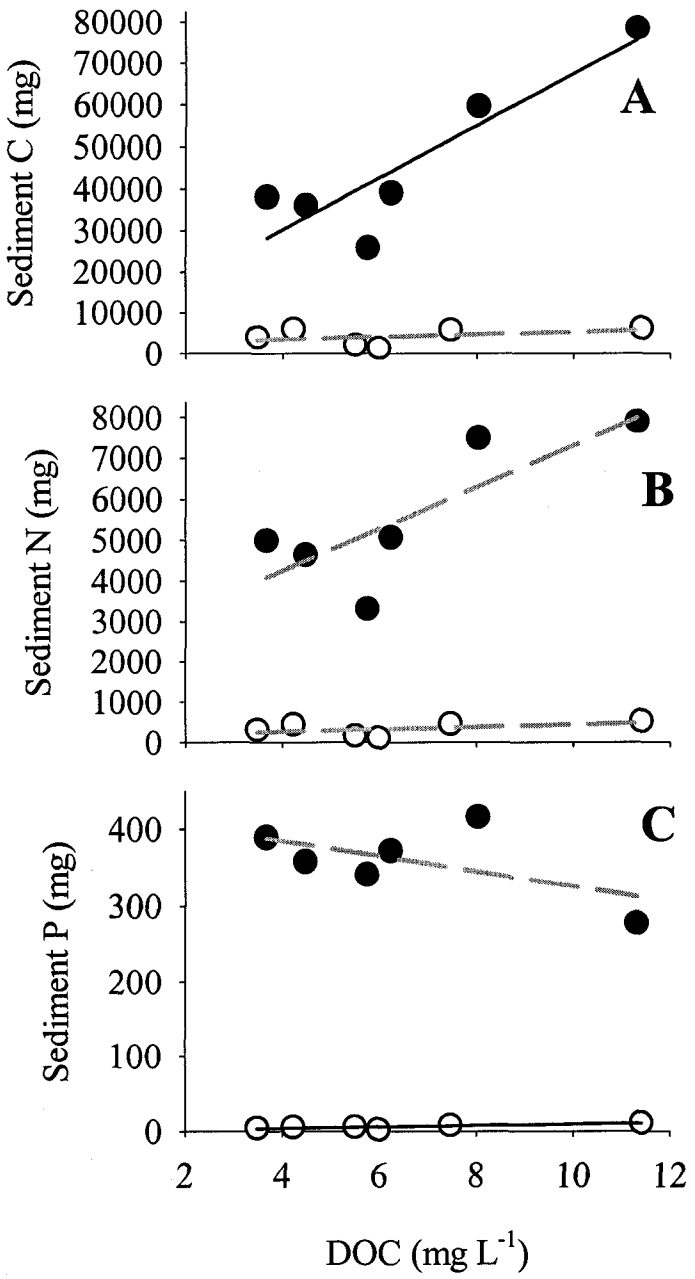


Figure 3.4. Sediment carbon, nitrogen, and phosphorus dependence on DOC concentration. A. Sediment carbon (mg). B. Sediment nitrogen (mg). C. Sediment phosphorus (mg). The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black line represents a significant regression at $P < 0.05$. The grey dashed lines represent non-significant regressions

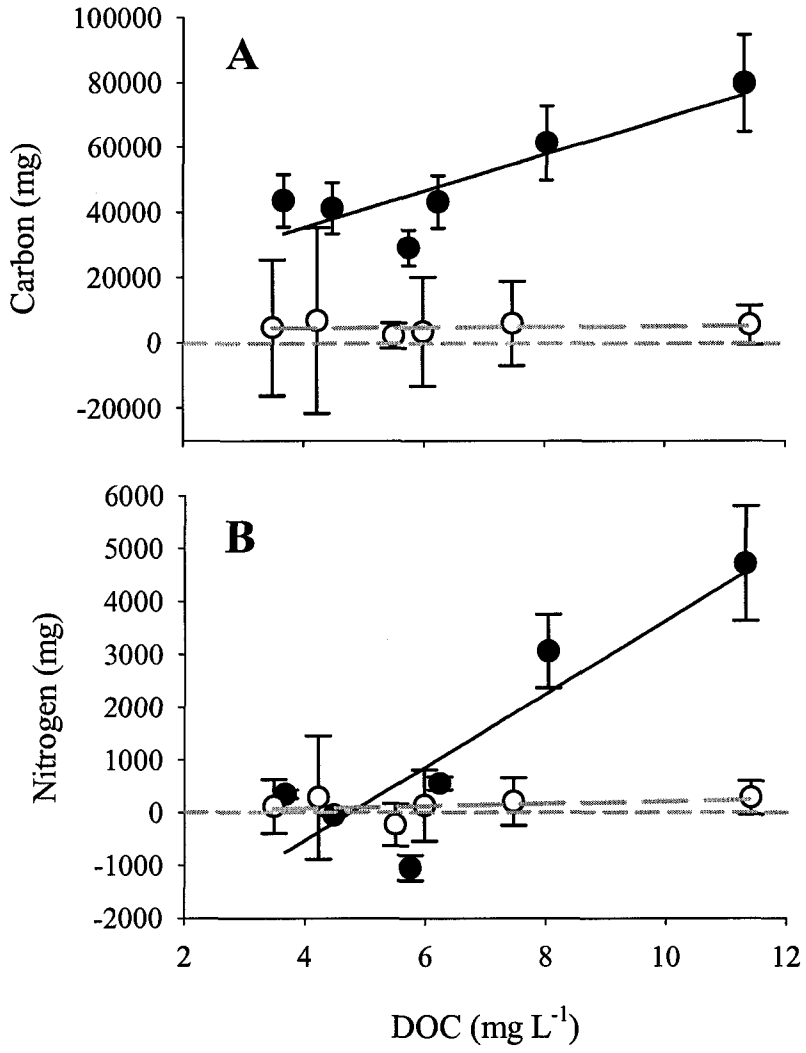


Figure 3.5. Carbon and nitrogen mass balances (the gain or loss from the water column of the lake enclosures) and DOC concentration dependence plotted with absolute errors calculated with a 95% confidence. A. Carbon (mg). B. Nitrogen (mg). The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black line represents a significant regression at $P < 0.05$. The long grey dashed lines represent non-significant regressions. The short grey dashed lines represent the zero or no change value.

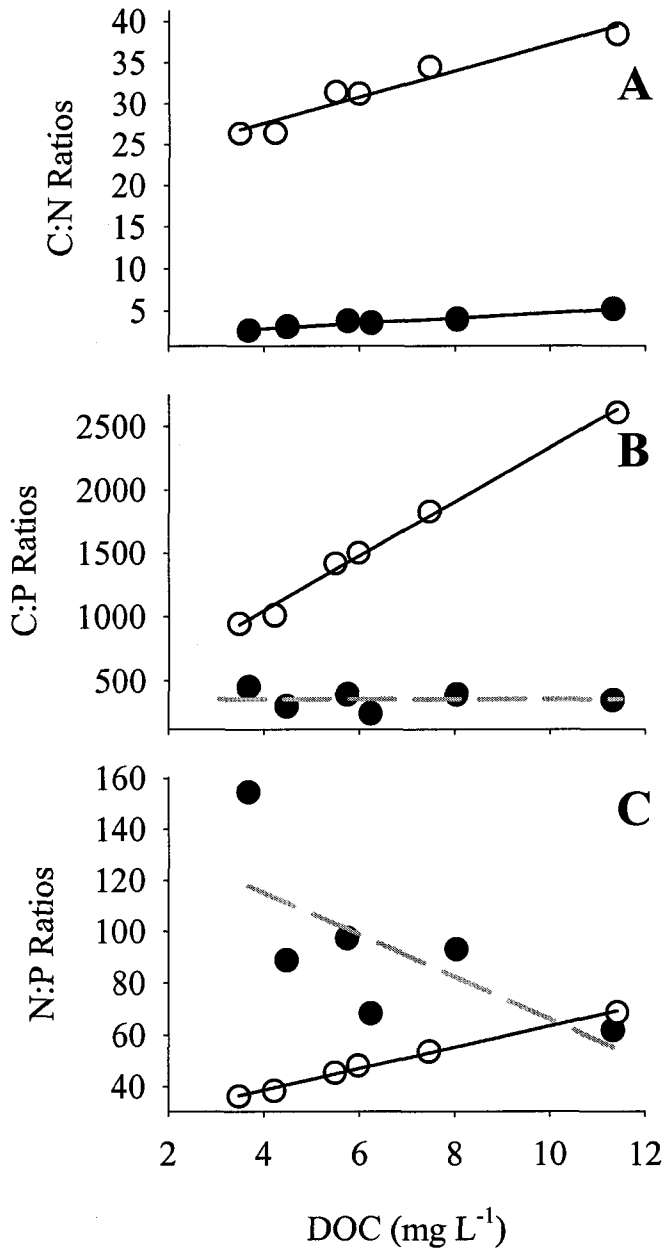


Figure 3.6. DOC dependence of dissolved water column element ratios. A. C:N element ratios. B. C:P element ratios. C. N:P element ratios. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent a significant regression at $P < 0.05$. The grey dashed lines represent non-significant regressions.

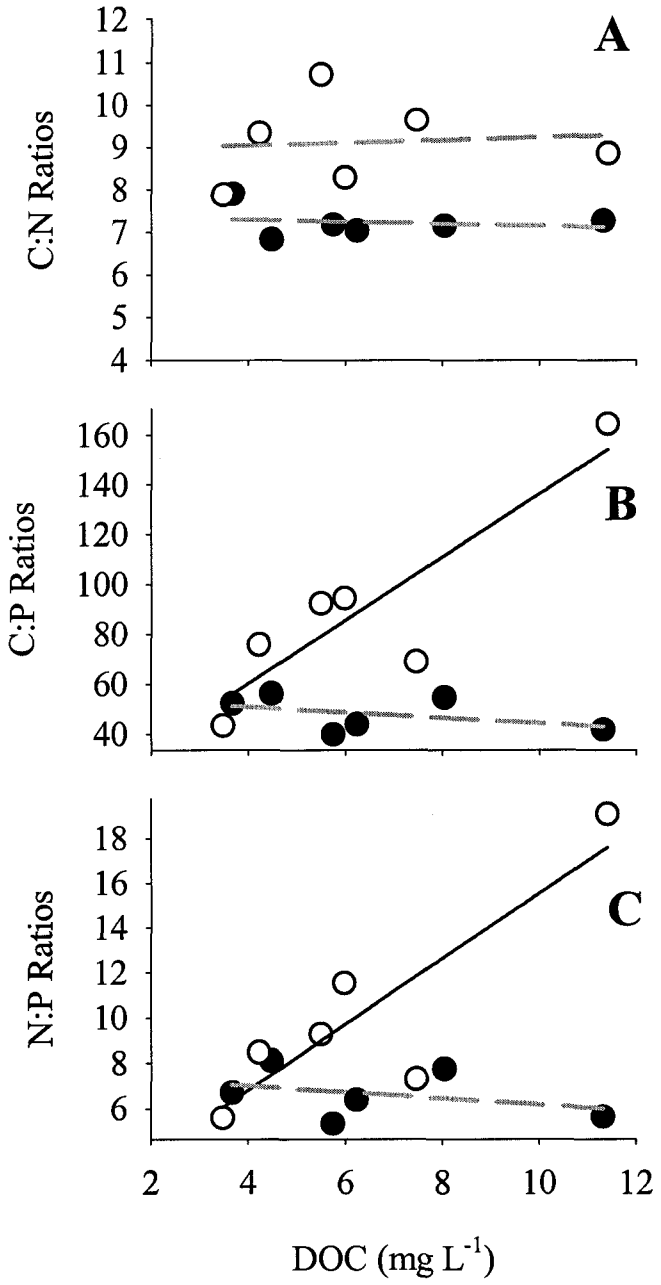


Figure 3.7. DOC dependence of seston element ratios. A. C:N element ratios. B. C:P element ratios. C. N:P element ratios. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent a significant regression at $P < 0.05$. The grey dashed lines represent non-significant regressions.

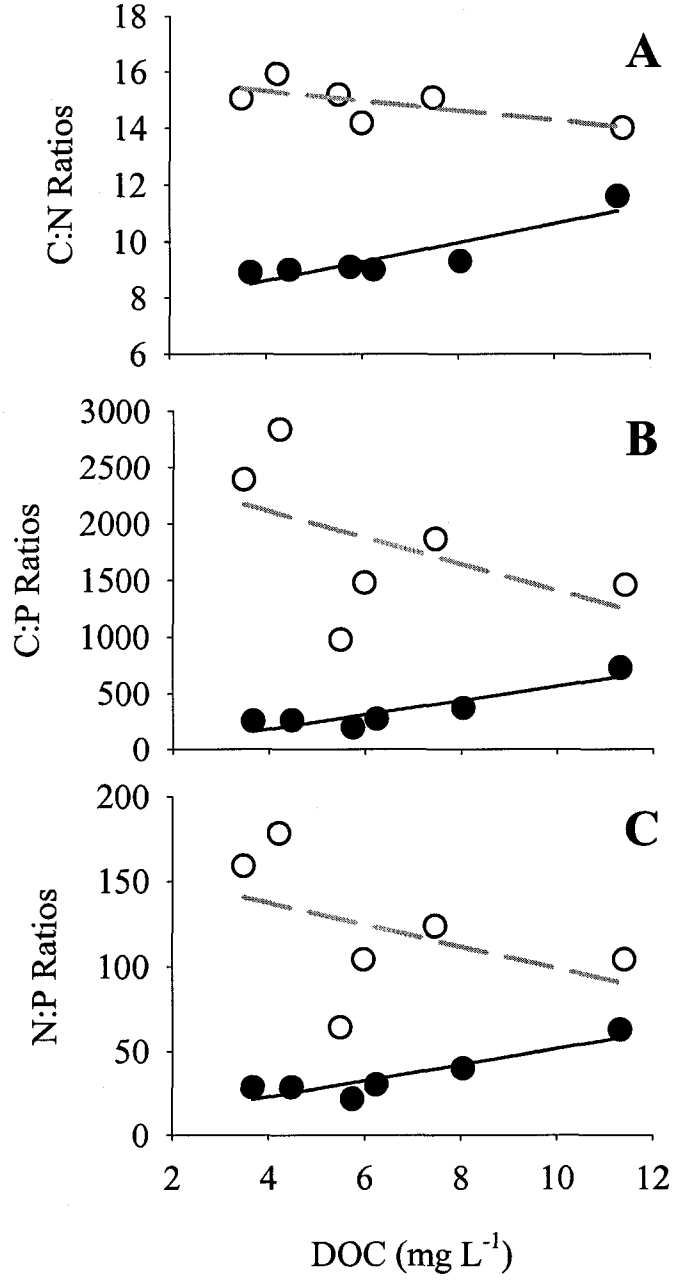


Figure 3.8. DOC dependence of sediment element ratios. A. C:N element ratios. B. C:P element ratios. C. N:P element ratios. The open circles represent the reference enclosures and the closed circles represent the nutrient enriched enclosures. The solid black lines represent a significant regression at $P < 0.05$. The grey dashed lines represent non-significant regressions.

4. Summary and Conclusions

Autotrophic lakes dominate our understanding of food web and nutrient cycling dynamics, whereas allotrophic lakes are far less understood, but they may represent a greater proportion of North American temperate lakes (Currie 1990; Cole et. al. 1994; Kirchman 1994). Autotrophic and allotrophic lakes differ with respect to the microbial metabolism that drives the system and the way nutrients are utilized (Jones 1992; Jansson 1998; Jansson et. al. 2000; Hakanson and Jansson 2002). Dissolved organic matter (DOM) and its ability to attenuate light and complex inorganic nutrients appears to be the primary mechanisms that differentiate these lake types (Jackson and Hecky 1980; de Haan et. al. 1990; Jones 1992; Shaw 1994; Scully and Lean 1994; Morris et. al. 1995; Bukaveckas and Robbins-Forbes 2000). Therefore, the objective of this research was to investigate the dependencies of food web and nutrient cycling dynamics on DOM and inorganic nutrients using lake enclosures.

The food web and nutrient cycling dynamics in the reference and nutrient enriched enclosures differed with DOM concentration and nutrient enrichment. A shift from autotrophic to allotrophic was observed in the reference enclosures across the DOM concentration gradient at approximately 6 mg L^{-1} of DOC, whereas autotrophy dominated in the nutrient enriched enclosures for all DOM concentrations.

Phytoplankton were likely light and nutrient limited in the reference enclosures and, therefore, bacterioplankton could outcompete phytoplankton for available nutrient concentrations. Bacterioplankton could also effectively access additional nutrients to drive productivity through the decomposition of organic matter. DOM stimulated bacterioplankton

growth by acting as a source of both carbon and nutrients. Furthermore, nutrients were recycled rapidly within the water column of the reference enclosures by biota resulting in nutrient deficient sediments.

Phytoplankton were the dominant producers in the nutrient enriched enclosures. Nutrient enrichment stimulated both phytoplankton and bacterioplankton growth, but bacterioplankton also depended on phytoplankton for labile autochthonous DOM production. High biomass turnover rates of both phytoplankton and bacterioplankton and rapid settling resulted in high retention of carbon, nitrogen, and phosphorus in the sediments. Sediments then acted as a source of nutrients to the water column.

Allotrophy likely becomes the dominant trophic state at DOM concentrations greater than the gradient established in this lake enclosure experiment, regardless of inorganic nutrient enrichment. Despite the food web and nutrient cycling differences observed in this study, DOM concentration becomes the primary factor regulating the dynamics at concentrations above 14 mg L^{-1}. Phytoplankton become limited by availability of solar irradiance and possibly inorganic carbon availability at high DOM concentrations. In contrast, bacterioplankton maintained a strong dependence on DOM in the nutrient enriched enclosures. Thus, the transition to allotrophy is a continuous function of DOC concentration, which supports the suggestion that dystrophy is not a discrete trophic state as previous thought (Fig 1.1). Therefore, most lakes likely fall along a continuous distribution of trophic states that are a function of DOC and nutrient concentrations (Fig 4.1).

4.1. Literature Cited

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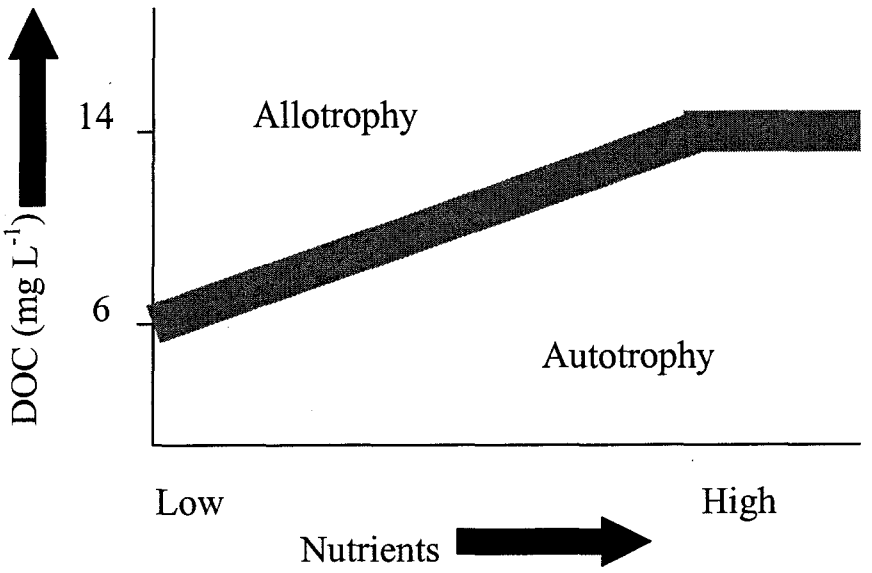

Figure 4.1. Revised autotrophy-allotrophy continuum graph.