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Relationship of nutrients, and mayfly (Ephemeroptera) abundance and diet to periphyton biomass in boreal streams

by

Mari Aurora Veliz ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science

in

Environmental Biology and Ecology
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Edmonton, Alberta

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In loving memory of my grandfather,
George William Harwood.

Abstract

Timber harvesting may increase nitrogen (N) and phosphorus (P) concentrations and mayfly (Ephemeroptera) abundance, three important determinants of stream periphyton biomass. *In situ* nutrient enrichment experiments and nutrient concentration and periphyton biomass surveys in sites upstream and downstream of small-scale logging activities in five streams on the Boreal Plain demonstrated temporal and spatial N limitation of periphyton biomass. To test the hypothesis that mayflies also constrained periphyton biomass, mayfly biomass, density, and diet were evaluated in the five streams. Mayflies were ubiquitous and *Baetis* sp. dominated their assemblage. Mayfly diet comprised mostly material produced instream. The combined surveys and *in situ* experiments demonstrated that both N concentrations and mayflies determined periphyton biomass in undisturbed and harvested sites in streams on the Boreal Plain. The increase of stream N concentrations and mayfly abundance, as seen elsewhere after watershed disturbance, will have important implications for boreal stream periphyton biomass.

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Chapter 1: Background to Study

1.0 Introduction

Many factors including nutrients (Grimm and Fisher 1986), irradiance (Triska et al. 1983; Hill and Knight 1988), velocity and discharge (Horner and Welch 1981; Humphrey and Stevenson 1992), and grazers (Hill and Knight 1987; Peterson et al. 1993) regulate periphyton biomass. However, recent experimental work has demonstrated that the nutrients nitrogen (N) and phosphorus (P) are often primary determinants of enhanced periphyton biomass (Stockner and Shortreed 1978; Grimm and Fisher 1986; Peterson et al. 1993). The development of regional empirical models (Lohman et al. 1992; Dodds et al. 1998) has further supported the role of N and P in determining attached algal abundance.

In addition to the effects on periphyton N and P enrichment of lotic systems typically results in responses at higher trophic levels. For example, the addition of P to rivers increased both periphyton biomass and the abundance of stream herbivores that use algae as a primary food source (Dubé et al. 1997; Harvey et al. 1998). However, in some experiments, increased rates of herbivory obscured nutrient enrichment responses of periphyton (Hill and Knight 1987; Peterson et al. 1993). In undisturbed streams on the Precambian shield, a nutrient gradient across streams was positively correlated with invertebrate abundance (i.e., higher invertebrate abundance in richer sites), but not periphyton biomass (Bourassa and Cattaneo 1998). Thus, increases in periphyton biomass are regulated by resources such as nutrient concentrations (i.e., bottom-up effects), whereas grazing contributes to periphyton biomass loss (i.e., top-down effects).

In streams on the Boreal Plain, little is known about the interaction between N and P concentrations, attached algae, and herbivorous insects, such as mayflies (Ephemeroptera). The paucity of information about potential nutrient enrichment effects in these northern streams is a concern as increased human activity (i.e., forest harvesting and oil and gas exploration) in the region may enhance P and N concentrations in receiving aquatic systems (P - Holopainen and Huttunen 1998; N - Likens et al. 1970). The increase in N and/or P concentrations may enhance the low periphyton biomass found in rivers on the Boreal Plain (Hickman et al. 1982) and in turn, increase stream macroinvertebrate densities, particularly mayflies, which rely on periphyton as a food resource.

This introductory chapter outlines the present understanding of the relationship between P and N, periphyton, and herbivores in streams. I review published literature about the effects of timber harvest on streams with respect to N and P concentrations, and periphyton and benthic macroinvertebrate standing crops. In addition, past survey findings on nutrient concentrations and mayfly biomass in northern streams and potential effects of nutrient enrichment on these systems are summarized. Finally, the main objectives and approaches of the thesis are defined.

1.1 Nutrients, periphyton, and grazers in streams

Periphyton require many nutrients (i.e., carbon, N, P, silica, etc.) for growth and maintenance, however, experimental addition of N and P to rivers has demonstrated the limiting tendency of these two elements in particular. Until recently P was thought to be the main element limiting freshwater algae in both lentic and lotic systems (Hecky and Kilham 1988). Some of the early nutrient enrichment experiments demonstrated P-limitation for stream periphyton (Stockner and Shortreed 1978; Elwood et al. 1981; Peterson et al. 1983). For example, the addition of N alone had no detectable effect on algal growth in streamside channels in Carnation Creek, BC, yet a five-fold increase in periphyton biomass over controls was observed following the tripling of P concentrations in these BC experiments (Stockner and Shortreed 1978). Likewise, the addition of P to the Kuparuk River in Alaska increased periphyton biomass three-fold (Peterson et al. 1983). However, both N and P (Allen and Hershey 1996; Wold and Hershey In press) and N limitation have been reported in some rivers (Grimm and Fisher 1986; Lohman et al. 1991; Bothwell 1992). For example periphyton biomass was three-fold greater in N-enriched channels compared to control or P-enriched channels in an Arizona stream. Similar increases in periphyton biomass were found for N-diffusing substrata (DS) compared to control or P-spiked DS in this Arizona stream (Grimm and Fisher 1986). In undisturbed rivers, N is typically the limiting nutrient for periphyton biomass only when P is in excess relative to N (Grimm and Fisher 1986; Lohman et al. 1991; Bothwell 1992).

Grazing insects contribute to periphyton biomass loss, and may obscure nutrient enrichment effects (see review by Feminella and Hawkins 1995). For example, removal of the mayfly *Ameletus validus* from *in situ* channels in a northern California stream resulted in a five-fold increase in periphyton biomass relative to treatments with four times the ambient density of *Ameletus* (Hill and Knight 1987). Simultaneous regulation of periphyton biomass by grazers and nutrients has been demonstrated in nutrient enrichment experiments in undisturbed streams. Experiments conducted in streamside channels beside a small, forested stream in Tennessee produced a two-fold increase in periphyton biomass in N+P+light enhanced channels that had no snails compared to ambient conditions; effects of single factors (i.e., light, or N+P, or grazers) were undetectable (Rosemond 1993). Peterson et al. (1993) also observed interacting effects of nutrient-addition and herbivory in a whole-stream P-enrichment study in the Kuparuk River, Alaska. Periphyton biomass was 20 times greater in a fertilized reach compared to the control reach for two years following P+N addition however, increased grazer (i.e., Trichopteran and the Ephemeropteran, *Baetis* spp.) densities mitigated periphyton biomass in years 3 and 4 of the experiment (Peterson et al. 1993). Thus, N and/or P concentrations are important determinants of periphyton accumulation, but the multitude of factors that contribute to periphyton standing crop, particularly grazing, may obscure the nutrient/periphyton relationship.

1.2 Forest harvesting effects on streams

Nutrient enrichment experiments conducted in undisturbed streams are typically intended to illustrate potential effects of human activity (i.e., forest cutting, agriculture, or urbanization). Timber harvesting has been known to change discharge, morphology, light availability, thermal regime, and sediment and N+P loading in streams on all continents (see Table 1.1 and reviews by Campbell and Doeg 1989; Keenan and Kimmins 1993). The introduction of N and/or P to streams as a result of timber harvest may enhance the abundance of primary producers and macroinvertebrates.

1.2.1 Nitrogen and phosphorus in streams after timber harvest

Nitrogen leaching into streams after harvest has been documented in many biogeographic regions in North America. Elevated stream N concentrations likely result from reduced vegetative uptake and enhanced organic matter (OM) decomposition (due to higher soil moisture, increased irradiance and temperatures) in the cut areas (Vitousek

1981). In the experimental drainage basin, Hubbard Brook, New Hampshire, nitrate-N concentrations in a head-water tributary draining a 100 % clear-cut and herbicide-treated catchment increased 41-fold one year after harvest. Studies conducted in the Pacific Northwest found comparatively modest increases in N (Feller and Kimmins 1984; Hartman and Scrivener 1990). In southwestern British Columbia, stream nitrate-N outputs were three-fold greater in the first post-clear-cut year compared to the unharvested site (Feller and Kimmins 1984). Hartman and Scrivener (1990) reported a two-fold increase in nitrate-N concentrations in Carnation Creek where 41% of the drainage basin was logged, and a seven-fold increase in nitrate-N concentrations where 100% of the basin of a small tributary was cleared. In the Experimental Lakes Area in northern Ontario, total dissolved N concentrations in streams draining one-year-old clear-cuts were twice the values from the unharvested streams, while concentrations for streams in four-year-old cuts were similar to reference streams (Nicolson et al. 1982). Differences in forestry practices, regional climate, and edaphic conditions contribute to contrasting results from these different regions. For example, litter decomposition may be slower in the drier and colder climate of New Hampshire than the Pacific Northwest, resulting in a forest floor rich in OM available for mineralization after harvest. Furthermore, fallen timber was not removed in the Hubbard Brook catchment, thereby providing material for mineralization. In addition, herbicides applied after logging at Hubbard Brook discouraged vegetative regrowth and the subsequent uptake of nitrate-N. Thus, N movement to aquatic systems following timber harvest depends on climatic conditions as well as forestry practices.

Phosphorus concentrations in North American streams affected by harvest are either not detectably different from reference conditions (Feller and Kimmins 1984; Hartman and Scrivener 1990), or show small increases only during the first year following harvest (Aubertin and Patric 1974; Nicolson et al. 1982). For example, total P (TP) export doubled the first year after harvest in a 100% cut compared to an undisturbed basin in West Virginia (Aubertin and Patric 1974). Mean total dissolved P (TDP) concentrations were also twice as high during July and August in streams with a one-year-old cut (100% cut and ploughed) in northern Ontario compared to streams in uncut and four-year-old cut areas (Nicolson et al. 1982). Dissolved and fine particulate P output were twice as high in a 100% logged and herbicide-treated Hubbard Brook, NH stream compared to an undisturbed basin, but P output increased 10-fold if bedload losses (due to erosion) were included (Hobbie and Likens 1973). Because P can be strongly bound to mineral particles, it has lower mobility and is less susceptible to leaching in most soils than N (Wood et al. 1984; Frossard et al. 1989). However, soil erosion by water may move particulate P to nearby aquatic systems (Hobbie and Likens 1973). In contrast to the North American studies, TP concentrations were five times higher and TDP eight times higher in clearfelled (58% of basin cut) areas compared to preharvest values in Finland (Holopainen and Huttunen 1998). Scarification (ploughing) of 16% of the harvested area likely enhanced P movement. However, scarification of soils in Northern Ontario did not yield dramatic increases in dissolved P as observed in Finland (Nicolson et al. 1982). Thus, differences in basin characteristics, particularly the thin soils and frequent granitic bedrock outcrops in Northern Ontario compared to organic rich material in peatlands in Finland, must contribute to the variability in stream P response to timber harvest.

1.2.2 Periphyton and macroinvertebrate responses to logging

Increased periphyton biomass is typically reported in streams following logging. This increase is assumed to be due to higher irradiance, N and P concentrations, and water temperatures. For most wooded landscapes, photosynthetically active radiation (PAR) is a primary limiting factor for periphyton. For example, nutrient enrichment did not detectably increase periphyton biomass in a shaded low-nutrient stream in Coweeta Experimental Forest, North Carolina. However, in the open-canopied stream, with similar nutrient concentrations, DS had seven-fold greater periphyton biomass than DS in the shaded site. Moreover, there was a trend for nutrient-enriched DS at the open site to have greater periphyton biomass than control DS, indicating light was primarily limiting algal growth in these streams (Lowe et al. 1986). Increased total N (TN), TP, and TDP was reported in two streams subjected to clearfelling in Finland, yet an increase in algal biomass (a 46-fold increase in late summer biomass) was only observed in the stream that had no riparian canopy (Holopainen and Huttunen 1998). In a streamside (i.e., full light conditions) trough experiment, algal biomass was eight-fold greater in a trough enriched with N+P compared to a control trough (Stockner and Shortreed 1978). However, periphyton did not grow in either treatment or control troughs when irradiance was reduced, suggesting light is paramount in determining epilithic biomass. Yet increased light intensity to streams following the removal of streamside vegetation does not ensure increased periphyton biomass. Low nutrient concentrations may ultimately limit epilithic biomass, even when PAR values increase, as in Carnation Creek, British Columbia, where periphyton biomass was not detectably different after harvest, presumably due to limiting P concentrations (Shortreed and Stockner 1983). Increased light availability (due to the removal of streamside vegetation) may increase periphyton biomass in streams where nutrient concentrations are not the limiting factor.

Most studies report higher densities of stream macroinvertebrates within the first five years following timber harvest. Comparison of invertebrate densities for logged and references sites from eight worldwide studies showed a weak trend of increased numbers at logged sites ($P = 0.06$; Figure 1.1). For example, macroinvertebrate densities in logged streams (10 to 70% of basins) of New Zealand, northern California, Alaska, and New England were two to four times greater than reference sites (Graynoth 1979; Newbold et al. 1980; Duncan and Bresven 1985; Noel et al. 1986). In northern California, total fauna abundance was 10-fold higher in the basin that was 100% logged compared to an unlogged basin (Newbold et al. 1980). These studies suggest that increased irradiance, water temperature, and nutrient concentrations following harvest enhance periphyton accumulation which in turn, increase macroinvertebrate numbers. Furthermore, rapid recolonization of certain pioneer species, like *Baetis* spp. and Chironomidae may inflate densities following harvesting (Newbold et al. 1980; Noel et al. 1986; Wallace and Gurtz 1986). After logging in a southern Appalachian hardwood catchment, the density of *Baetis* spp. exceeded that of the reference stream by 20 times. An increase in mayfly numbers was also noted in Carnation Creek (Culp and Davies 1983) and Coweeta Experimental Forest (Tebo 1955), despite the overall negative response of the total macroinvertebrate assemblage to harvest. High fecundity, a multivoltine life cycle (Wallace and Gurtz 1986), and mobility (i.e., drift and swimming capabilities) of *Baetis* spp. likely enable this genus to exploit the increased abundance of periphyton following timber harvest.

Few studies report lower macroinvertebrate densities after forest harvest and of those that did, increased fine sediment loading and associated scouring were implicated. In Coweeta Experimental Forest, a site upstream of an incoming tributary with 100% of its basin logged had twice the abundance of macroinvertebrates compared to the site downstream of the tributary. Unstable fine sediment resulted in turbidity values that were four-fold greater in the site below the harvested tributary, and this likely contributed to the reduction in macroinvertebrates (Tebo 1955). Likewise in Carnation Creek, British Columbia, benthic fauna were two-fold greater in a pre-harvest site compared to an unbuffered site affected by harvest (i.e., 12-41 % of the basin logged). The reduction was ascribed to the 5% increase in fine sediments from streambank erosion during high discharge periods (Culp and Davies 1983). Fine sediments that bounce and abrade the substrate have been found to reduce benthic invertebrate numbers by >50% as compared to areas where fine sediments are deposited (Culp et al. 1986). Newbold et al. (1980) suggested that fine sediment movement may initially reduce invertebrate densities, but stimulated algal production combined with insect recolonization strategies may subsequently increase densities. However, if forestry activities dramatically increase fine sediment concentrations as a result of terrestrial mass wasting and channel destabilization, long-term reductions in invertebrate numbers may result.

1.3 Predicted nutrient enrichment in streams on the Boreal Plain

Generalizations about stream abiotic and biotic responses to timber harvest are confounded by site characteristics such as drainage basin geomorphology, climate, stand composition and age, and edaphic attributes. Proximity of the cutblock to the stream, the size of the logged area, and forestry practices also contribute to variations in stream response. For example, an increase in the channel-forming peak flow after timber harvest was not observed in a Californian basin (67% logged) (Wright et al. 1990) because this region does not experience snowmelt events that typically exacerbate peak flows in other areas affected by logging (Alberta - Swanson and Hillman 1977). Thus, impacts of logging in northern Alberta can only be predicted after considering regional climate and vegetation characteristics, and forestry practices.

Cutting practices and northern Alberta climate and topography are hypothesized to minimize certain effects of harvest on streams in this region. The relatively dry climate and rapid regeneration of aspen (*Populus tremuloides*) might offset any changes in water yield (Richardson and Lund 1975). In addition, post-harvest erosion may not occur because winter logging practices do not disturb the surface layer and relatively small clear-cuts may also minimize basin soil exposure. Furthermore, the relatively low relief in the region may mitigate the effects of soil saturation and mass wasting observed in high relief areas, such as coastal British Columbia (Hartman et al. 1996). Reduced erosion means decreased fine sediment loading to streams. These open-canopied streams are not expected to experience higher irradiance and temperatures. Nevertheless, there is a potential for N+P enrichment of aquatic systems. Experiments conducted on soil from boreal forests have found an increase in soil nitrate concentrations in sites affected by cutting (Walley et al. 1996; N. Carmosini, University of Alberta, unpublished data), and high dissolved P concentrations in undisturbed sites (Evans 1999). The potential for movement of dissolved N+P from soil to streams is at present unclear. The uncertainty of the effects of forest clearing on stream N and P concentrations in this region highlights the need for comprehensive study.

In the open-canopied, undisturbed Boreal Plain streams, periphyton biomass is typically low (2.5 Chlorophyll *a* $\mu g{\cdot}cm^{-2}$, Hickman et al. 1982), much lower than aesthetic nuisance concentrations of 10 to 15 Chlorophyll *a* $\mu g{\cdot}cm^{-2}$ (Welch et al. 1988; Dodds et al. 1998). Although, dissolved nutrient concentrations are high in some Boreal Plain streams compared to other northern systems (Table 1.2), a low molar ratio of dissolved inorganic N:soluble reactive P (<4, Cooke 1996) suggests epilithic algal abundance may be N-limited in these systems. A molar ratio of N:P ratio of ≤ 10 typically signifies a transition from P to N limitation for algal cells (Redfield 1958; Schanz and Juon 1983). In these instances, if nitrate-N concentrations increase after harvest (Likens et al. 1970; Nicolson et al. 1982; Feller and Kimmins 1984), algal growth would likely be stimulated. However, low N:P ratios do not assure N-limitation (Allen and Hershey 1996; Wold and Hershey In press) and some rivers on the Boreal Plain have comparatively low P concentrations (Munn and Prepas 1986, Table 1.2), or have P-limited stretches

(Scrimgeour and Chambers 1997). Thus, N and/or P enrichment from timber harvesting may enhance periphyton biomass in streams.

Alternatively, low periphyton biomass in streams on the Boreal Plain may be related to the dominance of grazing Ephemeroptera, particularly *Baetis* spp. Benthic invertebrate surveys conducted in northern streams indicate that Ephemeroptera is a numerically dominant insect Order. For example, Ephemeropteran species typically comprised between 25 and 55% of the total standing crop of benthic invertebrates from sites in the Vindelälven River basin in northern Sweden and *Baetis rhodani* accounted for 50% of all mayfly nymphs collected (Ulfstrand 1968). In a pristine river in Norway, 10 times more Ephemeropterans were collected compared to either Plecopterans or Trichopterans. Again *Baetis* spp. dominated total numbers, comprising approximately 84% of the samples (Aagaard et al. 1997). In a Rocky Mountain foothills stream of Alberta, mayflies comprised 50% of the total fauna (Zelt 1970). In the Bigoray brown-water stream in Alberta, four taxa, chironomids, ostracods, and the ephemeropterans *Leptophlebia cupida* and *Baetis tricaudatus*, made up 61% of the total yearly fauna (Clifford 1978). A further increase of *Baetis* spp. following timber harvest, as indicated in other logging studies (Newbold et al. 1980; Noel et al. 1986; Wallace and Gurtz 1986), may overwhelm any stimulation of primary producers. Thus, nutrient enrichment effects attributable to timber harvest might only be seen at the grazer trophic level.

1.4 Summary and research objectives

Previous survey work in streams on the Boreal Plain has shown relatively low periphyton biomass (Hickman et al. 1982), potentially N or P limiting conditions (Cooke 1996, Scrimgeour and Chambers 1997), and high mayfly numbers (Clifford et al. 1973; Clifford 1978). To date, there has been no attempt to establish whether N or P concentrations or grazer densities determine periphyton standing crop in these northern systems. Furthermore, increased forest clearing in this region may enhance stream N and P concentrations (Likens et al. 1970; Holopainen and Huttunen 1998) which may increase periphyton biomass, and the standing crop of herbivorous stream insects. Thus, the goals of this study were to evaluate potential N and/or P limitation of periphyton (Chapter Two), and to determine the role of the dominant primary consumer (mayflies) in regulating periphyton abundance of boreal streams (Chapter Three). To address potential timber harvesting effects, these relationships were examined in five streams that had small adjacent clearcuts. The specific objectives and hypotheses of the projects are found in Table 1.3. My project is a part of a larger project, TROLS (Terrestrial, Riparian, Organisms, Lakes, and Streams) that evaluated the effects of timber harvest on lakes, streams, and the terrestrial ecosystems in northern Alberta.

Table 1.1: Abiotic effects of timber harvest on adjacent streams. [a]PAR = Photosynthetically Active Radiation

Effect	Location	Reference
Hydrology		
Stream water yield increase	Review	Bosch and Hewlett 1982
	New Hampshire	Likens et al. 1970
Peak flow increase	Oregon	Harr 1986
	Western Alberta	Swanson and Hillman 1977
Loading of Fine Sediments		
Basin soils	Review	Gibbons and Salo 1973
	British Columbia	Hartman et al. 1996
Streambank erosion	British Columbia	Hartman et al. 1996
Roads	Pennsylvania	Tebo 1955
	Oregon	Beschta 1978
Light and Temperature		
Decreased canopy cover	New England	Noel et al. 1986
Higher PAR[a] levels	Alaska	Duncan and Brusven 1985
	Northern California	Hill and Knight 1988
Higher temperatures	Pennsylvania	Lynch et al. 1984
	United Kingdom	Gray and Edington 1969
Higher degree days	British Columbia	Culp and Davies 1983
Organic Matter		
Leaf litter decreased	British Columbia	Culp and Davies 1983
	Washington	Bilby and Bisson 1992
Higher dissolved organic carbon values	Finland	Holopainen and Huttunen 1998
Elements		
Cation increase	New Hampshire	Likens et al. 1970
Nitrogen increase (see section 1.2.1)		
Phosphorus increase (see section 1.2.1)		

Table 1.2: Nitrogen (N) and phosphorus (P) concentrations during the openwater season in undisturbed northern streams. n = number of streams. [a] interpolated from graph [b] N and P concentrations may be inflated due to analytical problems (Mitchell and Prepas 1990). ELA is Experimental Lakes Area. n.d. = not detected. NO_2+NO_3 is nitrite+nitrate, NH_4 is ammonium, DIN is ammonium +nitrite+nitrate, TN is total nitrogen, SRP is soluble reactive phosphorus, TDP is total dissolved phosphorus, and TP is total phosphorus.

Region	Study	Notes	NO_2+NO_3 ($\mu g \cdot L^{-1}$)	NH_4 ($\mu g \cdot L^{-1}$)	DIN ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)	SRP ($\mu g \cdot L^{-1}$)	TDP ($\mu g \cdot L^{-1}$)	TP ($\mu g \cdot L^{-1}$)
<u>Boreal Plain</u>									
Baptiste Lake Tributaries, AB (*n* = 2)	Cooke 1996	range in monthly mean	21 - 62	11 - 92			12 - 87	24 - 100	80 - 239
Athabasca River Tributaries, AB (*n*=2)	Munn 1984	P – range in monthly mean N - 2 dates	1 - 3			460 - 769		9 - 23	14 - 48
Athabasca River Tributaries, AB (*n*=5)	Hickman et al. 1982[b]	annual mean	214					60	
<u>Boreal Shield</u>									
Québec Streams (*n* = 4)	Naiman 1982	ranges presented	1 - 199			49 - 623	1-17		5 - 29
Montmorency, Québec (*n*=1)	Bechara et al. 1992	range in monthly mean	63 - 69				3 - 8		
ELA Streams, ON (*n* = 2)[a]	Nicolson et al. 1982	range in monthly mean	20 - 40	15 - 21				9 - 13	
North Superior Streams, MN (*n* = 1)[a]	Allen and Hershey 1996	range in monthly mean			12 - 150		3 - 9		
<u>Alaska</u>									
Kuparuk River (*n*=1)	Peterson et al. 1993	mean values	10	20			n.d	10	
Covered Creek (*n*=1)	Duncan and Brusven 1985[b]	range in monthly mean	<10 - 30				<10		

Table 1.3: Specific objectives and hypotheses tested on the effects of nutrient limitation, grazing, and their interactions on periphyton biomass in streams on the Boreal Plain.

Objective	Description	Hypotheses	Location in the Thesis
Nutrients			
Contribute to existing information about nutrient (nitrogen, N and phosphorus, P) concentrations and periphyton biomass in boreal streams.	Nutrient concentrations were measured in 5 streams during the openwater season in 1996 and 1997.		Chapter 2
Determine an appropriate methodology for nutrient limitation testing.	A pilot nutrient enrichment experiment with diffusing substrata (DS) was conducted.	Reduced grazing pressure (via insecticide application) will increase periphyton biomass on DS.	Appendix A
Establish if periphyton are limited by N and/or P in Boreal Plain streams.	Periphyton field surveys and *in situ* nutrient enrichment experiments with DS were conducted concurrently.	Periphyton biomass will be greater on N and N+P DS compared to control DS.	Chapter 2
Determine if timber harvest (<3% of watershed logged) 1) caused nutrient enrichment, 2) enhanced periphyton biomass, or 3) alleviated nutrient limitation.	Nutrient concentrations and periphyton biomass were compared upstream and downstream of harvested areas. Results from the nutrient limitation experiment were also compared upstream and downstream of harvested areas	1+2) N and P concentrations and periphyton biomass will be greater in downstream sites compared to upstream sites. 3) In sites upstream of harvested areas, periphyton biomass will be higher on N-DS compared to control DS; in sites downstream of harvested areas, periphyton biomass will not be detectably different between treatments.	Chapter 2

Table 1.3 (continued)

Objective	Description	Hypotheses	Location in the Thesis
Mayflies			
Describe the abundance and assemblage composition of mayflies in streams of the Boreal Plain.	Field survey was conducted in 5 streams in October 1996 and 1997.		Chapter 3
Relate mayfly abundance to nutrient limitation response (Chapter Two results).	Field survey was conducted in 5 streams (October 1997).	There will be more mayflies in sites where a nutrient enrichment response was not detected.	Chapter 3
Determine abundance of *Baetis* sp.	Field survey was conducted in 5 streams in October 1996 and 1997.		Chapter 3
Identify food preferences of mayflies.	Carbon (C) and N stable isotope ratios of mayflies and their potential foods in 5 streams over 3 seasons were compared.	Periphyton will be more important than terrestrial detritus in mayfly diet.	Chapter 3
Determine if mayfly families have similar diets.	Stable isotope ratios of C and N were compared for the 4 mayfly families.		Chapter 3
Determine if timber harvest (<3% of watershed logged) 1) enhanced individual weight of *Baetis* sp., 2) increased mayfly and *Baetis* sp. abundance and biomass, or 3) altered mayfly assemblage composition.	1) Weight of *Baetis* sp. compared for upstream and downstream sites in one stream. 2) Mayfly and *Baetis* sp. abundance and biomass compared upstream and downstream. 3) Mayfly genera composition and abundance compared upstream and downstream.	1) Individual *Baetis* sp. will be larger in sites downstream of harvested areas. 2) Biomass and density of mayflies, particularly *Baetis* sp., will be higher in sites downstream of harvested areas. 3) Less diverse mayfly assemblage in sites downstream of harvested sites due to a higher proportion of *Baetis* sp.	Chapter 3

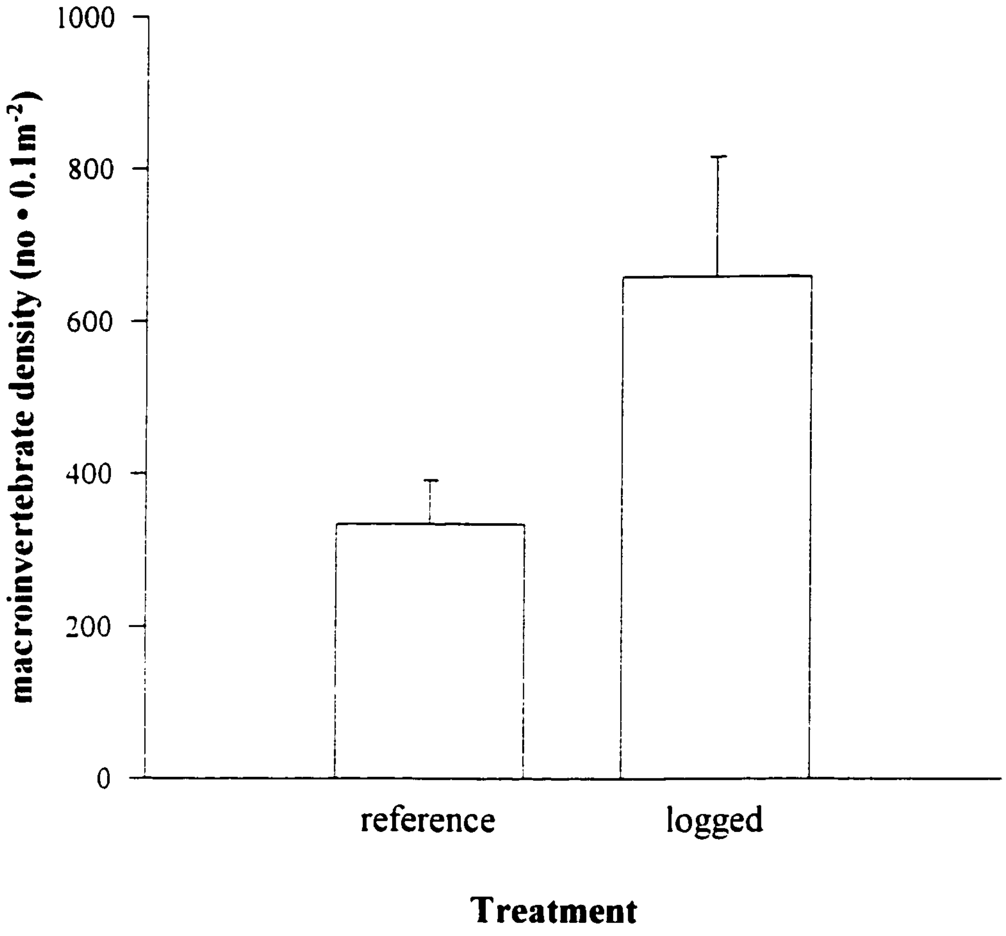

Figure 1.1: Mean (±1 *SE*) macroinvertebrate density (no • 0.1 m^{-2}) in reference (n=30) and logged sites (n=33) (t = 1.95; P= 0.06) (data from Graynoth 1979; Newbold et al.1980; Murphy et al. 1981; Culp and Davies 1983; Silsbee and Larson 1983; Duncan and Brusven 1985; Noel et al. 1986; Growns and Davies 1994).

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Chapter Two: Nutrient limitation in streams on the Boreal Plain

2.0 Introduction

In many streams, the nutrients nitrogen (N) and phosphorus (P) limit attached algal biomass (Stockner and Shortreed 1978; Grimm and Fisher 1986; Peterson et al. 1993). In remote northern streams of North America, relatively little is known about nutrient limitation. Increased algal biomass from P addition to a river in Alaska (Peterson et al. 1993) and the relatively low concentration of P found in streams on the Boreal Shield in Québec (Naiman 1982) suggest that P limitation is prevalent. However, recent work in northern Minnesota has found that stream primary producers experience both P and N limitation (Allen and Hershey 1996; Wold and Hershey In press). In two forested streams on the Boreal Plain of north central Alberta, high soluble reactive phosphorus (SRP) concentrations (~60 $\mu g \cdot l^{-1}$) and low molar dissolved inorganic N to SRP ratios (DIN:SRP ~ 3) were observed (Cooke and Prepas 1998). Previous work in aquatic systems has indicated that when molar inorganic N:P ratios fall below 10, algae experience N-limitation (Schanz and Juon 1983; Grimm and Fisher 1986), suggesting that attached algae in streams on the Boreal Plain are potentially N-limited. However, other work conducted in the region has focused on P-export (Munn and Prepas 1986) and indicated potentially P-limiting conditions (Scrimgeour and Chambers 1997). Thus, the little information that does exist about nutrient limitation in streams on the Boreal Plain is contradictory.

Understanding the relationship between the nutrients (N and P) and primary producers in streams on the Boreal Plain is long overdue particularly as forests in this region are increasingly harvested (Alberta Environmental Protection 1996). Across North America, forest harvest has been associated with increased N leaching due to soil disturbance and reduced vegetative uptake of nitrate (Likens et al. 1970; Nicolson 1982). Furthermore, the low relief in the Boreal Plain may enhance post-harvest dissolved P concentrations in surface waters because of the longer leaching period of P rich soils (D'arcy and Carignan 1997; Evans 1999). Although no studies have been undertaken on nutrient export to streams on the Boreal Plain following forest harvest, surveys conducted for Saskatchewan boreal forest soils demonstrated that soil nitrate concentration was more than double in a four-year-old clear-cut site compared to an unharvested aspen-dominated control stand (Walley et al. 1996). Presently, it is unclear if there will be changes in soil or stream P concentration after harvest, or if the increase in soil nitrate concentration after harvest found by Walley et al. (1996), will result in higher stream N and P concentrations and subsequent increased periphyton biomass in streams on the Boreal Plain.

The aim of this study was to test the hypothesis that periphyton are nutrient limited in streams on the Boreal Plain. Two approaches were undertaken: (1) N and P concentrations and periphyton biomass were surveyed in five streams on the Boreal Plain, and (2) *in situ* experiments with nutrient diffusing substrata were conducted to test for N, or P limitation (Fairchild and Lowe 1984). The hypothesis was tested in undisturbed sites and sites subjected to relatively small-scale logging activities (i.e., <3% of the catchments were logged).

2.1 Methods

2.1.1 Study sites

The study streams, Meadow, Cottonwood, Kettle, Pony, and Waddell are located in northern Alberta in the Christina River basin which drains into the Athabasca (Figure 2.1) and, ultimately, the Mackenzie River. All streams are located on the Forestry Management Area of Alberta-Pacific Forest Industries, Inc. Streams ranged from second to fourth order and watershed areas ranged from 36 to 264 km^2. The streams are open-canopied, low-relief, and highly coloured (Table 2.1). Summer (May - mid August) precipitation for the region, based on 1996 and 1997 data from Conklin and Stony Mountain look-out towers, averaged 371 mm (based on Alberta Environmental Protection precipitation data collected from Conklin and Stony Mountain lookout towers in 1996 and 1997). Annual mean discharge for Pony, the only stream with a long-term hydrographic record, is 1.24 m^3•s^{-1} (based on Environment Canada water survey data, n = 12 years). In this stream, high flows occur in response to summer rain events; minimum discharge occurs during winter when the stream freezes solid (Ferguson and Glozier, National Water Research Institute, Environment Canada, Saskatoon, personal communication 1996). Bedrock underlying the basins is sedimentary of La Biche formation (Cretaceous) (Green 1972), overlain by approximately 76 m of glacial till (Alberta Environmental Protection 1997). Soils in the uplands vary from a eutric brunisol in northern basins (Meadow and Cottonwood) to a dystric brunisol in southern basins (Kettle, Pony, and Waddell). In the poorly drained areas in the southern basins, peat deposits are the dominant organic soil parent materials (Masyk, Alberta Research Council, personal communication 1999; Lindsay 1957; Wynnyk 1963). Dominant vegetation is trembling aspen (*Populas tremuloides*); poorly drained sites in the southern basins are also vegetated by black spruce (*Picea mariana*) (Strong and Leggat 1992). The Boreal Plain forest is criss-crossed by seismic cutlines, interspersed with gas and oil wells, and increased logging activities disrupt forest continuity.

My project is a part of a larger project, TROLS (Terrestrial, Riparian, Organisms, Lakes, and Streams), that evaluated the effects of timber harvest on lakes, streams, and the terrestrial ecosystems in northern Alberta. Two aspen stands, each approximately 47 ha and extending approximately 1975 m along the stream, were harvested beside each stream in January 1997. Alberta-Pacific Forest Industries, Inc completed harvesting. The upstream cutblock had an approximate 30-m buffer and the lower block was immediately adjacent to the stream. The area cleared amounted to less than three per cent of basin areas, measured at the most downstream site. Sampling was conducted from three riffles at two sites on each stream: upstream and downstream of the two cutblocks. *In situ* nutrient enrichment experiments were conducted in one riffle at these same sites.

2.1.2 Nutrient and periphyton survey

During the open-water season, water samples were collected monthly at each site on all streams (i.e., 10 sites total) in 1996 (pre-harvest), and every 20 days in 1997 (post-harvest). Samples for soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), nitrite and nitrate-N (NO_2+NO_3) and ammonium-N (NH_4^+) were filtered within 24 h, through pre-rinsed 0.45-µm membrane filters. Samples for TDP were digested by Menzel and Corwin's (1965) potassium persulfate method. All P samples were analysed following the molybdenum blue colorimetric procedure (Murphy and Riley 1962), as modified by Prepas and Rigler (1982). Samples for NO_2+NO_3 and NH_4^+ were analysed on an autoanalyser following the cadmium-copper reduction method of Stainton et al. (1977), and Solorzano's (1969) phenolhypochlorite method, respectively. Dissolved inorganic N was calculated as the sum of nitrite, nitrate, and ammonium. All water samples were analysed at the University of Alberta's Meanook Biological Research Station (Athabasca) or Limnology Laboratory (Edmonton).

Periphyton biomass was determined for samples collected in September and October 1996 and July, August, and October 1997. With the exception of August 1997, biofilm material was scraped from a 9.6 cm^2 area of high biomass on four rocks randomly selected from each riffle. The scrapings from one riffle were pooled, giving a total of three samples (i.e., one sample/riffle), for each site. In August 1997, nine rocks in one riffle per site were sampled. The biofilm was stored immediately on dry ice and in the dark. In the laboratory, each periphyton sample was analysed for Chlorophyll *a* (Chl*a*) ($\mu g \cdot cm^{-2}$) with a Turner Designs®, model 10 fluorometer after a 90% ethanol extraction (Nusch 1980).

To determine timber harvesting effects, differences in SRP, DIN, and Chl*a* concentrations were evaluated in a three-way analysis of variance (ANOVA); test factors included year (1996 compared to 1997) and site (upstream sites compared to downstream sites), with stream as the blocking factor. If the three-way ANOVA yielded interactions ($P<0.05$) as was the case for the SRP data, two-way ANOVAs were performed for each stream (i.e., factors were site and year) at a more stringent significance level ($\alpha = 0.01$). Homogeneity of variance tests were performed for the dependent variable. DIN data were natural log transformed, nitrate data were $1/\sqrt{x+3/8}$ transformed, ammonium data were $\log_{10}$ transformed, and SRP data were not transformed to meet this assumption. Chlorophyll *a* data ($n=131$) did not meet the homogeneity of variance assumption even after $\log_{10}$, natural log, square root, $1/x$, or $1/\sqrt{x}$ transformations. With a large sample size, ANOVAs are considered robust (Underwood 1997) and therefore, non-transformed Chl*a* data were evaluated. Results are reported as means±1*SE*. Patterns between nutrient concentrations and discharge were evaluated with Pearson correlation analysis. Analysis of variance and correlation analyses were performed with SPSS (release 8.0, SPSS Inc. 1997).

2.1.3 Nutrient enrichment experiments

Post-harvest nutrient enrichment experiments with diffusing substrata (DS) were conducted in one stream in June (1997) and five streams in September 1997. A preliminary methods-development study was conducted in four streams in September 1996 (pre-harvest), and is described in Appendix 1. The DS consist of clay flower pots (6 cm high, 11 cm diameter, 325 mL volume) filled with agar (1% w/v) and one of four treatments (N, P, N+P, or control, C). The nutrient-releasing DS had a 0.5 M concentration of KH_2PO_4 (P treatments), or $NaNO_3$ (N treatments). A polypropylene disk (12 cm diameter, 4 mm thick) was sealed to the base of the DS with aquarium safe silicon. Diffusing substrata construction followed Scrimgeour and Chambers (1997).

Diffusing substrata were secured to the stream bottom with tent pegs (20 cm long, 2 cm wide). Forty DS, 10 replicates of each treatment, were randomly attached to the stream bed in one riffle above and another riffle below the harvest blocks in one stream (spring), and five streams (fall) 1997. Except for DS in Pony and Waddell during the fall experiment that were removed after 57 d, DS were incubated in the streams for approximately 29 d. Due to beaver activity, the DS from the upper Kettle site during the fall experiment were not retrieved. Periphyton was scraped from a 19.24 cm^2 area of the highest biomass of each DS and was analysed for Chl*a* following the procedure described above. Differences in Chl*a* concentration on DS were analysed for each site with a 2x2 factorial ANOVA (the presence or absence of N and the presence or absence of P), after data were $\log_{10}(x+1)$ transformed to meet the assumption of homogeneity of variance. Analysis of nutrient concentrations released over 24 h from N+P substrata incubated in 1 L of distilled, deionized water indicated no detectable difference in SRP release rates (P = 0.14) for DS retrieved after 29 compared to 57 d (mean±1*SE*, 3962±227 and 3702±574 $\mu mol \cdot L^{-1} \cdot d^{-1}$, respectively, n =12). Nitrite-nitrate release rates were lower (P = 0.008) for DS incubated for 57 d (2643±355, $\mu mol \cdot L^{-1} \cdot d^{-1}$, n = 12) compared to those in for 29 d (3913 ± 254, $\mu mol \cdot L^{-1} \cdot d^{-1}$, n =12). Nevertheless, DS still contained substantial N and P after 57 d *in situ*.

Stream physical, chemical, and biological variables were measured at the beginning and end of each experiment. Current velocity and water depth were measured 10 cm upstream of each DS except at the lower Waddell site where these data were not collected. Current velocity (measured at 0.6 times water depth) was measured with a model 2000 Marsh Mc-Birney® meter. A Price 1205 mini type current meter was used during the fall 1997 DS removal period. Canopy cover and photosynthetically active radiation (PAR) was recorded twice for each riffle with a Spherical Densiometer (Model

A) and a Licor Quantum Sensor meter (LI 1000), respectively. Light attenuation coefficients were determined as the slope of the relationship between natural log transformed PAR values and 3 water depths: 2 mm and 5 cm below the surface and substrate depth (substrate depth plus 5 cm – light meter base). Due to technical difficulties, PAR was not measured at the Waddell sites during the fall experiment and Cottonwood sites during the spring experiment. Water temperature was measured every hour during all experiments; data were stored on an Onset, one channel temperature logger. Degree days were determined as the sum of the mean daily water temperatures accumulated during each experiment. A modified Type F water level recorder logged water level hourly at downstream sites on three streams (Meadow, Kettle, and Pony) from June to October. Hourly stage was related to manual discharge measurements taken throughout the summer (June-October). Discharge data from the lower Kettle site provided surrogate data for the lower Cottonwood site because both basins were similar in size (121 and 106 km^2, respectively) and manual discharge readings from the 1997 open-water season were similar (P=0.34, 13 readings). Based on drainage basin size, Pony and Waddell discharge were assumed to be similar (264 and 208 km^2, respectively). Water was sampled from each site at the beginning and end of each experiment and analysed for SRP, TDP, NO_2+NO_3 and NH_4, as described previously. Differences in stream physical and chemical factors during the DS experiment were compared among sites with one factor (i.e., site) ANOVAs and post-hoc least significant differences (LSD) comparisons. Data were transformed where necessary and possible to meet the homogeneity of variance assumption (see Appendix B - Table B1). Background periphyton biomass, as Chl*a* concentration (see above), was determined from a random sampling of nine cobbles per riffle at the beginning and end of the experiments and compared to periphyton biomass on the N-enriched DS for each site. Background Chl*a* data were $\log_{10}$ or natural log transformed to meet the assumption of homogeneity of variance. All analyses were completed on SPSS (release 8.0, SPSS Inc. 1997).

2.2 Results

Discharge regimes during the open water season were similar for Pony and Kettle in 1997 (Figure 2.2). Flows peaked at 4.36 and 3.86 $m^3 \bullet s^{-1}$, respectively, following

September rain events. Discharge in Meadow peaked only after spring rains (maximum = 0.79 $m^3 \cdot s^{-1}$). Minimum flows occurred during July: 0.39, 0.22, and 0.08 $m^3 \cdot s^{-1}$ for Pony, Kettle, and Meadow, respectively.

2.2.1 Nutrient and periphyton survey

Nutrient concentrations tended to peak in July, however, high DIN concentrations were also found in the spring of 1996 (Figure 2.3). DIN concentrations were higher in 1996 compared to 1997 (64 ±4.7 and 28±1.9 $\mu g \cdot l^{-1}$, respectively; $P = 0.001$) and DIN:SRP ratios were greater in 1996 (P=0.001; Table 2.1). Similarly, NO_3 and NH_4 concentrations both showed greater concentrations in 1996 compared to 1997 (P=0.02 and 0.04, respectively). Soluble reactive phosphorus concentrations were not detectably different (P >0.05) between upstream and downstream sites for the five streams in 1996 and 1997 and averaged 43±1.5 $\mu g \cdot L^{-1}$. Nutrient concentrations did not appear to track discharge (r=-0.41, P=0.11 and r=–0.42, P=0.09 for DIN and SRP, respectively). Average Chl*a* biomass was low and not detectably different among streams and sites in 1996 and 1997 (1.9±0.4 $\mu g \cdot cm^{-2}$; P >0.05). Thus, temporal trends were found in nutrient concentrations but there were no detectable spatial patterns (upstream compared to downstream sites or among streams) in DIN or SRP.

2.2.2. Nutrient enrichment experiments

During the fall 1997 experiment, periphyton biomass in three of the five streams responded positively to N-enrichment whereas in two streams periphyton biomass did not respond to nutrient enrichment. In N-limited sites (i.e., both upper and lower Waddell, lower Pony, and lower Kettle), N-enriched DS supported greater Chl*a* than Control or P-enriched DS (N factor P<0.001 for each site; Figure 2.4). Although periphyton biomass on the N-enriched DS at the upper Pony site was not significantly higher than biomass on control DS (P=0.11), there was a weak indication of N-limitation at this site (Figure 2.4). Both sites in Meadow and Cottonwood showed no evidence of nutrient limitation in the fall experiment (N and P factors P>0.05 for each site). Thus, periphyton biomass in these five streams varied spatially in N-limitation.

Except for the lower Kettle site where periphyton biomass on N-enriched DS were lower than background values (P<0.001), N-enriched DS in N-limited sites supported higher Chl*a* concentrations than the adjacent substrata ($P<0.05$) (Figure 2.4). Except for the lower Meadow site where background concentrations of Chl*a* were not detectably different from N-enriched DS ($P=0.12$), periphyton biomass on N-amended DS was lower than background concentrations collected from rocks in the no response streams. In the two no response streams, background biomass of periphyton was therefore not enhanced by nutrient addition.

Differences in discharge, water depth and velocity, irradiance and canopy cover during the fall DS experiments, may have contributed to the variable response of periphyton in the different streams. Mean daily discharge was faster in Pony (1.65 $m^3 \bullet s^{-1}$) than Kettle (0.58 $m^3 \bullet s^{-1}$), or Meadow (0.13 $m^3 \bullet s^{-1}$) ($P<0.001$, Figure 2.2). Water depth and velocity were not detectably different among treatments at any site ($P>0.05$), however comparisons amongst sites (August base flow data only) indicated that DS were in deeper water at two no response sites (i.e., downstream Cottonwood and upstream Pony sites; $P<0.005$; Table 2.2). Fastest velocities were recorded at an N-limited site (i.e., downstream Kettle, 0.75 $m^2 \bullet s^{-1}$; $P<0.001$); lower velocities were measured at two no response sites, the upstream Pony and Meadow sites (0.28 and 0.33 $m^2 \bullet s^{-1}$, respectively; $P=0.04$) (Table 2.2). Degree days were higher for DS incubated in Pony and Waddell due to the extended period of this experiment, but did not differ between the lower Kettle and Cottonwood and Meadow sites (Table 2.2). Except for the most covered site (upper Meadow, a no response site) and the most open site (upper Waddell, an N-limited site), canopy cover did not detectably differ amongst sites ($P>0.09$; Table 2.2). With the exception of the lower Meadow (a no response site), where more light was attenuated ($P = 0.007$; Table 2.2), comparison of PAR attenuation coefficients indicated that all sites attenuated light at a similar rate. For all sites, DIN: SRP ratios were <4. DIN concentrations were lower in all Pony and Waddell sites (N-limited sites) compared to Cottonwood (no response sites) and downstream Kettle sites (an N-limited site) ($P<0.04$). Correspondingly, Pony and Waddell sites had lower DIN:SRP ratios than the Cottonwood sites ($P<0.03$). From these comparisons, no pattern in physical or chemical conditions was apparent between all of the no response sites (Cottonwood and Meadow), or between all of the N-limited sites (lower Kettle, upper Pony, and both Waddell sites).

In the spring 1997 assays, periphyton biomass in one no response stream, Cottonwood, was N-limited (N factor $P<0.001$ and 0.03 upstream and downstream, respectively; Figure 2.5). Unfortunately, nutrient enrichment experiments were only conducted in this one stream during the spring. Periphyton in this stream responded positively to N-enrichment in the spring and there was no response in the fall, suggesting that N-limitation in these streams also varies temporally.

2.3 Discussion

Both survey and experimental findings indicated potentially N-limiting conditions in streams on the Boreal Plain. Stream water DIN:SRP ratios were consistently <4. During fall 1997, periphyton in three of five streams were N-limited. One no response stream during the fall was N-limited in the spring. Thus, nutrient enrichment experiments conducted in the five streams demonstrated that, with the exception of one stream (Meadow), periphyton biomass was N-limited at some time in the year. Nitrogen-limitation has been found in streams across North America (Ozark Plateau - Lohman et al. 1991; Arizona Desert - Grimm and Fisher 1986; northern California - Hill and Knight 1988; Triska et al. 1983; central Oregon - Bothwell 1992). To date, N limitation in undisturbed sites from Arizona to Missouri and Oregon has been attributed to relatively high stream P concentrations (Grimm and Fisher 1986; Lohman et al. 1991; Bothwell 1992). Previous work on aquatic systems on the Boreal Plain attributed their relatively high P concentrations to the underlying sedimentary geology and high proportion of organic soils (Mitchell and Prepas 1990; Cooke and Prepas 1998). Thus, relatively P-replete waters in the undisturbed Boreal Plain region may contribute to N-limitation.

Survey findings and experimental results were not always consistent. During the fall experiment the DIN: SRP ratios for all streams were low (<4), indicating potential N-limitation, yet periphyton biomass was enhanced on N-diffusing substrata in only three of five streams. Inconsistency in nutrient limitation status determined from stream water N:P ratios and *in situ* enrichment experiments has been found in other nutrient limitation studies (Munn et al. 1989; Allen and Hershey 1996; Wold and Hershey In press). For example, DIN: SRP ratios for the French River, Minnesota during fall 1991 indicated N-limitation conditions however, bioassay results indicated periphyton were not detectably limited by N or P (Allen and Hershey 1996). Further study of six streams in northern Minnesota found that according to DIN:SRP ratios, *in situ* experiments should have indicated N-limitation however, N+P co-limitation was found in 36 of 72 bioassays and N limitation was only observed in three experiments (Wold and Hershey In press). Similar conflicting interpretation in nutrient limitation status as determined by water chemistry compared to enrichment experiments are also reported for phytoplankton. The dominance of cyanobacteria (7 to 88% of total algal biomass) and low inorganic N concentrations ($<10\ \mu g \bullet l^{-1}$) in lakes on the Boreal Plain indicated potentially N-limiting conditions. Yet, a combination of laboratory bioassays and limnocorrals demonstrated that phytoplankton Chl*a* increased only with the addition of N+P, not N alone (Prepas and Trimbee 1988). Likewise, in a study of Minnesota lakes DIN: SRP ratios did not corroborate nutrient limitation findings (Axler et al. 1994). The failure of N:P ratios to predict periphyton nutrient limitation status may be due, in part, to the fact that this indicator does not consider biological, physical and other chemical factors (i.e., algal community interactions, grazers, irradiance, discharge, silica concentrations etc.) that may overwhelm a nutrient enrichment effect (Lowe et al. 1986; Munn et al. 1989). Findings from the present study and others (Prepas and Trimbee 1988; Allen and Hershey 1996) highlight the importance of experimental procedures that manipulate nutrient availability to determine nutrient limitation of algae.

The lack of an N-enrichment response in two streams in fall 1997 suggests that some factor, or combination of factors may override N-limitation in the Boreal Plain. Factors such as irradiance (Triska et al. 1983; Hill and Knight 1988), velocity and discharge (Horner and Welch 1981; Humphrey and Stevenson 1992), water column nutrients (Stockner and Shortreed 1978; Grimm and Fisher 1986), and grazers (Hill and Knight 1987; Peterson et al. 1993) may control periphyton standing crop. These factors were measured during fall experiments (the importance of grazers at the different sites is discussed in Chapter Three). The most obvious difference among experiments was the incubation period (~29 d for Meadow, Cottonwood, and Kettle compared to 57 d for Pony and Waddell). Periphyton in the 57 d trials and one of the 29 d trials (lower Kettle) responded to N-enrichment, indicating that the 29 d experiment was of adequate duration to detect change. Others have also found that 29 d nutrient enrichment trials were sufficient (Hill and Knight 1988; Winterbourn 1990; Scrimgeour and Chambers 1997), indicating that it is unlikely that differences in incubation period contributed to the different nutrient-enrichment response amongst the streams. Low light values have been implicated as a factor restricting periphyton standing crop in other nutrient enrichment experiments (Lowe et al. 1986; Hill and Knight 1988). In the current study, patterns in canopy cover and PAR attenuation were not consistent between no response and N-limited sites. However, the upper Meadow site was the most densely covered site and PAR was attenuated more rapidly in the lower Meadow site, suggesting reduced irradiance cannot be discounted as a factor limiting algal standing crop in this stream.

Increased velocity and discharge may enhance or reduce periphyton standing crop (Horner and Welch 1981; Horner et al. 1990; Humphrey and Stevenson 1992). In the present study there was no evidence of algal-scouring. Periphyton in streams that had faster discharge (lower Pony and Kettle sites) responded to N addition whereas periphyton did not respond to nutrients in lower discharge conditions (Meadow sites). Furthermore, low water velocity and deep water in the upper Pony site may have confounded an N-limitation response. However, discharge and velocity differences do not adequately explain the lack of N-enrichment in Cottonwood. Discharge in Cottonwood (based on Kettle discharge) was relatively high, yet periphyton did not respond positively to N addition.

There were water column differences in N concentrations among sites. Cottonwood sites typically had greater DIN concentrations, but these concentrations were not detectably higher than the lower Kettle site (an N-limited site). In addition, concentrations in Waddell (N-limited sites) did not differ from Meadow (not nutrient limited) and DIN:SRP were <4 in all sites. Furthermore, there was a trend for no response sites to have lower Chl*a* on N-enriched DS than *in situ* Chl*a*, suggesting that nutrients were not the main factor restricting epilithic biomass in these streams. Survey results of physical and chemical background conditions suggest that low irradiance and low discharge and velocity rates cannot be discounted from overriding N-limitation in one stream. However, no one factor consistently explains the discrepancy between N -limited and no response streams. Further study, particularly involving manipulation of light and discharge rates, is needed to determine the role of other factors in determining periphyton biomass in these streams.

Nutrient concentrations found in these Christina River streams are similar to values observed in forested tributaries of Baptiste Lake (Cooke and Prepas 1998). It is therefore tempting to conclude that N-limitation exists throughout the larger Boreal Plain region. However, the inconsistent response to nutrient-enrichment within the Christina River basin and fluctuating N and P limitation conditions across six streams throughout the openwater season in northern Minnesota (Wold and Hershey In press) suggest that variability in nutrient limitation in undisturbed streams is more common than previously thought. Nutrient enrichment findings from one stream during one season are frequently extrapolated to an entire region (Stockner and Shortreed 1978; Grimm and Fisher 1986; Lohman et al. 1991). Findings from this study and that conducted by Wold and Hershey (In press) indicate that regional-scale extrapolation of a one season, one stream nutrient enrichment experiment may lead to inaccurate conclusions about the factors that limit periphyton standing crop.

Insights into the response of periphyton to nutrient enrichment has management implications for the forests of the western Boreal Plain. Because the streams are open-canopied and drain relatively flat terrain, typical responses of streams to harvest, such as increased light availability and sedimentation (Noel et al. 1986; Hartman et al. 1996), are expected to be minimal after harvest. However, an increase in soil nitrate concentrations following logging as shown in Saskatchewan (Walley et al. 1996) and in coniferous stands in northern Alberta (N. Carmosini, University of Alberta, unpublished results), may be important for stream periphyton. In the current study <3 % of the drainage area of the five basins was logged. In other areas, stream hydrologic responses to harvest are typically observed when 20% or more of a drainage basin is cut (Bosch and Hewlett 1982). Currently no review has been conducted to determine the proportion of a drainage basin that should be logged to result in a stream nutrient-enrichment effect, however it appears that 3% of a drainage basin is below this threshold. Increased human disturbance over a greater proportion of these basins on the Boreal Plain may increase stream N concentrations, with potential for a subsequent enhancement in periphyton biomass.

Nitrogen limitation, in part, explains the relatively low periphyton biomass (~2 $\mu g \cdot cm^{-2}$) found in these streams. However, the variable response of periphyton to N-addition suggests some other factor, or combination of factors overrides N-limitation. The role of dense canopy cover, rapid light attenuation, and low discharge in one stream needs further investigation. N-limitation was demonstrated by the bioassays, and suggested by DIN:SRP ratios but because chemical indicators do not incorporate physical and biological or other chemical factors, ratios did not adequately predict periphyton response to nutrient enrichment. Conclusions from the current study and the study conducted in northern Minnesota (Wold and Hershey In press), indicate that nutrient-limitation may be more variable than previously recognized. Spatial and temporal variability in nutrient-limitation and the inability of chemical indicators to predict enhancement conditions, suggest that nutrient enrichment experiments need to be conducted in different seasons and different streams before results are extrapolated to a larger similar geographic region.

Table 2.1: Summary of physical and chemical attributes for five boreal streams (pre- and post-harvest). All measurements were taken from site downstream of harvested areas. n.a. = not applicable. n.r. = not recorded.

Stream Attribute	Pre-harvest (1996) Mean (±1*SE*)	Post-harvest (1997) Mean (±1*SE*)
Basin Area (km^2)	147 (40)	n.a.
% of Basin Logged	n.a.	1.08
Slope	0.01 (0.002)	n.a.
Bankfull Width (m)	6.9 (0.5)	n.r.
Depth (cm)	23.5 (1.4)	20.1 (2.8)
Attributes that may be affected by harvest		
Water Velocity ($m \cdot s^{-1}$)	0.51 (0.04)	0.49 (0.03)
Canopy Cover (%)	35 (8)	28 (7)
Total Dissolved Phosphorus ($\mu g \cdot L^{-1}$)	54.8 (2.5)	47.9 (2.5)
DIN:SRP ratio	3.7:1	1.5:1

Table 2.2: Mean (±1SE) background physical and chemical conditions for 1997 nutrient enrichment experiments with diffusing substrata. [a]Spring experiment is missing the first day of temperature data; fall experiment is missing the last day of temperature data. [b]Spring experiment is missing the first 2 days of temperature data; fall experiment is missing the last 2 days of temperature data. n.r. is not recorded. DIN is ammonium+nitrite+nitrate and SRP is soluble reactive phosphorus.

Stream	Site	Incubation Period (d)	Average Depth (m)	Average Velocity (m•s^{-1})	Average Daily Temperature (°C)	Degree Days	Canopy Cover (% covered)	Light Attenuation Coefficient	DIN (µg•L^{-1})	DIN:SRP
<u>Spring</u>										
Cottonwood	above	30	0.28 (0.01)	0.39 (0.0001)	10.3^a (0.6)	308^a	18	n.r.	25 (7.4)	2.8
	below	29	0.23 (0.01)	0.42 (0.0002)	10.8^b (0.5)	302^b	42	n.r.	18 (0.04)	1.6
<u>Fall</u>										
Meadow	above	29	0.22 (0.01)	0.33 (0.0002)	12.1 (0.4)	363	74	n.r.	21 (1.9)	1.4
	below	31	0.23 (0.01)	0.52 (0.0002)	11.8 (0.4)	378	8	3.44	29 (1.9)	1.5
Cottonwood	above	29	0.22 (0.004)	0.37 (0.0002)	11.3 (0.5)	339	12	1.32	37 (6.3)	3.5
	below	29	0.30 (0.01)	0.58 (0.0001)	11.3 (0.5)	338	29	1.69	35 (9)	2.3
Kettle	above			DS not	removed					
	below	28	0.22 (0.01)	0.75 (0.0004)	12 (0.4)	346	30	1.78	38 (2)	1.8
Pony	above	57	0.31 (0.01)	0.28 (0.0002)	n.r.	n.r.	50	1.52	17 (0.3)	1.0
	below	57	0.26 (0.01)	0.48 (0.0003)	$9.6 (0.5)^a$	545^a	24	1.18	17 (0.4)	0.9
Waddell	above	57	0.26 (0.01)	0.59 (0.0004)	$10.7 (0.7)^a$	609^a	3	n.r.	19 (1.6)	1.1
	below	58	n.r	n.r.	$10.9 (0.5)^b$	632^b	23	n.r.	18. (7.7)	0.9

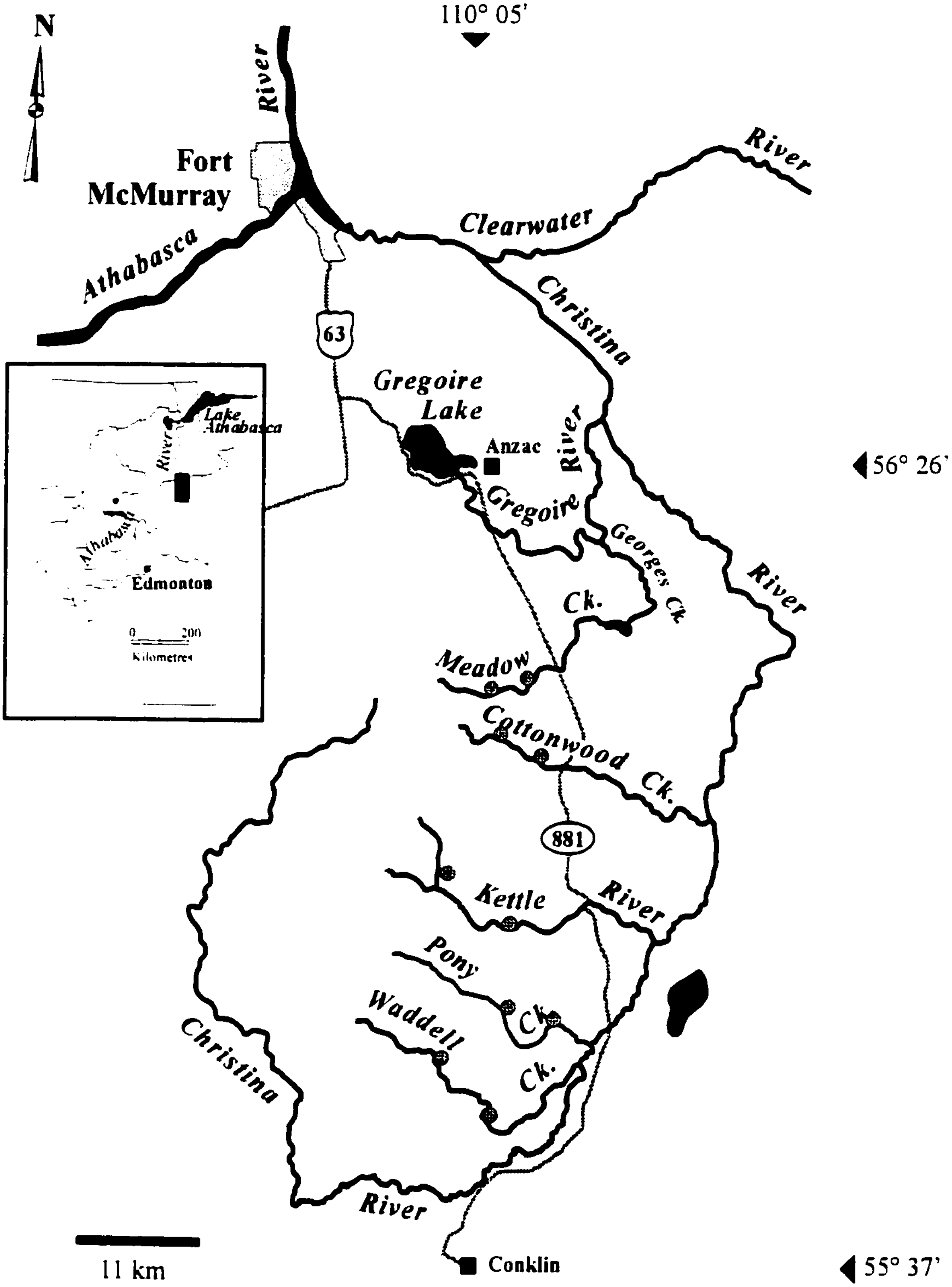

Figure 2.1: Map of Alberta (inset) and location of study streams. Sites (●) are upstream and downstream of harvested areas.

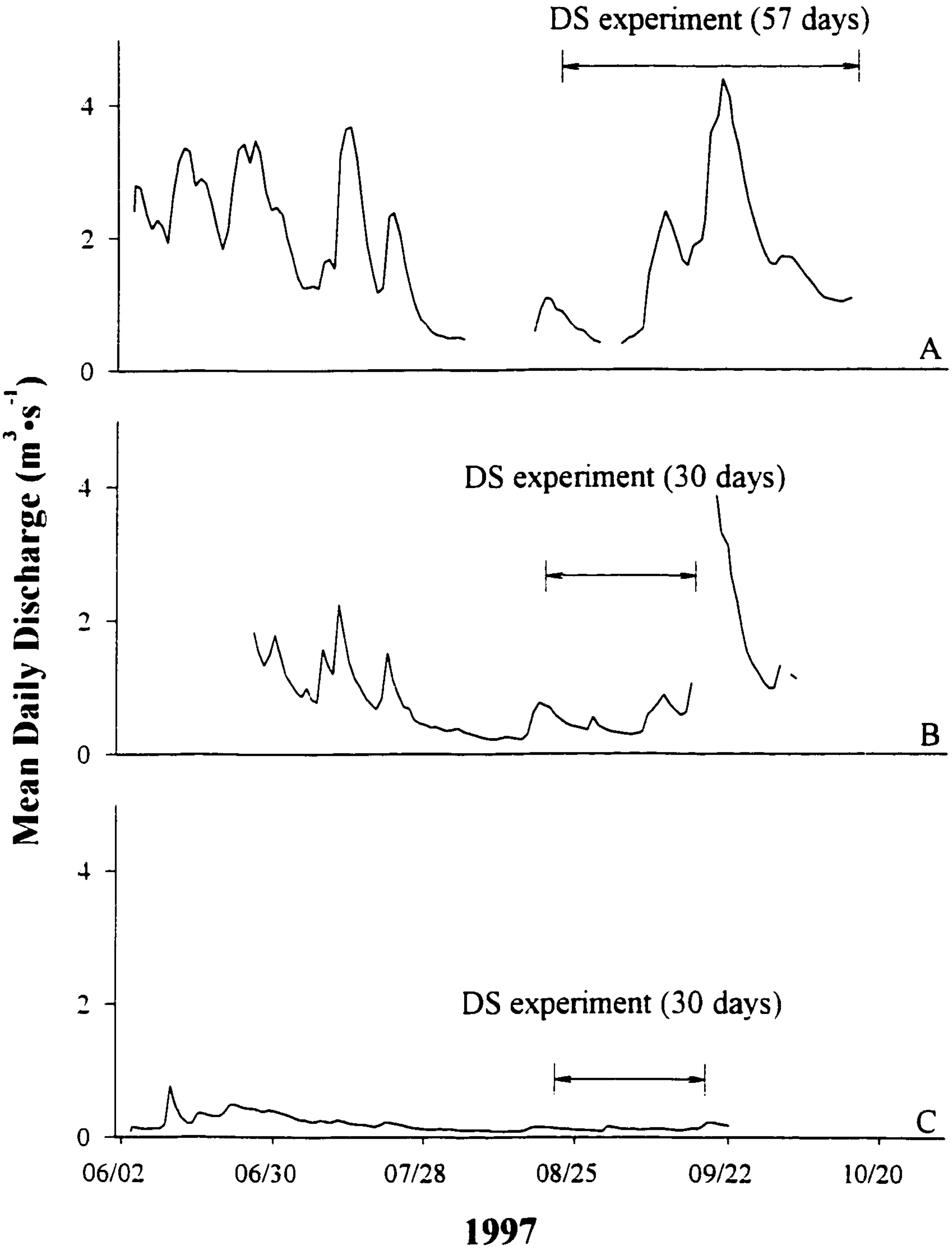

Figure 2.2: Mean daily discharge (m^3•s^{-1}) in three boreal streams, Pony (A), Kettle (B), and Meadow (C) 1997. Lines indicate period of nutrient enrichment experiments, with diffusing substrata (DS). Periods where discharge was not measured are shown as a broken line.

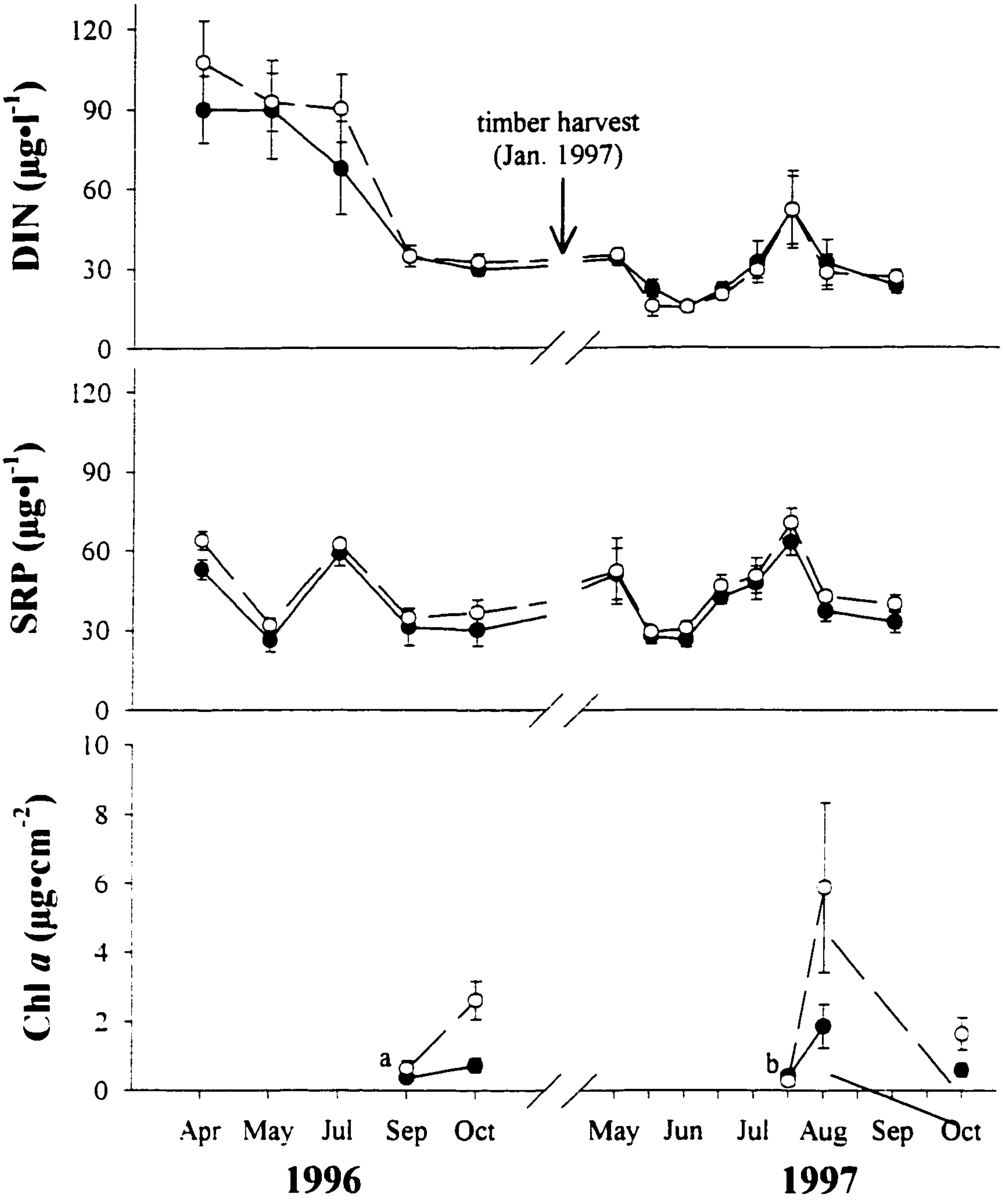

Figure 2.3: Mean (±1*SE*) dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP) concentrations and periphyton biomass (expressed as Chlorophyll *a* (Chl*a*) concentration) for sites upstream (●) and downstream (○) of harvested areas in five boreal streams in 1996 (pre-harvest) and 1997 (post-harvest). In all cases *n*=5 streams, except for [a] where Pony was not sampled (*n*=4 for both upstream and downstream sites and [b] where the upstream site at Kettle was not sampled resulting in *n*=4 for upstream sites.

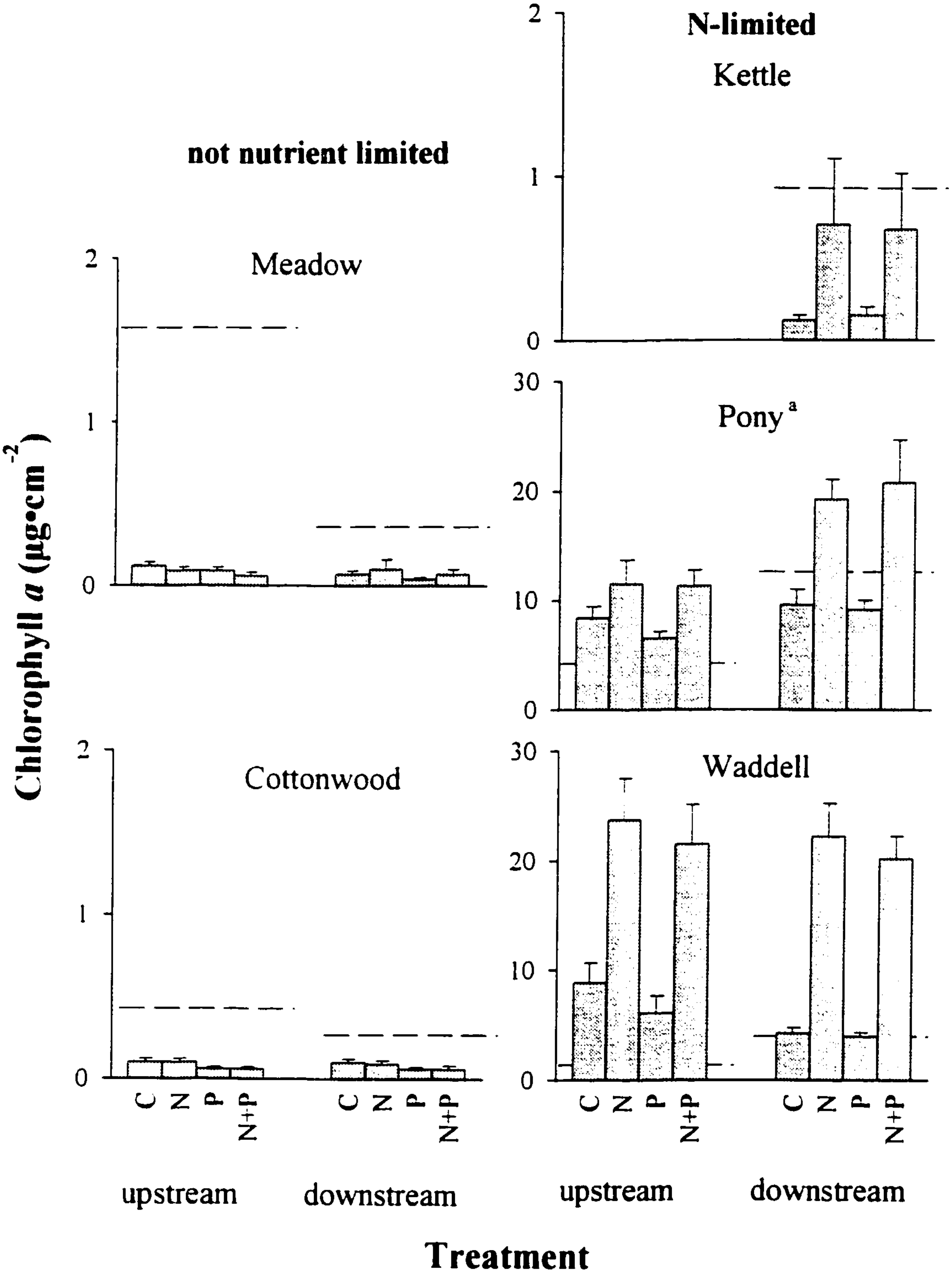

Figure 2.4: Periphyton biomass (expressed as Chlorophyll *a* concentration, mean±1*SE*) on four nutrient diffusing substrata treatments in five boreal streams in fall 1997 (post-harvest). C = Control, N = Nitrogen, and P = Phosphorus. — — — indicates periphyton Chlorophyll *a* concentration on nearby rocks. [a] Periphyton not N-limited at upper Pony.

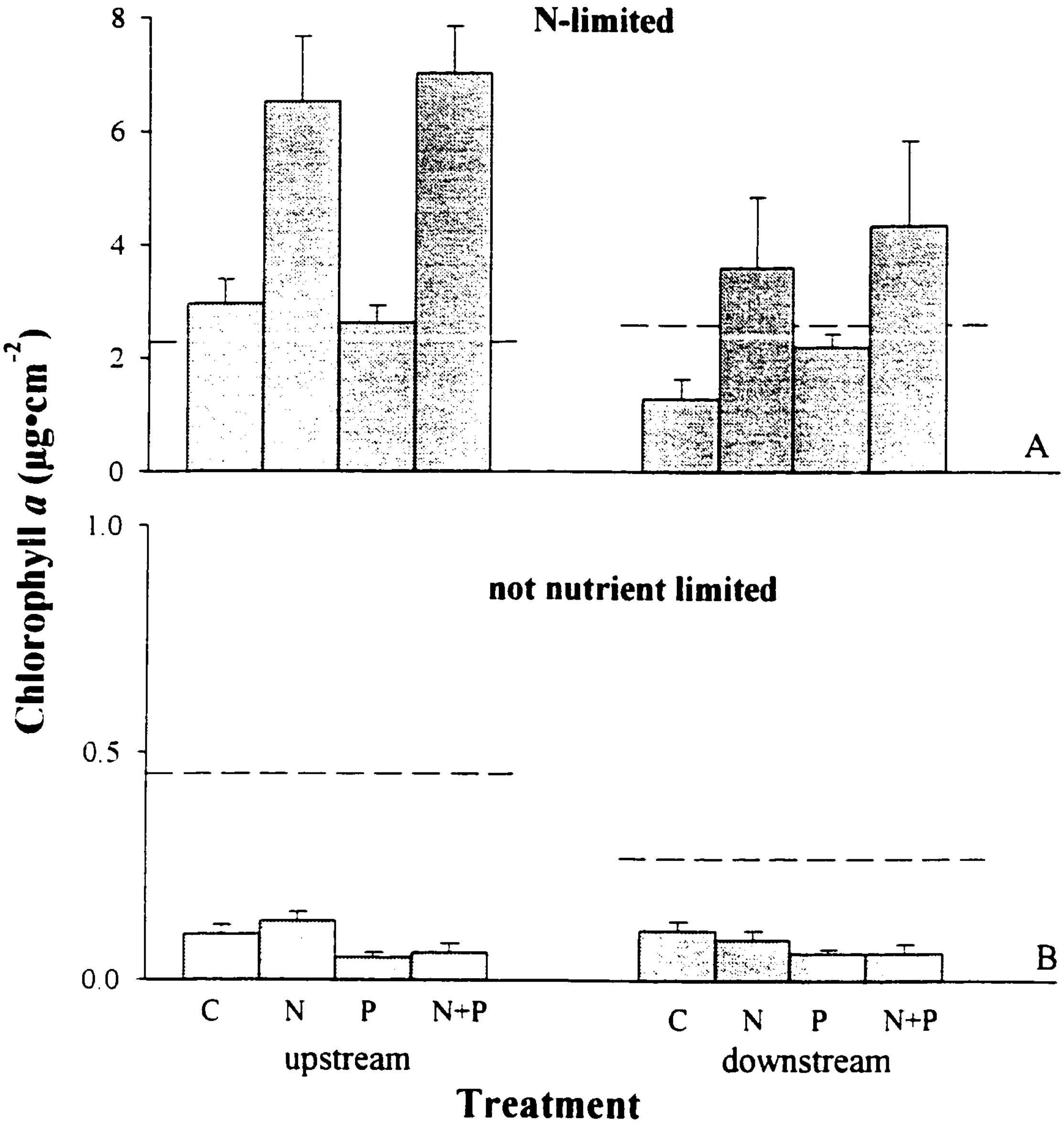

Figure 2.5: Periphyton biomass (expressed as Chlorophyll *a* concentration, mean±1*SE*) on four nutrient diffusing substrata treatments placed upstream and downstream of harvested areas in one boreal stream, Cottonwood. Experiments conducted in June (A) 1997 and September (B) 1997. C = Control, N = Nitrogen, P = Phosphorus. — — — indicates periphyton Chlorophyll *a* concentration on nearby rocks.

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Chapter Three: Mayflies (Ephemeroptera) in streams on the Boreal Plain

3.0 Introduction

Low periphyton biomass typical of undisturbed northern streams may be related to bottom-up limitation by low nitrogen (N) or phosphorus (P) availability (Chapter 2; Allen and Hershey 1996; Peterson et al. 1983), or by top-down grazing pressures from invertebrate herbivores (see Feminella and Hawkins 1995 for review). Currently, little is known about the relationship between periphyton and dominant primary consumers such as mayflies (Ephemeroptera) in northern streams. In an Alaskan stream, enhanced periphyton standing crop following P-enrichment was mitigated by an increase in a grazing insect guild that included mayflies (Peterson et al. 1993). Similarly, a positive relationship between total P and periphyton biomass was obscured by grazers in Québec streams (Bourassa and Cattaneo 1998). In streams on the Boreal Plain, results from my nutrient enrichment experiments suggested that a combination of factors masked N-limitation spatially and temporally (Chapter 2). In particular, the dominance of mayfly grazers in northern benthic communities (Ulfstrand 1968; Clifford et al. 1973) may make it difficult to detect N-limitation.

Previous research on mayflies in streams on the Boreal Plain focused primarily on life history characteristics and diet (Clifford 1978; Hamilton and Clifford 1983). These diet studies were qualitative because amorphous material in the gut, the dominant food item, could not be separated into terrestrial (allochthonous) or instream produced (autochthonous) organic matter. To date, a comprehensive study on the abundance of mayflies and their effect on periphyton standing crop in streams on the Boreal Plain has not been attempted. In addition, observations of increased mayfly density (particularly of *Baetis* spp.) following logging (Newbold et al. 1980; Noel et al. 1986; Wallace and Gurtz 1986) suggest that disturbance in Boreal Plain forests may influence the relationship between Ephemeroptera and periphyton. Higher mayfly abundance following timber harvest has been attributed to increased biomass of periphyton which is assumed to be caused by the multiple factors of increased water temperatures, irradiance, and N and P concentrations (Newbold et al. 1980; Noel et al. 1986). However, the causal mechanisms for increased periphyton biomass following forest disturbance are seldom examined.

This study examines the relationship between periphyton and grazers in streams on the Boreal Plain. Specifically, I tested the hypothesis that periphyton was a more important food source for mayflies than terrestrial detritus. Mayfly diet was determined by measuring the stable isotopes of N and carbon (C) in the tissues of mayfly families. Stable isotope analysis (SIA) has been used in aquatic systems to determine the importance of terrestrial and autochthonous carbon sources to stream food webs (Rounick et al. 1982; Bunn et al. 1989; Doucett et al. 1996). Because previous work in Boreal Plain streams could not separate the origin of organic carbon-based food resources, SIA should complement earlier research. The second goal of this study was to evaluate effects of small-scale logging (i.e., <3% of the watershed logged) on mayflies. In the potentially N-limited streams on the Boreal Plain, N-enrichment from forest harvesting may enhance periphyton biomass, and increase the abundance of stream herbivores such as mayflies that potentially use periphyton as a food resource. Increased food for grazers may affect individuals by increasing their growth or producing a numerical response at the population level (Hart and Robinson 1990). I hypothesized that in sites affected by a harvesting-related increase in nutrients, mayflies (particularly *Baetis* spp.) would be larger and/or have higher abundance at sites relative to reference sites. Furthermore, I predicted that the higher proportion of *Baetis* spp. would result in a less diverse mayfly assemblage in sites affected by harvest.

3.1 Methods

3.1.1 Mayfly survey

Benthic invertebrates were sampled with a U-frame net (mesh size, 250-µm) (Scrimgeour et al. 1993) from one site upstream and one site downstream of harvested areas on five streams (Meadow, Cottonwood, Kettle, Pony, and Waddell), for a total of 10 sites (see Chapter Two for further site description). Sampling occurred in October 1996 and 1997. In 1996, four samples were collected from each of three riffles at each site, except the Pony sites where three samples were collected. In 1997, four samples were collected from each of three riffles at each site, except in the upper Meadow, lower Kettle, and the Pony sites where three samples per riffle were collected. Replicate samples from each riffle (for each year) were pooled, except at the lower Kettle site, where replicates were kept separate. (Numbers from each replicate per riffle at the lower Kettle site were eventually pooled.) In the field, inorganic material was separated from organic material by swirling the entire sample in a pail of water, then decanting the suspended invertebrates and detritus into a 250-µm mesh. The samples, comprised predominantly of organic material, were preserved in 10% formalin.

In the laboratory, samples were sieved into >4 mm, >1-4 mm, and >0.25-1 mm fractions. Invertebrates from the largest fraction were sorted by eye. A dissecting microscope (12X magnification) was used when sorting invertebrates from the >1-4 mm fraction. The fine fraction was subsampled (Wrona et al. 1982) and insects sorted under 12X. Mayflies were identified to genus where possible, dried to a constant mass at 55 °C for 48 h, and weighed on a Cahn C-31 microbalance. *Baetis* sp. from one riffle in both the upper and lower Meadow sites were individually weighed and measured to obtain length/weight regressions. The thorax lengths of larval *Baetis* sp. were measured with the aid of a camera lucida and digitizing pad.

Regression analysis was used to relate thorax length to weight (both variables were $\log_{10}$ transformed to meet the assumption of normality) for *Baetis* sp. from sites upstream and downstream of logging in the Meadow basin for October 1996 and October 1997 (pre- and post-harvest, respectively). Differences in slopes and y-intercepts between the upstream and downstream sites, in both 1996 and 1997, were tested by analyses of covariance (ANCOVA). Regressions and ANCOVAs were performed with SPSS (release 8.0, SPSS Inc. 1997).

Analyses of biomass and density of mayfly assemblages and, specifically *Baetis* sp., were conducted to address: (1) the effects of timber harvest, and (2) site differences during the 1997 nutrient enrichment experiments. To detect a timber harvest effect, differences in total mayfly and *Baetis* sp. biomass and density were evaluated with a three-way analysis of variance (ANOVA) with year (1996 vs. 1997) and site (upstream vs. downstream) as fixed factors and stream as the blocking factor. If the blocked ANOVA yielded significant interactions ($P<0.05$) between stream and site or year, two-way ANOVAs (i.e., test factors were site and year) were conducted for each stream; in these cases the significance values were reduced to 0.01 (a Bonferroni adjustment for the five streams). A Levene's homogeneity of variance test was conducted for all analyses. In the three-way ANOVA, total density, total biomass, and *Baetis* sp. density data did not meet this assumption, ANOVAs were still conducted, however, caution was used when interpreting data. A square-root transformation of *Baetis* sp. biomass data yielded homogenous variances. To determine differences in these same four variables amongst the ten sites during the 1997 nutrient enrichment experiment, a one way ANOVA (i.e., test factor was site) was conducted, followed by Least Significant Differences (LSD) post-hoc comparisons. A *t*-test was used to compare the proportion of *Drunella* spp., a relatively large mayfly, in the total mayfly biomass between 1996 and 1997 after arcsine transformation of data. All analyses were performed with SPSS (release 8.0, SPSS Inc. 1997).

The mayfly assemblage was analysed with principal component analysis (PCA) and comparison of dominance diversity curves for upstream and downstream sites (pre- and post-harvest). PCA was used to summarize and describe sites based on genus composition; it was performed with Minitab (release 11.0, Minitab Inc. 1996). A taxa abundance index incorporating density and biomass was calculated for each genus as:

$$I_{ik} = (D_{ik}+B_{ik})/2,$$

where I_{ik} is the abundance of taxa i in site k, and D_{ik} and B_{ik} are the standardized density and standardized biomass of taxa i at site k, respectively. The abundance indices were used in the PCA. For the dominance diversity curves, the abundance index for each genus was calculated as described above, except total, not standardized, density and biomass values were used. The relationship between the index and the rank of that genus is a dominance diversity curve. The dominance diversity curves for all upstream and downstream sites in both 1996 and 1997 were compared. A Kolmogorov-Smirnov test was used to test for goodness of fit between the two curves (i.e., all upstream sites compared to all downstream sites in both 1996 and 1997). If logging were to have an effect, I expected a greater dominance of *Baetis* sp. and lower assemblage evenness (i.e., genera are less equally abundant) in sites affected by logging. Lower evenness would be indicated by a dominance diversity curve that is skewed to the left (Hawkins et al. 1982).

Physical, chemical, and biological stream variables were measured during the October surveys in 1996 and 1997. Water depth and current velocity were measured at each benthic sampling location, canopy cover, and photosynthetically active radiation (PAR) were measured and periphyton were sampled for Chlorophyll *a* (Chl*a*) analysis at each site, as described in Chapter Two. An Onset, one channel temperature logger recorded water temperature every hour from spring until fall (this period varied according to site and year). Degree-days were determined as the sum of the mean daily water temperatures accumulated from July 1 to October 9, for 1996 and 1997. In 1996, degree-days were calculated for July, August, and September for the upper and lower Meadow sites (i.e., sites where individual *Baetis* sp. length/weight relationships were determined). Differences in monthly degree-day values in the two sites were determined with χ^2 analysis. Nitrite+nitrate+ammonium (dissolved inorganic N - DIN) and soluble reactive phosphorus (SRP) concentrations were measured on five and eight occasions during the open-water season of 1996 and 1997, respectively, as described in Chapter Two. Except for temperature variables (i.e., average daily temperature and degree-days), differences in the background conditions were determined with a one way ANOVA (i.e., factor was site) followed by LSD post-hoc comparisons (release 8.0, SPSS Inc. 1997). All data were tested for homogeneity of variance; transformations were applied where data did not conform (see Appendix B - Table B2). In an attempt to explain the high abundance of *Baetis* sp. in specific sites in 1996 and 1997, physical, chemical, and biological characteristics measured during October surveys were analysed for each year separately.

3.1.2. Diet - Stable Isotope Analysis

Mayflies and their potential food sources (i.e., periphyton, bryophytes, and terrestrial detritus) were collected during the open water seasons of 1996 (pre-harvest) and 1997 (post-harvest). Benthos was sampled from three riffles per site in 1996 and one riffle per site in 1997. Upper sites were above all logging on all five streams; lower sites were immediately below the logging (see Chapter Two for further site description). Invertebrates, and their potential food sources, were collected in May, July, and September. Sampling the ten sites depended on access and stream conditions (particularly discharge); thus, some sites were sampled in all seasons and others were sampled only once a year. At each site, approximately 15 mg (freeze-dried weight) of periphyton was scraped from at least 9 rocks per site. Before freezing, invertebrates were removed from the composite periphyton sample. A D-frame net was used to collect bryophytes, terrestrial detritus, and mayflies (mesh size, 250-µm). The sample was rinsed in a 400-µm mesh bag. The apical meristems of the bryophytes were harvested, rinsed in deionized, distilled water (DDW), and frozen. Stream-conditioned terrestrial material (i.e., deciduous leaves) was also removed, rinsed in DDW, and frozen. Animals were removed from the detritus within 12 h of collection and placed, according to family, in containers filled with stream water. Invertebrates remained in the aerated stream water at ambient water temperatures for at least 8 h to allow them to void their guts. Following gut clearance, animals were rinsed in DDW, and frozen. In the laboratory, periphyton, terrestrial detritus, and bryophytes were acidified with a 2% v/v hydrochloric acid solution to a pH of 2 to remove adhering inorganic carbon (i.e., carbonates associated with the sediment) (Boutton 1991). All mayflies and food materials were freeze-dried.

Samples were analysed for stable C and N ratios with a Micromass Optima continuous flow mass spectrometer (CF-IRMS) coupled to a Carlo Erba NA1500 elemental analyser (EA) at the National Water Research Institute (NWRI) in Saskatoon. Whole mayflies, leaf pieces, bryophyte tips, or periphyton were packed into 5x8 mm tin capsules. One mg of mayfly material and 3 mg of periphyton, terrestrial plants, or bryophytes were analysed. In 1997, three replicates of all mayfly and potential food samples were analysed; in 1996 only one replicate of all samples was analysed. In some cases, particularly for periphyton or terrestrial detritus, N peaks were very low and these samples were re-analysed at a higher weight (5 mg). When samples were re-analysed, a mean $\delta^{13}C$ value was calculated from the three replicates and the $\delta^{15}N$ value was determined from the 5 mg sample. The packed samples were loaded into the EA autosampler, combusted at 1100 °C, desiccated with magnesium perchlorase, and then the N_2 and CO_2 gases were separated chromatographically. A helium gas carrier transported N_2 and CO_2 to the mass spectrometer. The magnetic deflector in the mass spectrometer separated heavy from light molecules of the N_2 reference gas, then the sample N_2. Following integration of the reference and sample N_2 peaks, the CO_2 gases were ionized. The sample CO_2 peak was integrated followed by an injection and integration of the reference CO_2. Every 10 samples, a laboratory standard of caffeine was analysed.

Standard reproducibility for both carbon and nitrogen was ± 0.2 $^0/_{00}$.

Stable isotope data are presented as the relative difference between ratios of the sample and standard gases. Delta (δ) is used to express the relative differences:

$$\delta R(^0/_{00}) = [(R_{sample}/R_{standard})/R_{standard}] \text{ X } 10^3$$

where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. $\delta^{13}C$ or $\delta^{15}N$ is the permille ($^0/_{00}$) deviation of that sample from the recognized isotope standard, PeeDee Belemnite (PDB) limestone for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$ (Gearing 1991). Higher (i.e., less negative) $\delta^{13}C$ or $\delta^{15}N$ values denote enrichment in the heavier isotope relative to the lighter isotope (i.e., enrichment in ^{13}C relative to ^{12}C); lower (i.e., more negative) $\delta^{13}C$ or $\delta^{15}N$ values reflect depletion in the heavier isotope. Generally, there is small enrichment (0-1 $^0/_{00}$) of ^{13}C in a consumer compared to its prey; thus, $\delta^{13}C$ of organisms indicate their carbon source (DeNiro and Epstein 1978). There is also greater enrichment of ^{15}N in predators relative to prey (2-5 $^0/_{00}$) because organisms preferentially excrete the lighter isotope (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987). Results are presented as means±1*SE*.

To compare isotopic values of the three resources (i.e., periphyton, terrestrial detritus, and bryophytes) two one-way ANOVAs (i.e., factor was resource) were conducted. The first ANOVA compared $\delta^{13}C$ values (after a $1/\delta C^{13}$ transformation to meet the assumption of homogeneity of variance); the second ANOVA compared $\delta^{15}N$ values (these data did not meet the assumption of homogeneity of variance). The isotopic values of the four mayfly families (i.e., Baetidae, Heptageniidae, Ephemerellidae, and Ameletidae) were compared with two one-way ANOVAs (i.e., factor was family). $\delta^{15}N$ data were squared to meet the assumption of homogeneity of variance. To determine spatial and temporal differences for each mayfly family a three-way ANOVA for $\delta^{13}C$ and $\delta^{15}N$ was conducted. Site (upstream vs. downstream sites) and season (May, July, and September) were fixed factors, and stream was the blocking factor. Mayfly samples from 1996 (n=25) and 1997 (n=213) were pooled. When the three-way ANOVA yielded significant interactions (P<0.05), two-way ANOVAs (i.e., factors were site and season) were run for each stream and the significance level was reduced to 0.01 (a Bonferonni adjustment). For this analysis, a Levene's test to meet the assumption of homogeneity of variance was employed. In cases where data did not conform to this assumption, even after transformation, ANOVA results that suggested marginal differences were treated with caution (see Appendix B - Table B3). ANOVAs were performed with SPSS (release 8.0, SPSS Inc. 1997).

3.2 Results

Sites differed in physical, chemical, and biological characteristics measured during October surveys. In 1996, there were differences in water depth and velocity, canopy cover, light attenuation coefficients and Chl*a* concentrations ($P<0.05$; Table 3.1) among the 10 sites, but no detectable differences in SRP and DIN. Water was deeper at the upper Waddell site compared to the other nine sites ($P<0.02$). Except in the lower Kettle, water speeds were greatest at the Waddell sites ($P<0.04$). With the exception of the lower Meadow, Kettle, and Waddell sites, the upper Meadow site had a denser canopy cover than the other six sites ($P<0.04$). Light was most rapidly attenuated at the upper Kettle site ($P<0.05$). Monthly degree-days between the upper and lower sites at Meadow in 1996 were indistinguishable ($\chi^2 = 0.5$; $P>0.5$). Except in the lower Cottonwood and Waddell sites periphyton biomass, as Chl*a*, was greatest in the lower Pony site compared to the other seven sites ($P<0.004$). In October 1997, water depth, canopy cover, light attenuation coefficients, and DIN concentrations differed amongst the sites ($P<0.05$). Again, water depth was greatest ($P<0.02$) at the upper Waddell site. Except for the upper Pony site, canopy cover was most dense at the upper Meadow site compared to the other eight sites ($P<0.001$). Light was attenuated more rapidly at the lower Meadow site compared to the other nine sites ($P<0.007$). DIN concentrations were lower in all Pony and Waddell sites compared to the Cottonwood and downstream Kettle sites ($P<0.04$). With the exception of water depth (deepest at the upper Waddell site in both years) and canopy cover (densest at the upper Meadow site in both years), site differences in physical, chemical, and biological factors were inconsistent between 1996 and 1997, and did not demonstrate any trends consistent with the effects of forest harvesting.

3.2.1 Mayfly Survey

Baetis sp. weight was positively related to thorax length for upstream and downstream Meadow sites in 1996 and 1997 (Figure 3.1). The length/weight relationship between sites for each year was not detectably different (*P*>0.05; Table 3.2). However, in 1996, *Baetis* sp. for a given length weighed less in the upper compared to the lower Meadow site (*P* <0.001). This result may be confounded as the slopes of the two regression lines approached a detectable difference (*P*=0.052) that year; thus, it is difficult to be certain that mayflies from the upper Meadow site are heavier for a specific thorax length. No detectable difference (*P*=0.11) was found in individual *Baetis* sp. weights between upper and lower Meadow sites in 1997.

Baetis sp. was the only genus of Baetidae, and it dominated mayfly assemblages at all sites. *Baetis* sp. was also the most abundant mayfly collected during seasonal sampling for SIA. In 1996, *Baetis* sp. comprised 82% and 73 % of total mayfly density and biomass, respectively (Figure 3.2). However, in 1997 *Baetis* sp. accounted for 70% and 40% of the total mayfly density and biomass, respectively. The reduced percentage of *Baetis* sp. in the total biomass was due to the increased biomass of the ephemerellid, *Drunella* spp. (*t*=-21.15, *P*<0.001) in 1997 samples compared to 1996. *Drunella* spp. comprised approximately 24% of the total mayfly biomass in 1997 and 8% of the biomass in 1996.

The four mayfly variables (i.e., total mayfly density, total mayfly biomass, *Baetis* sp. density, and *Baetis* sp. biomass) indicated inconsistent trends across streams, sites (upstream compared to downstream), and years (1996 and 1997) and no detectable timber harvest effects. Total mayfly biomass was marginally greater in 1997 than in 1996 (*P* = 0.04) (Figure 3.2). Neither year, stream, nor site (i.e., upstream vs. downstream sites) yielded detectably different total mayfly density values (*P*>0.05). *Baetis* sp. density depended on stream. In Meadow, *Baetis* sp. density was greater in 1996 compared to 1997 (*P*<0.001), and at the upstream than the downstream site (*P*=0.001). In contrast, in Cottonwood *Baetis* sp. density was greater in 1997 compared to 1996 (*P*=0.004). Differences in *Baetis* sp. biomass also depended on stream, such that the upper Meadow had greater *Baetis* sp. biomass than the lower Meadow site (*P*<0.001). Overall, there were no detectable differences in total mayfly density and biomass amongst streams, or sites. Differences in *Baetis* sp. density and biomass did, however, depend on stream.

Comparison of four mayfly variables among the 10 sites during the 1997 nutrient enrichment experiment (Chapter Two), also showed inconsistent results. There was no detectable difference in total mayfly biomass among sites (P=0.2), but sites differed in total density, *Baetis* sp. density, and biomass (P=0.01, 0.02, and 0.02, respectively). Although mayfly density and biomass were not consistently higher for the Cottonwood sites compared to all sites, these sites tended to support greater total mayfly and *Baetis* sp. densities, and greater *Baetis* sp. standing crop (Table 3.3).

Baetis sp. density and biomass also resulted in different assemblages for the five streams. A PCA conducted on the standardized combination of density and biomass values for the different mayfly genera demonstrated that Cottonwood and Meadow streams had a greater abundance of *Baetis* sp. and fewer *Ephemerella* spp. than Pony and Waddell, based on Axis 1 which explained 32% of the variance (Figure 3.3). The second axis explained an additional 22% of the variance and separated the 1996 upper Meadow site from all the other sites. The separation of the upper Meadow site in 1996 was due to the increased *Baetis* sp. and *Cinygmula* spp. abundance. There was no pattern associated with harvesting effects in the PCA (i.e., harvested sites did not cluster together). Similarly, dominance diversity curves for mayfly assemblages were indistinguishable in upstream compared to downstream sites in both the pre- and post-harvest years (D_{obs}=5.4, D_{crit}=53, P»0.1 and D_{obs}=14.3, D_{crit}=60, P»0.1, respectively) (Figure 3.4). Thus, mayfly assemblage composition was not detectably affected by streamside logging activities.

3.2.2 Diet - Stable Isotope Analysis

Mean $\delta^{13}C$ and $\delta^{15}N$ differed amongst potential food sources (P<0.001 and P=0.002, for $\delta^{13}C$ and $\delta^{15}N$ respectively), such that bryophytes were more enriched in ^{15}N and depleted in ^{13}C than periphyton or terrestrial material (Table 3.4). Terrestrial detritus values were typically enriched in ^{13}C and depleted in ^{15}N (Figure 3.5). The $\delta^{13}C$ and $\delta^{15}N$ of epilithon was variable (Table 3.4), but intermediate between that of terrestrial material and bryophytes.

Unlike the potential food sources, mean $\delta^{13}C$ and $\delta^{15}N$ values of the Ephemeropteran families (i.e., *Baetis* sp., Heptageniidae, Ephemerellidae, and Ameletidae) were close (Figure 3.5). However, Ephemerellidae were more enriched in ^{13}C than heptageniids and *Baetis* sp. (P<0.001), and *Baetis* sp. were more enriched in ^{15}N than heptageniids (*P*<0.001), ephemerellids (*P*<0.001), and ameletids (*P*=0.02). Ephemerellids were also more enriched in ^{15}N than heptageniids (*P*<0.001). Mayfly isotopic values are between potential food source values, which indicates that these insects did not specialize on any one measured resource.

Baetis sp. and Heptageniidae $\delta^{13}C$ and $\delta^{15}N$ values varied amongst sites, streams, and seasons (*P*<0.05), and Ephemerellidae, and Ameletidae isotopic signatures were not detectably different (*P*>0.05) spatially or temporally. In Meadow, heptageniid $\delta^{15}N$ values were greater (*P*<0.001) in summer than spring (5.29 and 3.63, respectively). In Cottonwood, *Baetis* sp. had the most depleted $\delta^{13}C$ values in the summer compared to the spring or fall (-32.3, -31.2, and -29.9, respectively, *P*<0.003), and downstream was greater than upstream (-31 and -33.4, respectively, *P*=0.006). Similarly, ^{13}C values of *Baetis* sp. in Kettle were more depleted in the summer compared to the spring (-32.6 and -30.6, respectively, P <0.001) and downstream was greater than upstream (-31.4 and -33.4, respectively, *P*=0.001). Thus, in two streams, *Baetis* sp. $\delta^{13}C$ values were more depleted in the summer and in upstream sites.

3.3 Discussion

Mayfly assemblages were dominated by *Baetis* sp. in Boreal Plain streams that I studied. This genus was clearly the most numerous in all sites in the October surveys, accounting for between 40 and 80% of total Ephemeropteran biomass and density values, respectively. *Baetis* sp. were also a major component of the qualitative samples collected in May, July, and September of both 1996 and 1997 for SIA. Other streams in the region also support benthic invertebrate communities dominated by *Baetis* sp. In the La Biche River, *Baetis* sp. was second in abundance to *Hyalella azteca* or *Gammarus lacustris* amphipods in June and July but reached its population peak in August and September (Robertson 1967). In Wandering River, *Baetis* sp. made up 28 % of the total macrobenthic fauna in June (Robertson 1967). In Wampus Creek, a Rocky Mountain Foothills stream, Ephemeroptera comprised at least 50% of the total benthos, with *Baetis* sp. being the most abundant mayfly for most of the year (Zelt 1970). Mayflies, particularly *Baetis* spp., are an important component of overall biomass in other northern streams. For example, in the Kuparak River in Alaska and a second-order tributary on the

Canadian Shield in Québec, *Baetis* spp. were among the dominant invertebrates in the benthos (Peterson et al. 1993; Bechara et al. 1992, respectively). Qualitative study of a pristine river in Norway showed high numbers of *Baetis* spp., compared to Plecoptera and Trichoptera (Aagaard et al. 1997). Extensive benthic invertebrate surveys of the Vindelälven River system in northern Sweden also demonstrated the important contribution of Ephemeropteran species to total biomass, as this Order typically comprised between 25 and 55 % of the benthic invertebrate communities (Ulfstrand 1968). The reasons for the trend of *Baetis* spp. dominance in northern streams are likely related to its relatively high fecundity and short generation times. Both traits would provide *Baetis* spp. with an advantage in northern streams, which have a relatively short open-water period and low regime of thermal degree-days.

In the PCA, sites generally clustered together, indicating that the studied streams have similar mayfly composition. Subtle assemblage differences were found for Pony and Waddell sites because of the presence of *Ephemerella* spp. and *Heptagenia* spp. and fewer *Epeorus* spp., *Cinygma* spp., and *Cinygmula* spp., than the Cottonwood and Meadow sites. Three sites are obviously removed from the cluster: the upper Meadow site in 1996 and the Cottonwood sites in 1997. Density and biomass values confirm that the separation of these sites is due to the high abundance of *Baetis* sp. Site differences in water depth and velocity, mean daily water temperature, periphyton biomass or factors that typically determine periphyton biomass such as light and nutrient concentrations did not explain higher *Baetis* sp. biomass in the upper Meadow site or the Cottonwood sites.

In 1996, the upper Meadow site had high *Baetis* sp. density; individuals appeared to weigh less than conspecifics collected at the lower site. If these insects are smaller, their size and higher abundance might be explained by density-dependent factors. Factors such as food supply and habitat availability are typically limited and only support a certain biomass, thus, if there are more individuals they tend to be smaller (Ricklefs 1990; Hart and Robinson 1990). However, overall *Baetis* sp. biomass was also greater at the upper Meadow site, suggesting other factors determined *Baetis* sp. standing crop at this site. One potential factor was temperature, however, no difference was detected in degree-days from July to October between Meadow sites in 1996. Unfortunately, temperature data were not collected in early spring (April through June), a period that may be critical for individual growth and population biomass (Brittain 1976; Sweeney and Vannote 1978; Hawkins 1986). Other physical, chemical, and biological variables (i.e., water depth, velocity, canopy cover, light attenuation, DIN, SRP, and Chl*a* concentrations) also do not account for the increase in *Baetis* sp. density and biomass in the upper Meadow site.

Isotopic values indicate that mayflies in these boreal streams are generalists. Assuming a 2-5 $^0/_{00}$ enrichment in $^{15}\delta N$ (Minagawa and Wada 1984) and a 0-1 $^0/_{00}$ $^{13}\delta C$ enrichment of consumers compared to their source (DeNiro and Epstein 1978), mean isotopic values for *Baetis* sp. indicate consumption of mainly material produced instream. In addition to periphyton, *Baetis* sp. were potentially eating a more ^{13}C depleted source such as bryophytes, or epiphytes associated with bryophytes (pers. observation). *Baetis* sp. collected in the fall from one stream, Cottonwood, have $\delta^{13}C$ and $\delta^{15}N$ values which indicate a greater reliance on terrestrial material, although *Baetis* sp. mean stable isotope values for the three seasons and five streams do not suggest such a relationship. Mean $\delta^{13}C$ values for Ephemerellidae suggest they relied heavily on periphyton. They also ate terrestrial detritus in all seasons. The four Ameletidae samples came from the upper Meadow site, and their isotopic signatures suggest that they consume both bryophytes and periphyton in the summer and fall. Isotopic signatures of Heptageniidae suggest that they consume all three resources. The slightly depleted $\delta^{15}N$ signatures of these mayflies may be attributed to the presence of terrestrial material in their diet, however, the proximity of $\delta^{13}C$ values to instream material suggests the reliance on an unmeasured source (i.e., epiphytes associated with moss, or a specific component of the periphyton). While not measured isotopically in the current study, purified algal cells were more ^{13}C depleted than the epilithon in two southern Ontario streams (Rosenfeld and Roff 1992). Thus, unmeasured resources may account for the depleted ^{13}C values of heptageniids. Across North America, gut content analysis (Chapman and Demory 1963; Gilpin and Brusven), growth experiments (Mihuc and Minshall 1995), and SIA (Rounick et al. 1982; Junger and Planas 1994) have documented generalist feeding habits of mayflies. Although my analysis supports past conclusions, the clustering of the four mayfly families near periphyton strongly suggests the importance of this food resource in all seasons.

The potential importance of bryophytes as food for mayflies is an unexpected result as it is generally accepted that aquatic invertebrates do not consume live moss (Cummins and Klug 1979; Suren and Winterbourn 1992). Although moss-covered substrata typically support greater insect densities than moss-free areas, the increase is not attributed to consumption of the macrophyte. Instead, bryophytes are thought to provide a substratum for periphyton colonization, trap detritus (Suren and Winterbourn 1992), and also provide refuge from predation (Brusven et al. 1990). Typically, herbivory on macrophytes is on the dead macrophyte material (Newman 1991), however, some insects avoid even decaying moss material (Davies 1975). Feeding experiments followed by gut content examination of ephemerellid mayflies fed moss and an associated diatom epiphyte demonstrated that bryophytes were consumed but not digested, whereas the diatom cells were always assimilated (Willoughby and Mappin 1988). Furthermore, there is rarely evidence of bryophytes in gut contents (Gilpin and Brusven 1970; Hamilton and Clifford 1983). Because bryophytes are not typically considered a potential resource, the suggested importance of bryophytes by SIA in this study needs further examination and confirmation. Alternative methodologies such as gut content analyses or growth experiments where mayflies are given different foods and weighed at the beginning and end of the experiment should be conducted to verify SIA findings of this study.

Cottonwood sites tended to have greater mayfly and *Baetis* sp. densities during the 1997 nutrient enrichment experiment. Periphyton biomass in this stream was not enhanced by N-enrichment, and diffusing substrata in this stream supported very low Chl*a* concentrations (Chapter Two). Unlike Meadow, the other stream where periphyton did not respond to N-enrichment, discharge and low irradiance were not factors in Cottonwood. Thus, in Cottonwood, the trend of greater mayfly (e.g., *Baetis* sp.) density and the potential for greater grazing pressures may have confounded an N-enrichment response.

Streamside logging did not detectably alter mayfly biomass, density, and assemblage characteristics. In contrast to findings from other North American streams where timber harvesting led to an increased abundance of *Baetis* spp. (Tebo 1955; Newbold et al. 1980; Noel et al. 1986; Wallace and Gurtz 1986), there were no detectable harvest effects on either total mayfly or *Baetis* sp. abundance in these boreal streams. Dominance diversity curves for both harvested and reference sites in the post-harvest year were indistinguishable, indicating that logging did not increase *Baetis* sp. dominance and lower assemblage evenness. Furthermore, site separation in the PCA bore no relationship to harvesting effects. Chapter Two results also indicated that DIN, SRP concentrations, and periphyton biomass were not detectably altered by the relatively small-scale streamside logging activities. In other geographic areas, hydrologic responses to harvest are observed when logging affects at least 20% of the basin (see review by Bosch and Hewlett 1982). Thus, logging that removes <3% of this low relief Boreal Plains basins has little effect on trophic-enrichment.

Results from this study demonstrated that five streams in the Boreal Plains ecoregion of northern Alberta have similar mayfly genera composition and assemblages dominated by *Baetis* sp. The high *Baetis* sp. abundance in a few sites (i.e., upper Meadow 1996 and the 1997 Cottonwood sites) was not attributable to periphyton biomass, water depth and velocity, water temperature or irradiance. However, high mayfly densities were found in the Cottonwood sites in 1997 and periphyton in these sites did not respond to N-enrichment (Chapter Two). The importance of instream material, particularly periphyton in the mayfly diet (as indicated by SIA), suggest that grazing may have confounded an N-enrichment effect in at least one stream on the Boreal Plain.

Table 3.1: Mean (±1*SE*) physical, chemical, and biological characteristics at each site during the fall 1996 and 1997 benthic invertebrate surveys. [a]Temperature recorded from July 1 to October 9 both in 1996 and 1997. [b]Dissolved inorganic nitrogen (DIN = ammonium+nitrite+nitrate) and soluble reactive phosphorus (SRP) concentrations are based on seasonal average. Chl*a* is Chlorophyll *a* concentration from October survey. n.r. is not recorded.

Stream	Site	Average Depth (m)	Average Velocity ($m \cdot s^{-1}$)	Average Daily Temperature[a] (°C)	Degree Days[a]	Canopy Cover %	Light Attenuation Coefficient	DIN[b] ($\mu g \cdot L^{-1}$)	SRP[b] ($\mu g \cdot L^{-1}$)	Chl*a* ($\mu g \cdot cm^{-2}$)
1996										
Meadow	above	0.18	0.50	10.1	1022	64	3.69	46	28	0.43
		(0.03)	(0.13)			(22)	(0.62)	(10)	(7)	(0.24)
	below	0.21	0.38	10.9	1106	53	2.82	50	45	0.74
		(0.02)	(0.13)			(15)	(0.2)	(10)	(6)	(0.11)
Cottonwood	above	0.29	0.25	n.r.	n.r.	32	3.09	91	37	0.07
		(0.02)	(0.07)			(5)	(0.7)	(21)	(11)	(0.02)
	below	0.15	0.49	11.3	1145	28	n.r.	96	39	2.84
		(0.02)	(0.12)			(9)		(24)	(9)	(0.26)
Kettle	above	0.21	0.39	11.6	1565	21	5.18	57	53	0.08
		(0.06)	(0.04)			(11)	(0.98)	(10)	(6)	(0.03)
	below	0.20	0.62	n.r.	n.r.	40	0.40	65	46	0.39
		(0.03)	(0.03)			(18)	(0.73)	(17)	(5)	(0.28)
Pony	above	0.26	0.4	n.r.	n.r.	55	n.r.	51	48	2.59
		(0.01)	(0.03)			(11)		(16)	(8)	(0.84)
	below	0.28	0.32	n.r.	n.r.	24	n.r.	76	48	7.89
		(0.04)	(0.005)			(2)		(20)	(9)	(1.14)
Waddell	above	0.37	0.74	13.1	1327	1	3.57	71	39	0.85
		(0.04)	(0.01)			(0.7)	(0.04)	(23)	(5)	(0.45)
	below	0.24	0.74	13.4	1348	49	0.08	65	49	6.78
		(0.03)	(0.06)			(6)	(0.24)	(15)	(8)	(4.02)

Table 3.1 (cont'd)

Stream	Site	Average Depth (m)	Average Velocity (m•s^{-1})	Average Daily Temperature^{a} (°C)	Degree Days^{a}	Canopy Cover (%)	Light Attenuation Coefficient	DIN^{b} (µg•L^{-1})	SRP^{b} (µg•L^{-1})	Chl*a* (µg•cm^{-2})
<u>1997</u>										
Meadow	above	0.12	0.44	12.3	1246	74	n.r	21	39	0.26
		(0.01)	(0.04)			(9)		(3)	(6)	(0.04)
	below	0.13	0.38	12.4	1249	8	3.44	34	49	0.04
		(0.02)	(0.05)			(4)	(0.18)	(6)	(8)	(0.03)
Cottonwood	above	0.24	0.40	12.1	1222	12	1.32	39	32	0.14
		(0.01)	(0.04)			(1)	(0.20)	(7)	(5)	(0.11)
	below	0.16	0.48	12.1	1222	29	1.69	36	34	1.35
		(0.02)	(0.06)			(3)	(0.34)	(8)	(4)	(1.33)
Kettle	above	0.21	0.37	12.7	1282	n.r.	n.r.	44	55	0.30
		(0.02)	(0.05)					(9)	(7)	(0.09)
	below	0.24	0.55	12.4	1256	30	1.78	29	46	2.96
		(0.02)	(0.06)			(9)	(0.38)	(6)	(4)	(2.89)
Pony	above	0.27	0.41	n.r.	n.r.	50	1.52	21	41	0.94
		(0.02)	(0.02)			(6)	(0.08)	(2)	(5)	(0.48)
	below	0.28	0.56	12.8	1296	24	1.18	20	47	3.13
		(0.02)	(0.08)			(1)	(0.36)	(2)	(6)	(0.34)
Waddell	above	0.30	0.37	14.7	1484	3	n.r.	20	38	4.12
		(0.02)	(0.07)			(2)		(3)	(3)	(2.44)
	below	0.19	0.44	13.9	1406	23	n.r.	20	51	3.13
		(0.02)	(0.04)			(3)		(4)	(7)	(0.67)

Table 3.2: Regression equations for relationship between thorax length (mm) and weight (mg) for *Baetis* sp. in the upper and lower Meadow sites in 1996 (pre-harvest) and 1997 (post-harvest). All equations were significant at $P<0.001$. [a] The low P may be a result of the slopes of the two lines approaching a detectable difference.

Site	n	r^2	Regression Equation	Slope Similarity P	Adjusted Y intercept P
1996					
upper	99	0.79	$\log_{10}$weight = 3.12 ($\log_{10}$thorax) -1.03	0.052	<0.001[a]
lower	131	0.90	$\log_{10}$weight = 3.14 ($\log_{10}$thorax) - 0.77		
1997					
upper	64	0.76	$\log_{10}$weight = 2.00 ($\log_{10}$thorax) - 0.83	0.68	0.11
lower	42	0.89	$\log_{10}$weight = 3.11 ($\log_{10}$thorax) - 0.74		

Table 3.3: Results from one-way analysis of variance (i.e., factor was site) of total mayfly and *Baetis* sp. density and biomass during the 1997 nutrient enrichment experiments.

Variable	Result	
mayfly density	Upper Cottonwood	>lower Meadow (*P*=0.03) >upper Kettle (*P*=0.03) >upper Pony (*P*=0.002) >both Waddell sites (*P*=0.02)
	Lower Cottonwood	>both Meadow sites (*P*<0.01) >both Kettle sites (*P*<0.03) >upper Pony (*P*<0.001) >both Waddell sites (*P*=0.002)
mayfly biomass	no significant difference (*P*>0.05)	
Baetis sp. density	Upper Cottonwood	>both Meadow sites (*P*<0.04) >upper Kettle (*P*=0.03) >upper Pony (*P*=0.003) >upper Waddell (*P*=0.04)
	Lower Cottonwood	>lower Meadow (*P*=0.01) >upper Pony (*P*=0.01)
Baetis sp. biomass	Upper Cottonwood	>lower Meadow (*P*=0.02) >upper Pony (*P*=0.008) >both Waddell sites (*P*<0.04)
	Lower Cottonwood	>lower Meadow (*P*=0.04) >upper Pony (*P*=0.02)

Table 3.4: Mean (±1*SE*) and range of $\delta^{13}C$ and $\delta^{15}N$ signatures ($^0/_{00}$) of mayflies and their potential food sources in five streams on the Boreal Plain in 1996 and 1997.

sample	*n*	Mean (±1*SE*)		Range (Minimum – Maximum)	
		$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Periphyton	66	-31.98 (0.23)	5.23 (0.37)	-35.41 to -25.67	-6.45 to 10.39
Detritus	70	-28.38 (0.16)	-0.49 (0.20)	-31.37 to -25.32	-5.93 to 4.62
Bryophyte	52	-38.47 (0.36)	6.53 (0.2)	-42.66 to -31.20	2.50 to 8.30
Baetidae	83	-33.44 (0.25)	6.39 (0.13)	-38.08 to -29.43	2.28 to 8.30
Heptageniidae	73	-34.08 (0.34)	4.71 (0.18)	-39.82 to -27.65	0.98 to 8.00
Ephemerellidae	80	-31.77 (0.29)	5.79 (0.11)	-40.14 to -26.97	3.80 to 7.90
Ameletidae	4	-33.92 (1.03)	4.98 (0.70)	-35.52 to -30.94	3.19 to 6.45

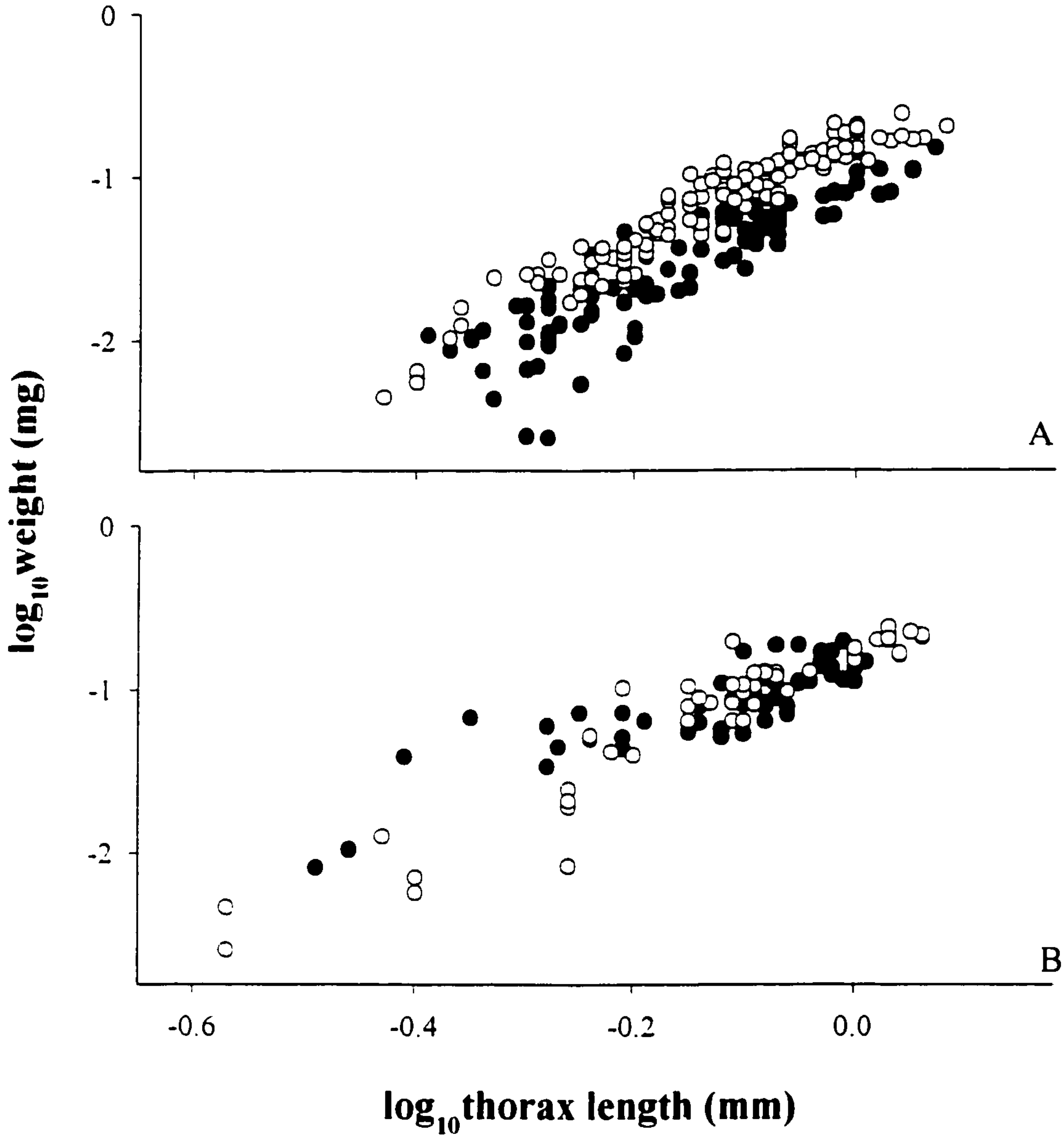

Figure 3.1: Relationship between $\log_{10}$thorax length (mm) and $\log_{10}$weight (mg) of *Baetis* sp. individuals in Meadow during 1996 (pre-harvest) (A) and 1997 (post-harvest) (B). Closed circles represent upper Meadow insects and open circles are lower Meadow insects.

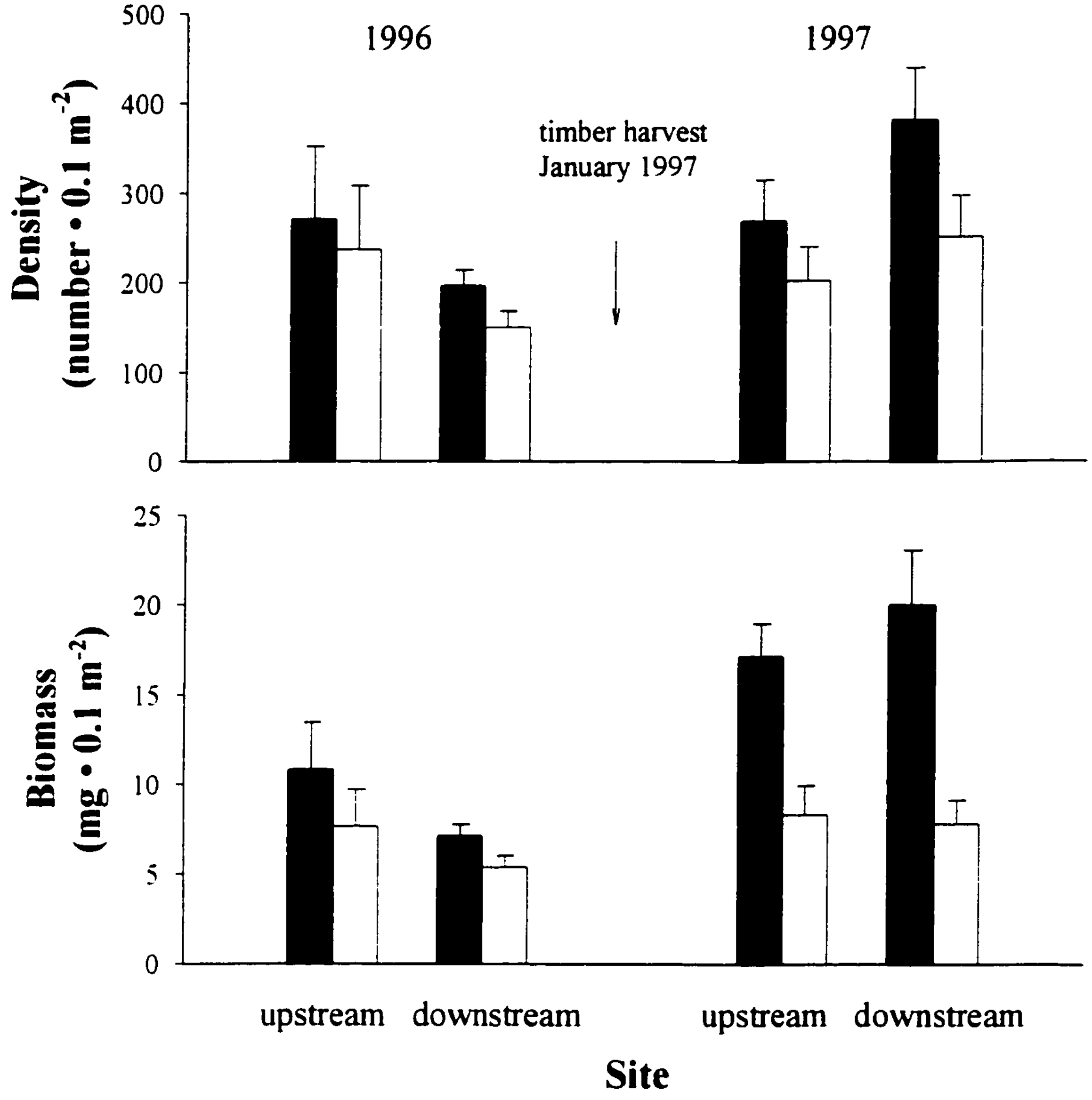

Figure 3.2: Mean density (±1 *SE*) and mean biomass (±1 *SE*) for all mayflies (closed bars) and *Baetis* sp. (open bars) in five streams on the Boreal Plain in 1996 (pre-harvest) and 1997 (post-harvest).

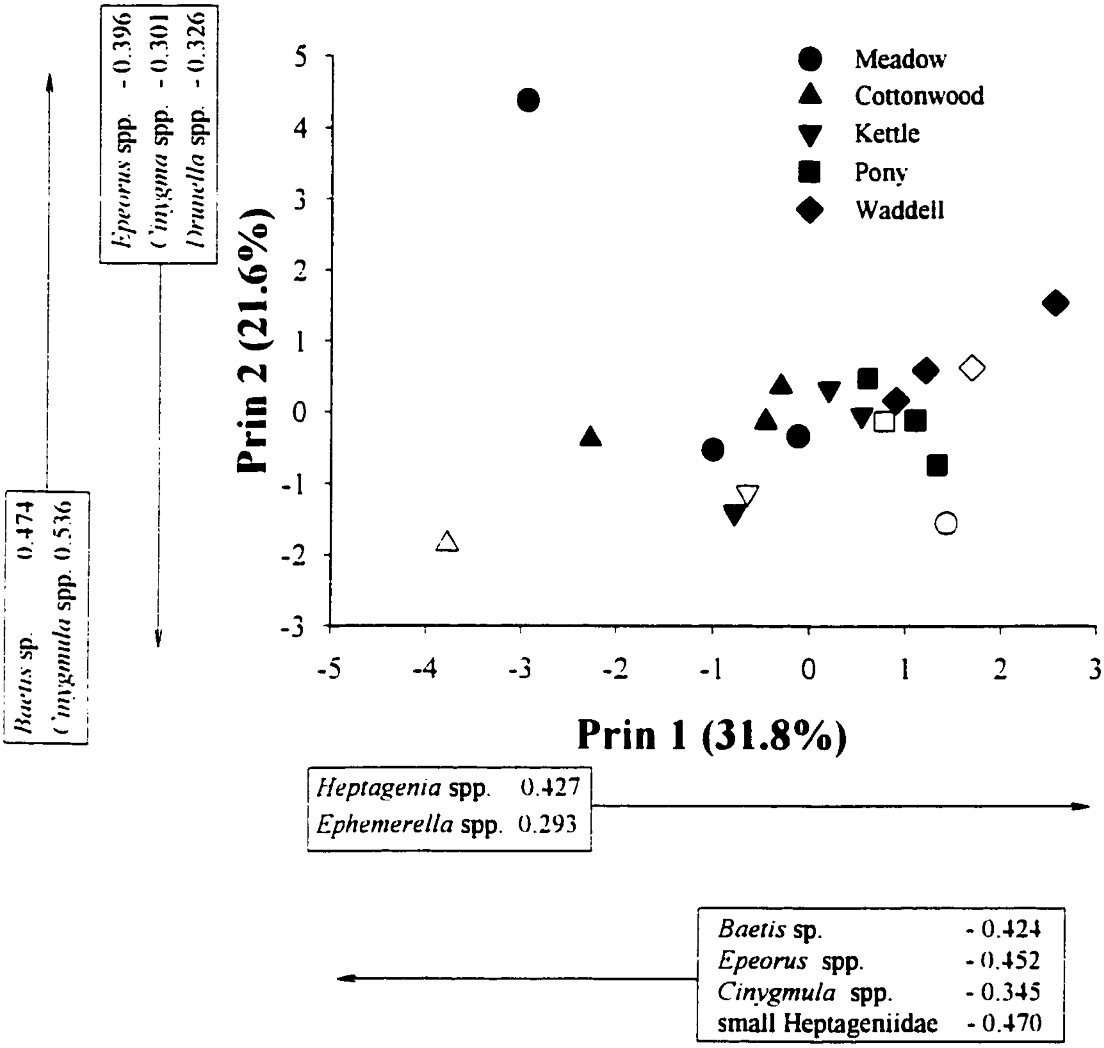

Figure 3.3: Ordination (PCA) of mayfly assemblages in five streams on the Boreal Plain in October 1996 (pre-harvest) and 1997 (post-harvest). Filled symbols are reference sites (both sites in 1996 and upstream sites in 1997), open symbols are the downstream sites in 1997. Taxa with high eigenvalues on both axes are indicated. The arrowhead indicates direction of increasing abundance.

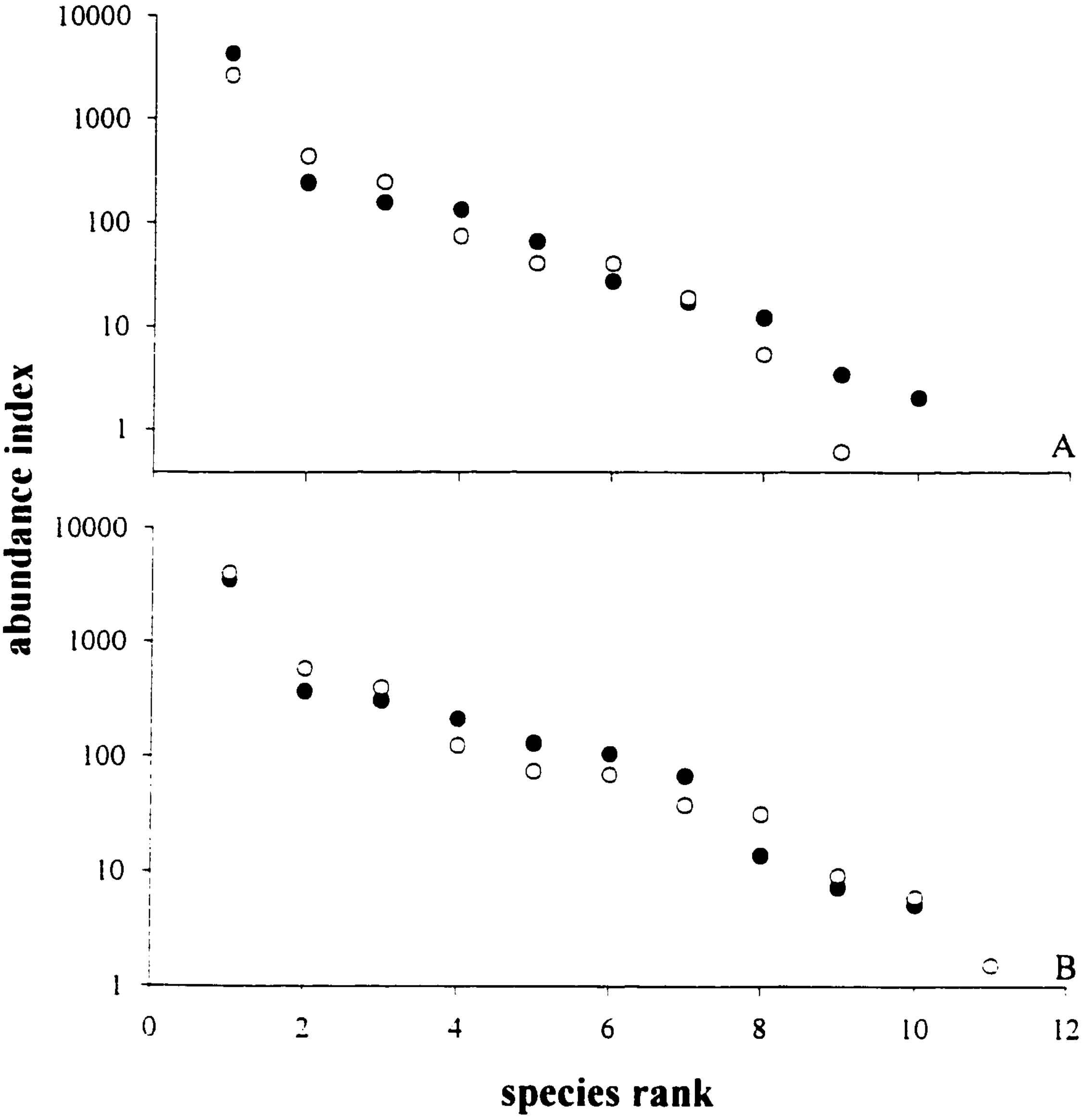

Figure 3.4: Dominance-diversity curves for mayfly assemblages in five streams on the Boreal Plain in 1996 (pre-harvest - A) and 1997 (post-harvest - B). Abundance indices for the upper sites (●) and (○) for the lower sites.

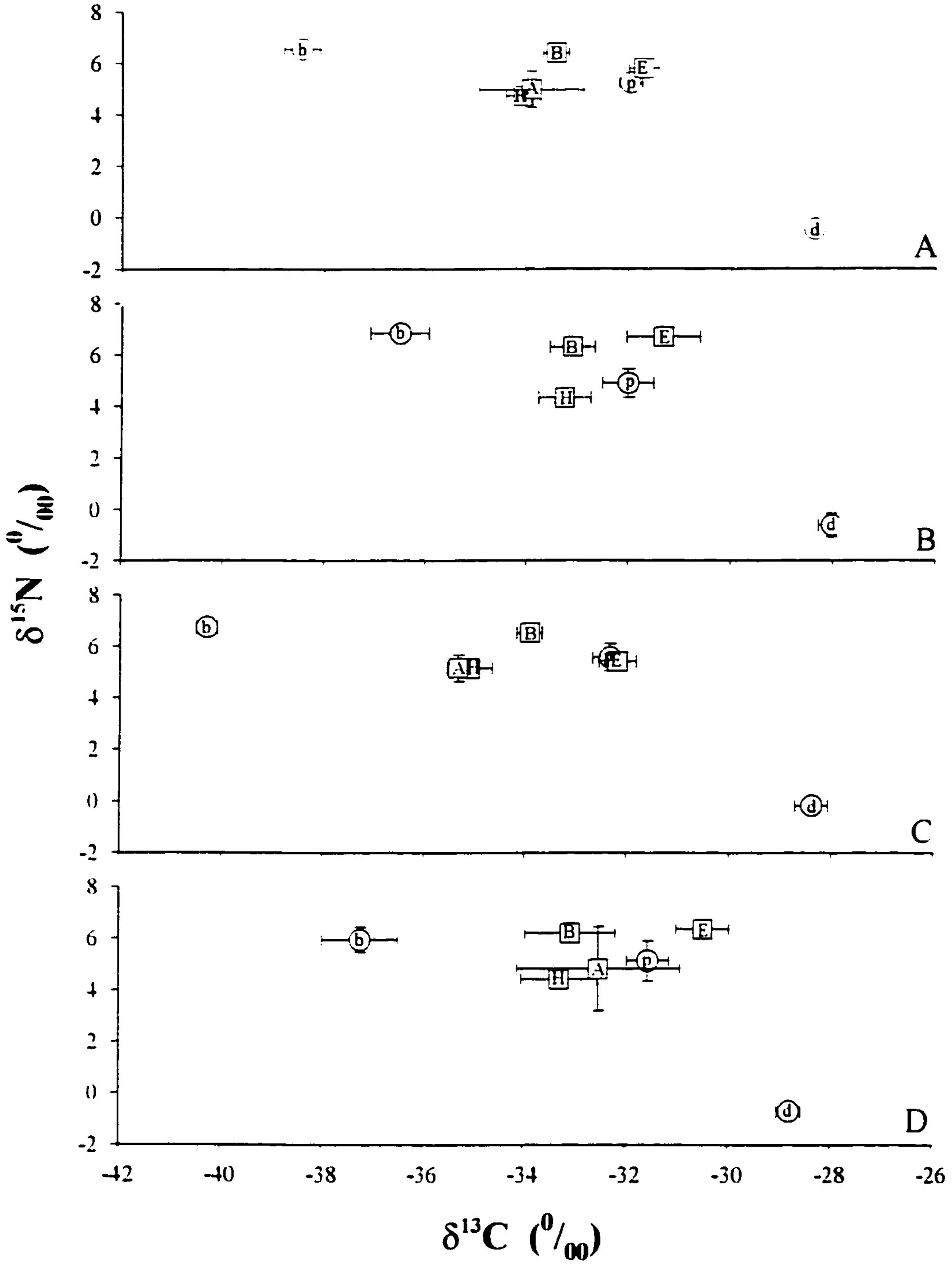

Figure 3.5: Mean (±1*SE*) δ^{13}C vs. mean (±1*SE*) δ^{15}N for three food sources (○) and four Ephemeropteran families (□) from five streams on the Boreal Plain overall (A), in spring (B), summer (C), and fall (D), 1996 and 1997. b = bryophyte, p = periphyton, d = detritus, B = *Baetis* sp., H = Heptageniidae, A = *Ameletus* sp., and E = Ephemerellidae.

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Chapter 4: Synthesis

4.0 Synthesis

The aim of this research was to examine the role of nitrogen (N) and phosphorus (P) concentrations and Ephemeropteran diet and abundance in determining periphyton biomass in Boreal Plain streams. Although the role of nutrients and grazers in regulating periphyton biomass in streams across North America is well established (Peterson et al. 1983; Grimm and Fisher 1986; Hill and Knight 1987; Feminella and Hawkins 1995), these processes are poorly understood in streams on the Boreal Plain. This paucity of data is problematic given that increased human activity in the region may enhance stream concentrations of N and P and, in turn, increase periphyton biomass. The increased periphyton biomass may increase abundance of macroinvertebrates, particularly Ephemeroptera (Fuller et al. 1986; Bechara et al. 1992). Alternatively, these insects may modify a nutrient enrichment effect by consuming the accumulated periphyton (Peterson et al. 1993).

Two projects were conducted to address the main objective. The first project examined periphyton limitation by N, P, or N+P in five streams on the Boreal Plain. N and P concentrations and periphyton biomass were surveyed, and *in situ* experiments with nutrient diffusing substrata were conducted (Chapter Two). The second project investigated mayfly diet by assessing the stable isotopes of N and carbon (C) in mayflies and their potential food sources. The abundance of each mayfly genera was also quantified in the five streams (Chapter Three). Finally, surveys and experiments were conducted in sites above and below streamside cutblocks, prior to and after small-scale forest harvesting (i.e., <3% of basins were logged). My project is a part of a larger project, TROLS (Terrestrial, Riparian, Organisms, Lakes, and Streams) that evaluated the effects of timber harvest on lakes, streams, and the terrestrial ecosystems in northern Alberta.

In streams on the Boreal Plain, periphyton biomass, measured as Chlorophyll *a* (Chl*a*) was very low (~ 2 Chl*a* $\mu g \bullet cm^{-2}$); this result is similar to findings from other undisturbed northern streams (Hickman et al. 1982; Peterson et al. 1993). Although these streams have relatively high soluble reactive P (SRP) concentrations (i.e., ~ 43 $\mu g \bullet l^{-1}$), the low algal biomass may result from the low molar ratio of DIN: SRP (<3). Alternatively, grazing pressures may regulate low periphyton standing crop. Survey results indicated that *Baetis* sp. is dominant in streams on the Boreal Plain. The high abundance of *Baetis* sp. is similar to findings from other northern streams in the region (Clifford et al. 1973), across North America (Peterson et al. 1993) and in Scandinavian countries (Ulfstrand 1968; Aagaard et al. 1997). Stable isotope analysis (SIA) indicated mayflies in these streams are generalists, but predominantly consume material produced in the stream (i.e., autochthonous material). Because mayflies are abundant and appear to rely on periphyton, they likely contribute to periphyton biomass loss in these boreal streams.

The *in situ* experiments with the nutrient diffusing substrata provided more direct information on nutrient limitation in these boreal streams. *In situ* experiments indicated that N-limitation explains in part, the relatively low periphyton biomass found in Boreal Plain streams. However, the variable response of periphyton to N-addition suggests some other factor, or combination of factors overrides N-limitation. In particular, high mayfly densities, particularly *Baetis* sp., were found in one of the streams that did not respond to nutrient enrichment compared to other four streams. This suggests that grazing pressure may have nullified periphyton accumulation due to N-enrichment. Although, low irradiance and discharge cannot be completely discounted as factors that contribute to low periphyton biomass in one stream, the *in situ* experiments generally indicated that N-limitation and grazer abundance determine periphyton biomass in these boreal streams.

I assessed the effects of harvesting by conducting experiments and surveys in sites above and below small-scale logging activities (<3% of the basins). Because harvest sites were subjected to relatively small clearcuts, I found no detectable effects of harvest on DIN and SRP concentrations, periphyton and mayfly biomass, or N-enrichment response (i.e., sites upstream and downstream of the harvested areas responded in the same way to N-addition). A hydrologic response to timber harvest is typically documented when >20% of a basin is disturbed (Bosch and Hewlett 1982); thus, disturbance that occurs in <3% of the basin might not be expected to elucidate a nutrient enrichment response. However, I found that periphyton is potentially N-limited and findings from another study conducted as a part of TROLS indicate greater soil nitrate concentrations in harvested mixed-wood plots compared to reference plots (N.Carmosini, University of Alberta, unpublished data).

Increased resource extraction (i.e., gas, oil, and timber) forecast for the region may enhance soil nitrate concentrations and potentially enhance stream nitrate concentrations. Thus, the potentially N-limited periphyton in these streams may increase in response to disturbance-related increase in stream N concentrations, although a biomass response will depend on primary producer-herbivore interactions.

After timber harvesting, mayflies are hypothesized to consume more autochthonous material than terrestrial material (Doucett et al. 1996; Rounick et al. 1982). The ability to detect a resource shift is only possible if C and N stable isotope ratios are distinct between terrestrial material and periphyton, as was the case in New Zealand (Rounick et al. 1982). Although N and C stable isotope signatures of autochthonous and terrestrial material found in my boreal streams were distinct, mayflies fed mostly on autochthonous carbon sources in reference sites. Therefore, it is unlikely that SIA would be able to measure harvesting effects as there may not be a resource shift after harvest. Furthermore, food webs in lakes on the Boreal Plain were difficult to interpret, due to the overlap of C isotope signatures of the different terrestrial and internal resources (Beaudoin 1998). Thus, in aquatic systems on the Boreal Plain, the future application of SIA to document consumer resource shifting after logging needs careful consideration.

Many abiotic and biotic factors determine stream periphyton biomass. Among these determinants are light (Triska et al. 1983), low nutrient concentrations (Peterson et al. 1983; Grimm and Fisher 1986), velocity and discharge (Horner and Welch 1981), and grazing pressures (Feminella and Hawkins 1995). The current study has demonstrated that for streams on the Boreal Plain, there is strong evidence for N-limitation and preliminary evidence for grazer control of periphyton. However, other physical factors such as light or discharge may also regulate periphyton biomass. Future experiments to determine periphyton biomass in these streams should incorporate discharge and light measurements but should test for interactive effects of mayfly grazing and N-limitation, specifically.

4.1 References

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Appendix A: Pilot nutrient enrichment experiments

A.0 Pilot nutrient enrichment experiments

To test the hypothesis that streams of the western Boreal Plain were nitrogen (N) or phosphorus (P) limited, nutrient enrichment experiments were conducted with diffusing substrata (DS). Diffusing substrata have been used successfully to demonstrate nutrient limitation in North America (Grimm and Fisher 1986; Dubé et al. 1997) and New Zealand (Winterbourn 1990). However, DS have also provided inconclusive and confusing results, possibly related to the high variability associated with artificial substrata (Hill and Knight 1988; Corkum 1996). A recent development in DS technology is the addition of organophosphorus-based insecticides. Malathion, a cholinasterase inhibitor, has been found to reduce invertebrate numbers and, thereby increase periphyton biomass on DS (Dubé et al. 1997). However, malathion addition to DS in a New Zealand stream caused a reduction of invertebrates but no subsequent increase in periphyton biomass (Winterbourn 1990). Variability in methods employed and results attained by different studies indicated that pilot experiments should be conducted to determine: (1) the number of replicates for each treatment, and (2) if malathion increased periphyton biomass on the DS (presumably by reduced grazer abundance).

Pilot nutrient enrichment experiments with DS were conducted in four streams in September 1996 (pre-harvest) and in one stream in May 1997 (post-harvest). The clay flower pot DS were constructed as described in Chapter 2, with the addition of a 0.05 M concentration of malathion added to the four treatments (N, P, N+P, or control, C), in the September 1996 experiment. In the May 1997 experiment, eight nutrient treatments were tested, four nutrient treatments without malathion (C, N, P, N+P), and four nutrient treatments containing malathion (MC, M+N, M+P, M+N+P).

In fall 1996, twenty-four DS, six replicates of each treatment, were randomly attached to the streambed in three riffles above and another three riffles below small scale logging activities (i.e., <3% logged) beside four streams. In spring 1997, eighty DS, ten replicates of each treatment, were randomly placed in one riffle above and another riffle below the harvest blocks in one stream (Cottonwood). The DS were incubated in the streams for approximately 30 d. Periphyton was scraped from a 19.24 cm^2 area on the downstream facing surface of each DS. Periphyton from the DS was analysed for

Chlorophyll *a* (Chl*a*) following the procedure outlined in Chapter Two.

In the fall 1996 experiment, differences in Chl*a* concentration on DS were analysed for each site with a 2x2 factorial ANOVA (i.e., the presence or absence of N and the presence or absence of P). Chlorophyll *a* data from lower Meadow, lower Cottonwood, both Kettle sites and lower Waddell were $\log_{10}(x+1)$ transformed to meet the assumption of homogeneity of variance. For the upper Kettle site data were $1/x+3/8$ transformed. In the spring 1997 experiment, malathion-treated DS were analyzed with a 2x2 factorial ANOVA (i.e., an N factor and a P factor) for each site. Likewise, DS not containing malathion were also analysed separately with a 2x2 factorial ANOVA. Because data were separated based on malathion content, a Bonferonni correction was employed (i.e., $\alpha = 0.025$).

Periphyton in streams on the Boreal Plain did not exhibit N or P limitation ($P>0.07$ for N and P factor at each site), in the fall 1996 experiment (Figure A1). It is possible that other environmental parameters overwhelmed a nutrient enrichment response, or that malathion, an organophosphorus-based insecticide, confounded a nutrient enrichment response. Furthermore, high variability was associated with these DS. Thus, more replicates of each treatment and the placement of DS in only one riffle (as opposed to three riffles) were considered for future experiments.

In the spring 1997 experiment, N-limitation was apparent in the upper Cottonwood site when malathion treated DS were removed from the analysis. ($P<0.001$ for the N factor; Figure A2). However, malathion confounded a nitrogen limitation response at this site, as the malathion treated DS did not exhibit N limitation (N factor, $P = 0.08$). No detectable N or P enrichment was found at the lower Cottonwood site (N factor $P= 0.15$, P factor $P= 0.32$; N factor $P= 0.03$, P factor $P= 0.1$, for malathion treated and non-malathion treated DS, respectively). Although, Chl*a* concentration on the insecticide-free N-enriched DS at the lower Cottonwood was not significantly higher than the Control DS ($P = 0.03$), there was a trend to higher Chl*a* concentrations on N-enriched DS at this site. Thus, N-enrichment response in both Cottonwood sites was confounded by malathion addition.

In contrast to previous studies that found malathion addition contributed to higher periphyton biomass on substrata containing the limiting nutrient (Peterson et al. 1993; Dubé et al. 1997), results from May 1997 suggest malathion confounded an N-enrichment effect. Malathion has been found to have a range of effects to freshwater algae, depending on algal species and insecticide concentration. Low concentrations (0.1, 0.5 μg•l^{-1}) of the insecticide has been found to stimulate Chl*a* production by *Chlorella* (Torres and O'Flaherty 1976). However, concentrations of malathion of 1 μg•L^{-1} inhibited Chl*a* production in many algal species (i.e., *Tribonema*, *Stigeoclonium*, *Vaucheria*, and *Oscillatoria*) (Torres and O'Flaherty 1976). Growth of two species of cyanobacteria, *Anabaena* and *Aulosira* was not altered by the addition of 0.01 μg•L^{-1} malathion (Tandon et al. 1988), yet cyanobacterial growth was inhibited at 0.05 and 0.1 μg•L^{-1} malathion immediately following exposure (Tandon et al. 1988). *Anabaena* returned to control growth rates after 20 d, while *Aulosira* growth remained low after 30 d (Tandon et al. 1988). Murray and Guthrie (1980) also observed that organophosphorus insecticides initially inhibit algal growth but algae return to control rates ultimately. Thus, the effects of malathion on freshwater algae are variable and are species and concentration specific. Experiments to determine how malathion confounded the N-enrichment response are beyond the scope of the current study. However, the results from the spring 1997 experiment indicated that the insecticide should not be used in future experiments.

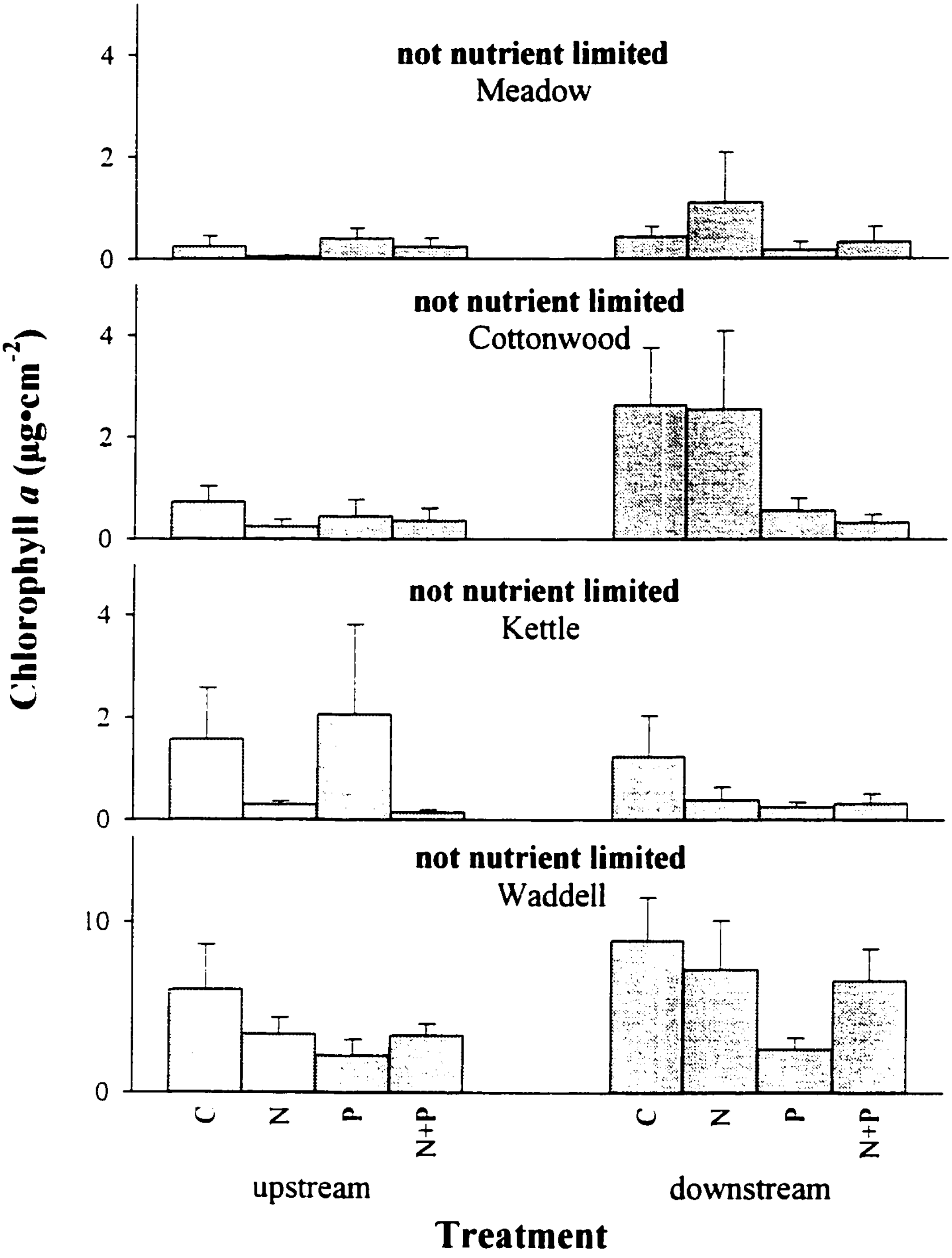

Figure A1: Periphyton biomass (expressed as Chlorophyll *a* concentration, mean± 1*SE*) on four nutrient diffusing substrata treatments in four boreal streams in the fall of 1996 (pre-harvest). C = Control, N = Nitrogen, and P = Phosphorus.

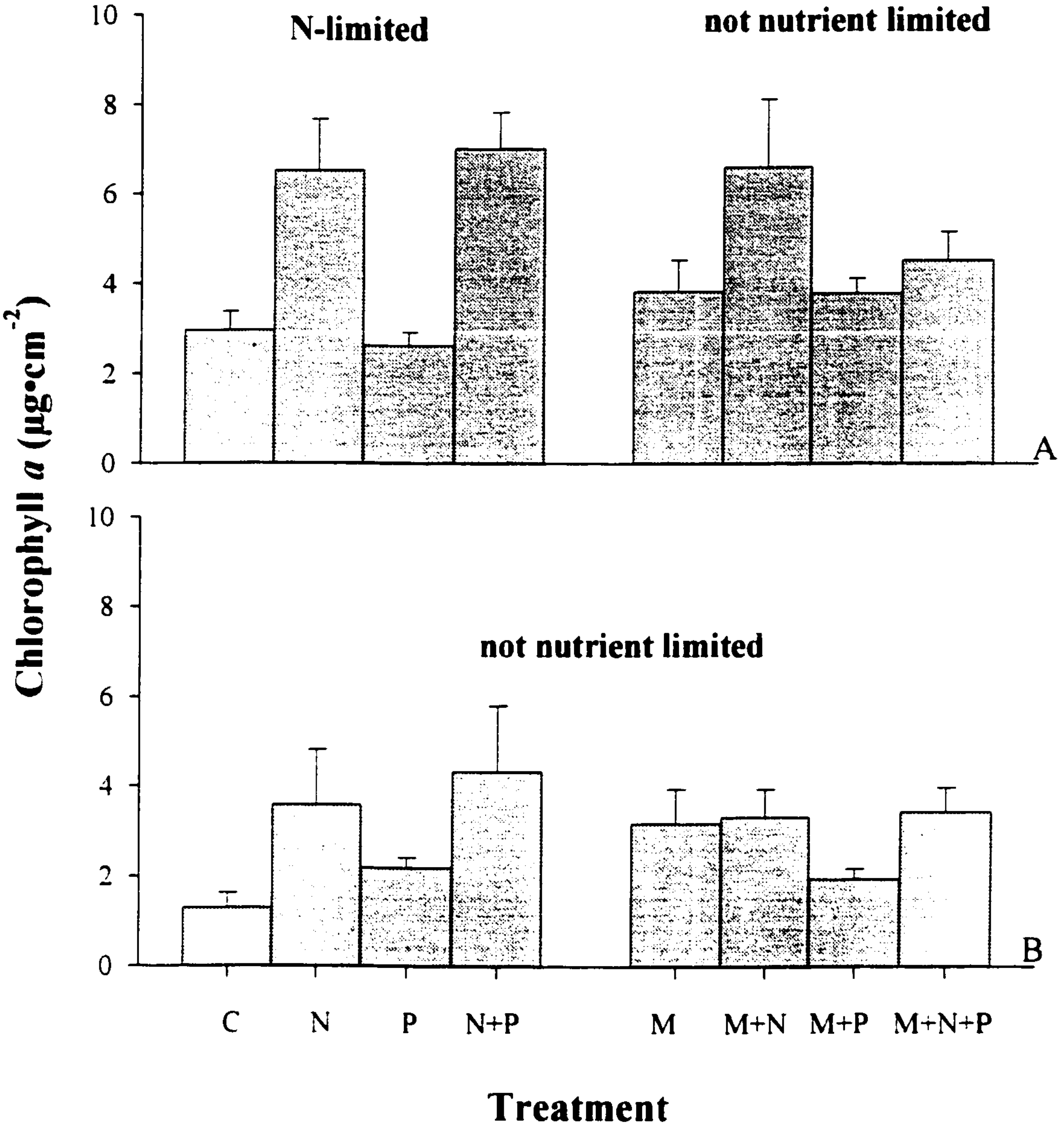

Figure A2: Periphyton biomass (expressed as Chlorophyll *a* concentration, mean±1*SE*) on eight nutrient diffusing substrata treatments placed upstream (A) and downstream (B) of harvested areas in one boreal stream, Cottonwood. Experiments conducted in June 1997. C = Control, M = Malathion, N = Nitrogen, P = Phosphorus.

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Appendix B: Data transformations

Table B1: Data transformations performed on physical and chemical variables during the 1997 nutrient enrichment experiments. Velocity and depth data did not meet that assumption of homogeneity of variance (Levene's Test) even after $\log_{10}(x)$, $\ln(x)$, $1/\sqrt{x+{}^3/_8}$, and x^2 transformations, x represents velocity or depth data. n.a. is not applicable.

Factor	Transformation	Levene's *P*	Meet Assumption
discharge	$1/\sqrt{x}$	0.07	y
velocity	none	0.00	n
depth	none	0.00	n
canopy cover	none	0.10	y
attenuation coefficients	none	0.37	n
DIN	only two measurements	n.a.	n.a.
DIN:SRP	only two measurements	n.a.	n.a.

Table B2: Data transformations performed on physical, chemical, and biological variables during the 1996 and 1997 benthic surveys. x represents the specific variable.

Factor	Transformation	Levene's *P*	Meet Assumption
velocity			
1996	x^2	0.06	y
1997	none	0.24	y
depth			
1996	none	0.14	y
1997	$\log_{10}x$	0.10	y
canopy cover			
1996	none	0.10	y
1997	arcsine	0.003	n
attenuation coefficients			
1996	none	0.33	y
1997	none	0.37	y
DIN			
1996	$\log_{10}x$	0.47	y
1997	$\log_{10}x$	0.34	y
SRP			
1996	none	0.04	n
1997	none	0.23	y
October Chlorophyll *a*			
1996	$\log_{10}x$	0.08	y
1997	none	<0.001	n

Table B3: Significance values of homogeneity of variance tests for $\delta^{13}C$ and $\delta^{15}N$ signatures of four mayfly families evaluated in a three way analysis of variance (ANOVA). A *P* of <0.05 indicates that the assumption of homogenous variance across sets were not met. Bolded values indicate transformation that was used in ANOVAs. x is $\delta^{15}N$ or $\delta^{13}C$. ^{a} x+10 for $\delta^{15}N$ prior to $\log_{10}$ or square root transformation. ^{b} –(x) for $\delta^{13}C$ prior to $\log_{10}$ or square root transformation.

type	none	1/x	$\log_{10}x$ ^{a,b}	$\sqrt{x}$ ^{a,b}	x^2
$\delta^{13}C$					
Baetis sp.	**0.05**	0.05	0.05	0.05	0.04
Heptageniidae	**<0.001**	<0.001	0.001	0.001	0.001
Ephemerellidae	**0.02**	0.08	0.02	0.02	0.02
$\delta^{15}N$					
Baetis sp.	**<0.001**	<0.001	<0.001	<0.001	<0.001
Heptageniidae	0.01	**0.09**	0.01	0.01	<0.001
Ephemerellidae	**0.38**	0.05	0.30	0.34	0.49