

Spatial variation of nitrogen fixation in lakes of the northern Great Plains

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Abstract

Cyanobacterial blooms are a regular feature of lakes in central North America, but little is known of their importance to the nitrogen (N) cycle and nutrition of aquatic food webs. We hypothesized that N₂-fixing cyanobacteria constitute a significant source of N to prairie lakes, that fixed N is effectively transferred to primary and secondary consumers, and that the importance of fixed N is structured on a landscape scale due to spatial gradients of lake chemistry and catchment characteristics. These hypotheses were tested using stable isotope analyses and mass budgets in six chained lakes of the northern Great Plains that were sampled biweekly during summers of 1994–2004. Mean annual N isotope signatures of particulate organic matter ($\delta^{15}\text{N-POM}$) were highly correlated to the abundance of N₂-fixing cyanobacteria ($r^2 = 0.75$, $p < 0.001$), but exhibited no marked spatial organization. Instead, the temporal variability of $\delta^{15}\text{N-POM}$ was greatest in downstream lakes where N₂-fixing cyanobacteria were abundant. Furthermore, seasonal declines in $\delta^{15}\text{N-POM}$ were correlated with reductions in $\delta^{15}\text{N}$ of individual zooplankton taxa (*Daphnia* spp., *Diacyclops thomasi*, *Leptodiptomus siciloides*, *Leptodora kindtii*), especially in downstream eastern lakes. N mass balances revealed that the importance of biological N₂ fixation relative to total N inputs (up to 77%) and initial N standing stock (up to 201%) were significantly linearly correlated with landscape position ($0.59 \leq r^2 \leq 0.83$, $p \leq 0.07$) and effective drainage area ($0.61 \leq r^2 \leq 0.98$, $p \leq 0.07$), as were volumetric and areal estimates of N₂ fixation during summer ($r^2 \geq 0.87$, $p \leq 0.007$). These patterns demonstrate that fixed N is spatially structured, highly predictable based on landscape position, and readily assimilated into aquatic food webs.

About 90 to 130 million metric tons of nitrogen (N) are biologically fixed on land each year, while an equivalent amount may be fixed in oceans (Vitousek et al. 1997). In lakes, the importance of biological fixation is extremely variable, with annual rates varying between 200 and 9,200 mg N m⁻² in eutrophic lakes (Howarth et al. 1988b). In general, N₂ fixation tends to be greater in the littoral or shallow regions of lakes than in pelagic zones where fixation rates usually increase from spring to late summer (Wetzel 2001). Recent studies of the importance of N₂ fixation in lakes are based on analysis of temporal variations of N₂-fixing cyanobacterial abundance (Havens et al. 2003), isotopic composition of primary producers (Rolff 2000; MacGregor et al. 2001), or acetylene to ethylene reduction (Tonno and Noges 2003; Ferber et al. 2004). However, most studies have been limited to a single site, and little is known of how N₂ fixation varies on a spatial scale. Because lake position within the landscape is known to strongly influence characteristics such as catchment area, nutrient status, production, and biological diversity (Magnuson and Kratz 2000), the relative impor-

tance of atmospheric N₂ fixation might be expected to vary in a spatially explicit fashion. Specifically, because the concentrations of conservative elements and nutrients often increase from upstream lakes to downstream lakes (Soranno et al. 1999), because lakes differ in the relative retention rates of N and phosphorus (P) (e.g., Kenney 1990), and because relative supply rates of N and P influence cyanobacterial abundance (Howarth et al. 1988a), the importance of fixed N to lake metabolism is hypothesized to vary along hydrologic gradients.

Heterocystous, potentially toxic cyanobacteria are primarily responsible for pelagic N₂ fixation, especially in eutrophic lakes with elevated concentrations of dissolved P, low N : P ratios, and high ambient irradiance (Millie et al. 1999) such as are common on the Great Plains of North America. For example, lakes of the prairie ecozone of Canada typically harbor species of the genera *Aphanizomenon*, *Anabaena*, and *Gloetrichia*, in part because of P-rich soils and high irradiance (Barica 1987). However, despite the prevalence of cyanobacteria in the phytoplankton of some lakes, there is little agreement on whether atmospheric N₂ is quantitatively incorporated into the aquatic food webs. On one hand, the colonial nature and potential toxicity of heterocystous cyanobacteria make them poor food for zooplankton (de Bernardi and Giussani 1990). On the other hand, strong empirical correlations between cyanobacterial abundance and stable isotope signals in plankton and lake sediments (Talbot 2001) suggest that fixed N₂ may be quantitatively important to some lake ecosystems. In particular, seasonal development of N₂-fixing cyanobacteria often results in decreased ratios of ¹⁵N to ¹⁴N (lower $\delta^{15}\text{N}$ values) of algae (e.g., Rolff 2000; MacGregor et al. 2001; Lehmann et al. 2004) because prokaryotes assimilate atmospheric N₂ with little fraction-

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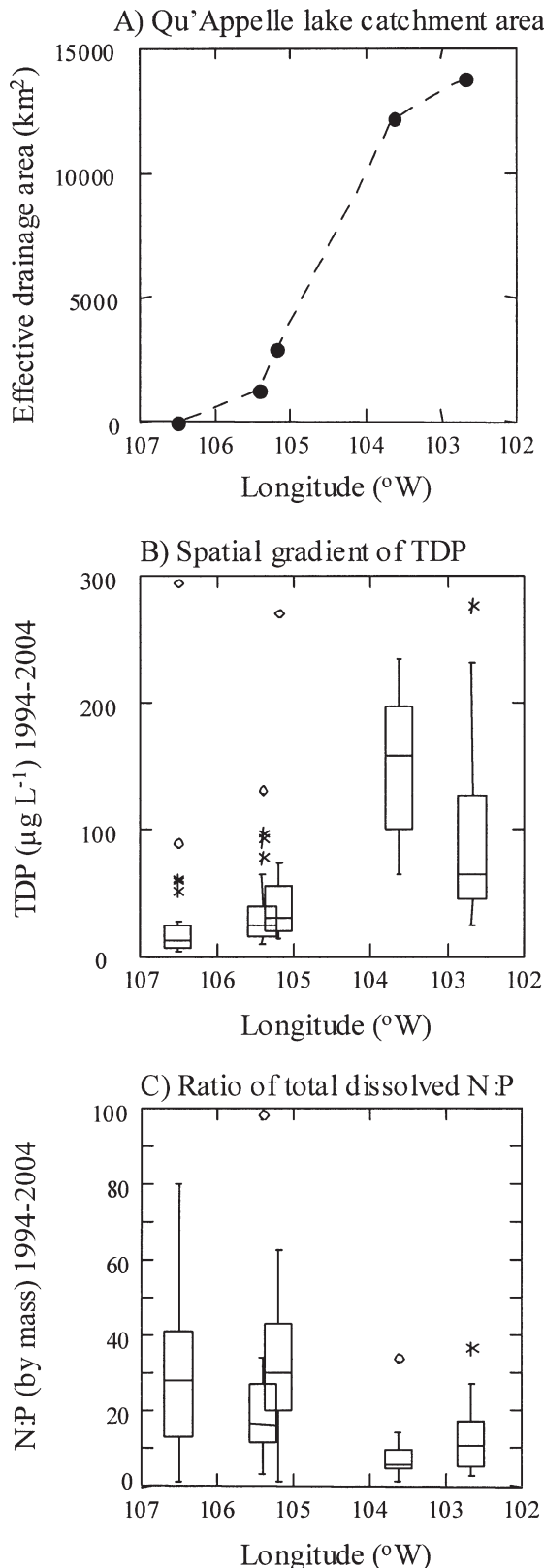


Fig. 1. (A) Spatial gradient of effective drainage area per lake, (B) mean total dissolved P concentration (DOY 130–234), and (C) mass ratio of total dissolved N : P for lakes receiving water from the Qu'Appelle River, 1994–2004. Wascana Lake lies on a separate tributary and is not included. Asterisks denote

values greater than $1.5 \times$ the interquartile range (IQR), while empty circles denote values greater than $3 \times$ IQR.

ation ($\delta^{15}\text{N}_{\text{atm}} = 0\text{‰}$) (Peterson and Fry 1987). Unfortunately, little is known of how the importance of this fixed N varies across landscapes. The primary objective of this study is to quantify how the importance of N_2 fixation to lake metabolism and aquatic food-web nutrition varies as a function of lake position within the landscape. Because of obvious spatial gradients of nutrient concentration and ratios within our prairie catchment (Soranno et al. 1999), we hypothesized that the importance of fixed N would increase from headwaters to downstream lakes. Further, we believed that this atmospherically derived N should be rapidly incorporated into the aquatic food webs. These hypotheses were tested using stable isotope analysis of particulate organic matter (POM) and individual zooplankton species, as well as whole-lake mass balance calculations, for six linked prairie lakes during summers from 1994 to 2004.

Material and methods

Study sites—The Qu'Appelle Valley river basin encompasses $\sim 52,000$ km² ($50^{\circ}00'N$ to $51^{\circ}30'N$) in southern Saskatchewan and extends ~ 400 km from its headwaters near Lake Diefenbaker to the confluence with the Assiniboine River in Manitoba (see Fig. 1 in Hall et al. 1999b). Land use within the Qu'Appelle basin is 75% cropland and 12% natural grasslands (Hall et al. 1999b) while main urban centers include the cities of Regina and Moose Jaw. Under natural conditions, the Qu'Appelle River originates near Eyebrow Lake, Saskatchewan, and flows through Buffalo Pound Lake, a central chain of four basins (Pasqua, Echo, Mission, and Katepwa lakes), and two downstream lakes (Crooked and Round). Water from Last Mountain Lake and the Wascana Lake reservoir flow into the Qu'Appelle River via Last Mountain Creek and Wascana Creek, respectively. Since the 1960s, flow of the Qu'Appelle River has been supplemented with water from the Lake Diefenbaker reservoir, which impounds the South Saskatchewan River draining the eastern Rocky Mountains, whereas Buffalo Pound Lake serves as a drinking water reservoir for Regina and Moose Jaw. Average annual flow in the Qu'Appelle River is $8.7 \text{ m}^3 \text{ s}^{-1}$ near the catchment midpoint north of Regina, 34% of which occurs during May–August (days of year [DOY] 123–241). Further, control structures are used to regulate water levels of most lakes, as well as to supply water to Last Mountain Lake during the spring hydrologic discharge maximum.

Landscape patterns of lake chemistry and biology have been described in detail by Hall et al. (1999a,b), Dixit et al. (2000), and Quinlan et al. (2002). Morphometric features of selected lakes are presented in Table 1. All lakes are polymictic in most years, yet central and eastern lakes exhibit pronounced deepwater anoxia by late summer. In addition, the effective drainage area, defined as the region supplying water to a lake during years of median river flow,

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values greater than $1.5 \times$ the interquartile range (IQR), while empty circles denote values greater than $3 \times$ IQR.

Table 1. Morphometric and catchment characteristics of lakes of the Qu'Appelle Valley drainage basin.

	Diefenbaker	Buffalo Pound	Last Mountain	Wascana	Katepwa	Crooked
Lake area (km ²)	500	29	227	0.5	16	15
Volume (10 ⁶ m ³)	9,400	87	1,807	0.68	233	121
Mean depth : maximum depth*	0.53	0.55	0.26	0.50	0.62	0.49
Flushing rate (yr ⁻¹)	0.77	1.43	0.08	64	0.75	2.0
N : P ratio, by total dissolved mass	30	20	31	6	8	12
Cyanobacterial species†	1	3, 4, 7, 8	2, 4	No data	2, 4, 5, 6, 7	2, 4, 5, 6, 7

* Mean depth to maximum depth ratio indicates lake shape, from conical (0.33), to parabolic (0.66), to flat-bottomed (1).

† Cyanobacteria species composition during 1995; 1. *Anabaena* sp., 2. *Anabaena spiroides*, 3. *Anabaena inaequalis*, 4. *Aphanizomenon flos-aquae*, 5. *Aphanocapsa delicatissima*, 6. *Lynbya birgei*, 7. *Microcystis aeruginosa*, 8. *Oscillatoria tenuis*.

increases predictably from west to east for lakes that receive Qu'Appelle River water (Fig. 1A), as do mean summer concentrations of total dissolved P (Fig. 1B) and chlorophyll *a* (Chl *a*; Soranno et al. 1999). In contrast, mean ratios of total dissolved N : P are generally greater in western headwaters (20–31, by mass) than in downstream lakes (6–12; Fig. 1C), as is Secchi-depth transparency (Soranno et al. 1999). Cyanobacteria are common in all lakes, with intense surface blooms of colonial and N₂-fixing taxa during mid to late summer (McGowan et al. 2005). Because of these spatial gradients, we hypothesized that the importance of fixed atmospheric N₂ to the food-web nutrition and overall lake N budget should increase on a landscape basis, from west to east.

Sampling—Diefenbaker, Buffalo Pound, Last Mountain, Katepwa, and Crooked lakes were sampled biweekly between 01 May (DOY 121) and 31 August (DOY 243) during 1995–2004. All lakes were also sampled 01 July–31 August 1994, whereas Wascana Lake was sampled during 1997–2004 only. Integrated water samples for phytoplankton enumeration were collected using a tube deployed over the entire water column, while water samples for chemical analyses were obtained from pooled Van Dorn casts taken each meter below the surface. Aliquots of integrated water for phytoplankton enumeration were preserved with Lugol's iodine solution. Prescreened (243- μ m mesh) integrated water samples were filtered onto prewashed GF/C glass fiber filters and were frozen (–10°C) before analysis for Chl *a* by standard trichromatic methods, algal and bacterial carotenoids, and chlorophylls by high-performance liquid chromatography (HPLC; Leavitt and Hodgson 2001), and elemental composition and stable isotope content of POM (Savage et al. 2004). Microscopic inspection confirmed that POM was composed primarily of algae and that zooplankton was absent. Zooplankton were collected using both 243- μ m and 750- μ m mesh conical nets towed to the surface from maximum depth. Taxonomic analyses were performed on 243- μ m mesh net samples preserved with sugared ethanol solution, whereas stable isotope analyses were performed on individual species isolated from formalin-preserved, 750- μ m mesh net samples that had been thoroughly washed to remove the preservative. Comparison of isotopic signatures of preserved (up to 1 yr) samples and fresh samples revealed no

systematic deviation in $\delta^{15}\text{N}$ signatures ($\pm 0.5\%$); therefore, no correction was applied to isotopic analyses.

Laboratory analyses—Quantification of P, orthophosphate, total dissolved nitrogen (TDN), nitrate, and ammonium were performed on 0.45- μ m filtered integrated water samples following standard procedures (D'Elia et al. 1977; Environment Canada 1979). In 1995 only, algal species composition and abundance were quantified at $\times 400$ magnification using inverted light microscopy (Graham 1997). Algal cells were identified to species and enumerated along randomly chosen 1-cm-long transects following sedimentation for 24 h using the Utermöhl technique (Utermöhl 1958). Counts were also performed on cyanobacterial species exhibiting heterocysts to estimate the absolute (cells mL⁻¹) and relative (%) abundance of N₂-fixing algae in 1995 phytoplankton communities (Graham 1997; Patoine et al. 2005).

Algal composition during 1994–2004 was also estimated by analyzing algal pigment concentrations in integrated water-column samples (Graham 1997; Hall et al. 1999b). Carotenoid, chlorophyll, and derivative pigment concentrations were quantified using a Hewlett Packard model 1050 HPLC system following standard procedures detailed in Leavitt and Hodgson (2001). Biomarker concentrations (nmol pigment L⁻¹) were estimated for pigments characteristic of total algal abundance (Chl *a*, pheophytin *a*, β -carotene), siliceous algae and some dinoflagellates (fucoxanthin), mainly diatoms (diatoxanthin), cryptophytes (alloxanthin), chlorophytes (Chl *b*, pheophytin *b*), chlorophytes and cyanobacteria (lutein-zeaxanthin), total cyanobacteria (echinenone), colonial cyanobacteria (myxoxanthophyll), Nostocales (canthaxanthin), and potentially N₂-fixing cyanobacteria (aphanizophyll). In this HPLC system, carotenoids from *Aphanizomenon* (aphanizophyll), *Anabaena* (4-keto-myxoxanthophyll), and the Oscillatoriaceae (oscillaxanthin) were incompletely resolved and were presented together as “aphanizophyll” (Hall et al. 1999b).

Stable isotope analyses of POM and individual zooplankton were conducted following the method described by Savage et al. (2004). POM was removed from dried (40°C) GF/C filters using forceps, whereas individual adult zooplankton were sorted by hand from the 750- μ m mesh net to the species level, including *Daphnia* spp. (primarily *Daphnia galeata mendotae* and some *Daphnia retrocurva*),

Leptodiatomus siciloides, *Diacyclops thomasi*, and *Leptodora kindtii*. All isolated samples were dried completely at 40°C and ~2 mg of dry mass was packed into tin capsules before combustion in an NC2500 Elemental Analyzer (ThermoQuest, CE Instruments) and introduction into a Thermoquest (Finnigan-MAT) Delta Plus^{XL} isotope ratio mass spectrometer (IRMS). Nitrogen stable isotope ratios are reported in the conventional δ notation with respect to atmospheric N ($\delta^{15}\text{N} = 0\text{‰}$). Analytical precision, expressed as the standard deviation on 12 wheat flour standard replicates, was 0.4‰.

Nitrogen mass balance—Lake-specific nitrogen budgets were developed to quantify the importance of N_2 fixation relative to other nitrogen sources (inflow, precipitation, others) and sinks (outflow, sedimentation), and to determine whether there was evidence of landscape patterns of N_2 fixation such as suggested by spatial patterns of lake P content and N : P ratios (Fig. 1).

An isotope balance equation (e.g., Hübner 1986; Yoshioka and Wada 1994) was used to estimate the net amount of N fixed during the course of the growing season, based on the lake's standing stock of N (product of N concentration and lake volume) and the N isotopic signature of POM ($\delta^{15}\text{N}$ -POM) at the beginning (defined as average date DOY 130) and end of the growing season (DOY 234). According to this equation, $\delta^{15}\text{N}$ -POM at the end of the season (δ_{t+1}) should equal the mass-weighted average of the background N isotopic signature at the beginning of the season (δ_t), and the signature of pure N_2 -fixing populations of cyanobacteria (δ_{fix}):

$$\delta_{t+1} = (M_t \delta_t + M_{\text{fix}} \delta_{\text{fix}}) / (M_t + M_{\text{fix}}) \quad (1)$$

where M_t and M_{fix} represent the N standing stock (in megagrams) at the beginning of the season (M_t) and the amount of N fixed (M_{fix}), respectively. We first solved for M_{fix}/M_t (the fractional increase in the starting N standing stock that can be attributed to N_2 fixation) as:

$$M_{\text{fix}}/M_t = (\delta_t - \delta_{t+1}) / (\delta_{t+1} - \delta_{\text{fix}}) \quad (2)$$

A tentative value for δ_{fix} was obtained by extrapolating the ordinary least-square regression line of $\delta^{15}\text{N}$ -POM as a function of the relative abundance of heterocystous cyanobacteria in the phytoplankton community. The corresponding $\delta^{15}\text{N}$ -POM was -2.8‰ when POM samples were composed entirely of N_2 -fixing cyanobacteria. However, because this value was obtained by extrapolating beyond the observed range of abundance for heterocystous cyanobacteria, we preferred to use a δ_{fix} value of 0‰ , which is more in line with previously reported values of $\delta^{15}\text{N}$ from N_2 -fixing cyanobacteria (e.g., -0.55‰ in Minagawa and Wada 1984; -1.7‰ in Rolff 2000).

Mass balances were calculated using two contrasting values for initial standing stock of N. First, we assumed that particulate (POM) and dissolved pools of N were in equilibrium and that the entire standing stock of N at the start of the growing season was labeled with atmospheric N

from N_2 -fixing cyanobacteria. In this scenario, we estimated the maximum possible mass of nitrogen fixed as:

$$M_{\text{fixedMax}} = (M_{\text{fix}}/M_t) \times \text{TN}_{\text{ss}} \quad (3)$$

where TN_{ss} is the total (particulate and dissolved) N standing stock on DOY 130. However, because it is unlikely that both dissolved and particulate pools of N were labeled with atmospheric N to the same extent or at the same rates, we also computed the minimum possible estimate of fixed N:

$$M_{\text{fixedMin}} = (M_{\text{fix}}/M_t) \times \text{PON}_{\text{ss}} \quad (4)$$

where PON_{ss} is the particulate organic nitrogen on DOY 130. PON concentration was estimated from Chl *a* concentration, assuming that PON sources other than algae were quantitatively negligible. Chl *a* and ash-free dry mass (AFDM) content of algal cells were assumed to be 1% and 50% of dry mass, respectively, whereas N content was assumed to be 7% of AFDM (Reynolds 1984). While the resulting ratio of 3.5 PON to 1 Chl *a* may vary somewhat (between 2 and 8) depending on the phytoplankton species composition and the ambient growth conditions, it is treated as a fixed constant across all lakes. In this scenario, we assumed that atmospheric N is incorporated solely into the PON pool. This calculation underestimates the true rate of net N fixation because it does not account for the rapid turnover of algal biomass during the summer or for transfers of fixed N to other pools (e.g., zooplankton, sediments, etc.). Consequently, by bracketing the true estimates of net ecosystem N fixation, we sought to focus on the spatial patterns of N fixation, rather than the absolute values of N fixed.

The mass-balance approach makes several additional assumptions. First we assumed that N pools are mixed rapidly within each lake and that pelagic samples also reflect littoral and benthic N transformations. Second, we assumed that temporal changes in $\delta^{15}\text{N}$ -POM primarily reflect the balance between uptake of atmospheric N_2 by biological fixation (which reduces $\delta^{15}\text{N}$ -POM) and losses or transformations of N by denitrification or nitrification (which increase $\delta^{15}\text{N}$ -POM) and, therefore, that declines in $\delta^{15}\text{N}$ -POM arise from a net influx of N_2 via biological fixation within the lake. Finally, because we lacked highly resolved (weekly) inputs of N from all sources, we assumed that other N inputs during the sampling period did not contribute to the N isotope balance, although we recognize that this is unlikely to be true. As a consequence, this mass-balance approach entails some uncertainty surrounding the true rate of biological N_2 fixation (see Discussion), but remains useful in quantifying the relative importance of N fixation among lakes and along spatial gradients.

Nitrogen loss through particulate sedimentation was computed using previously published, lake-specific estimates of permanent sediment accumulation ($\text{g cm}^{-2} \text{ yr}^{-1}$) and N content of sediments (% dry mass) during 1985–1995 (Hall et al. 1999a,b) and lake area (km^2). Comparison of 62 cores within reference Pasqua Lake revealed that these central cores were representative of lake-wide N deposition patterns (P. R. Leavitt, et al. unpubl.). Further, we assumed

that because sediment parameters related to N varied little during the past 30 yr (e.g., Hall et al. 1999a,b and unpubl.), they could be used to estimate N deposition rates during the period 1994–2004. Annual sedimentation rates were adjusted to represent the mean annual sampling period (DOY 130–234) by assuming that sedimentation was constant throughout the average ice-free period (mid-April to mid-October). Overall, this procedure overestimates true N export to sediments, but provides a reasonable value for comparison among lakes given that most basins lie in valley bottoms and share similar morphometry (steep littoral zones, large flat central regions) (Table 1).

Nitrogen loss through river outflow was estimated as the product of total nitrogen concentration (sum of the mean seasonal dissolved and particulate concentrations in mg L^{-1}) and outflow river discharge rates ($\text{m}^3 \text{s}^{-1}$) during the sampling period (DOY 130–234). Discharge rates were obtained from the Saskatchewan Watershed Authority Corporation and are based on weir measurements for 1994–2004. In contrast, N gains through river inflow were estimated directly from measurements of TDN concentrations and river inflow rates during DOY 130–234 for all years. While particulate N influx in the Qu'Appelle River is potentially significant in some circumstances (e.g., high flow events), too few data were available (1980–83; Munro 1986) to allow their use in the mass balances we constructed (1994–2004). Because this estimate of particulate N influx can reduce only the “other inputs” of N in the mass balance (see below), it does not affect spatial patterns of N_2 fixation. N deposition from the atmosphere was estimated at $2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Kochy and Wilson 2001). Other inputs of N (i.e., not fixed, not from main inflow river, not from precipitation) were estimated by mass difference so that total gains (N_2 fixation, river inflow, precipitation, other sources) and losses (sedimentation, outflow) were balanced for each lake, allowing for observed seasonal changes in a lake's standing stock of N.

Statistical analyses—Unreplicated linear regression was used to identify environmental correlates of variability in mean $\delta^{15}\text{N}$ -POM (‰), relative N_2 fixation (% initial standing stock, % total N inputs) and volumetric (g N m^{-3}) or areal (g N m^{-2}) estimates of N_2 fixation during DOY 130–234 for 1994–2004. As well, $\delta^{15}\text{N}$ -POM values from Diefenbaker, Buffalo Pound, Last Mountain, Katewpa, and Crooked lakes during DOY 130–234 in 1995 were regressed against absolute (cells mL^{-1}) and relative (%) abundances of cyanobacterial cells located in heterocystous filaments to estimate the effects of N_2 fixation by these taxa on N isotopic signatures. Potential predictors of spatial variation in N_2 fixation parameters were also analyzed by stepwise multiple regression using forward selection, and included longitudinal position of lakes (decimal degrees), effective catchment area (km^2), catchment : lake area ratios, mean flushing rate (yr^{-1} ; mean 1994–2002), lake depth (in meters), Secchi depth (in meters), water-column Chl *a* (mg L^{-1}), orthophosphate ($\mu\text{g L}^{-1}$; 1994–2001), total dissolved phosphorus ($\mu\text{g L}^{-1}$; 1998, 2002–2003), total dissolved nitrogen (mg L^{-1} ; 1994, 1998, 1999, 2002, 2003), nitrate + nitrite and ammonium

(mg L^{-1} ; 1995–2003), pH, dissolved organic carbon (DOC, mg C L^{-1}), salinity ($\text{mg total dissolved solids [TDS] L}^{-1}$), conductivity ($\mu\text{S cm}^{-1}$), major anion and cation concentrations (2003), mean cloud cover (% sky coverage on day of sampling), surface and deepwater temperatures ($^{\circ}\text{C}$), oxygen ($\text{mg O}_2 \text{ L}^{-1}$), thermal stratification ($\text{Temp}_{\text{top}} - \text{Temp}_{\text{bottom}}$), and deepwater anoxia ($[\text{O}_2]_{\text{top}} - [\text{O}_2]_{\text{bottom}}$). All environmental variables were mean values for the DOY 130–234 period, 1994–2004, unless otherwise noted. All statistical analyses were computed following appropriate transformation to normalize variance using SYSTAT v. 10.

Results

Spatial and temporal variations of $\delta^{15}\text{N}$ —Mean $\delta^{15}\text{N}$ -POM during DOY 130–234 varied from 4.7‰ in Buffalo Pound Lake to 9.4‰ in Katewpa Lake for the period 1994–2004, but exhibited little spatial structure within the Qu'Appelle Valley catchment, with elevated values in both headwater Lake Diefenbaker and downstream Katewpa Lake (Fig. 2A). In contrast, temporal variability of $\delta^{15}\text{N}$ -POM, estimated as the standard deviation of all the sampling events, increased as a function of the longitudinal position of the lake, from headwater to downstream (Fig. 2B). In general, this variation arose from progressive declines in mean $\delta^{15}\text{N}$ -POM during summer in Qu'Appelle lakes (Fig. 2C), particularly at eastern downstream sites (see following). When averaged over all lakes, annual $\delta^{15}\text{N}$ -POM varied in a sinusoidal pattern, with peaks in 1995 and 2001–2003 (Fig. 2D). This temporal pattern was not significantly correlated with a variation in the major climatic systems, indicated by indices of the El Niño–Southern Oscillation, North Atlantic Oscillation, and Pacific Decadal Oscillation (analysis not shown).

Variability of $\delta^{15}\text{N}$ -POM was strongly correlated to seasonal and interannual variability in the abundance of N_2 -fixing cyanobacteria (Fig. 3). For example, $\delta^{15}\text{N}$ -POM during both 1994 (see Graham 1997) and 1995 was strongly and negatively correlated with both the absolute ($r^2 = 0.75$, $p < 0.001$; Fig. 3A) and relative ($r^2 = 0.79$, $p < 0.001$; not shown) abundances of cyanobacterial cells associated with heterocystous filaments. Cyanobacteria species during 1995 included *Anabaena spiroides*, *Anabaena inaequalis*, *Aphanizomenon flos-aquae*, *Aphanocapsa delicatissima*, *Lyngbya birgei*, *Microcystis aeruginosa*, and *Oscillatoria tenuis* (Table 1). Similarly, values of $\delta^{15}\text{N}$ -POM during 1997–2003 were significantly and negatively correlated ($r^2 = 0.27$, $p < 0.001$) with water-column concentrations of aphanizopyll, a carotenoid complex indicative of potentially N_2 -fixing cyanobacteria (Fig. 3B), as well as with biomarkers of total cyanobacteria (echinenone), colonial cyanobacteria (myxoxanthophyll), and Nostocales forms (canthaxanthin) (all not shown).

Analysis of the $\delta^{15}\text{N}$ signature of individual zooplankton taxa revealed the expected pattern of trophic enrichment. When averaged over all lakes and years, mean ($\pm\text{SD}$) $\delta^{15}\text{N}$ -POM ($6.5 \pm 3.2\text{‰}$) was 3.8‰ less than that of *Daphnia* (~ 1 trophic level, Minagawa and Wada 1984; Post 2002), whereas daphnids were 2.6‰ less enriched than were omnivorous *D. thomasi*. In general, there was little

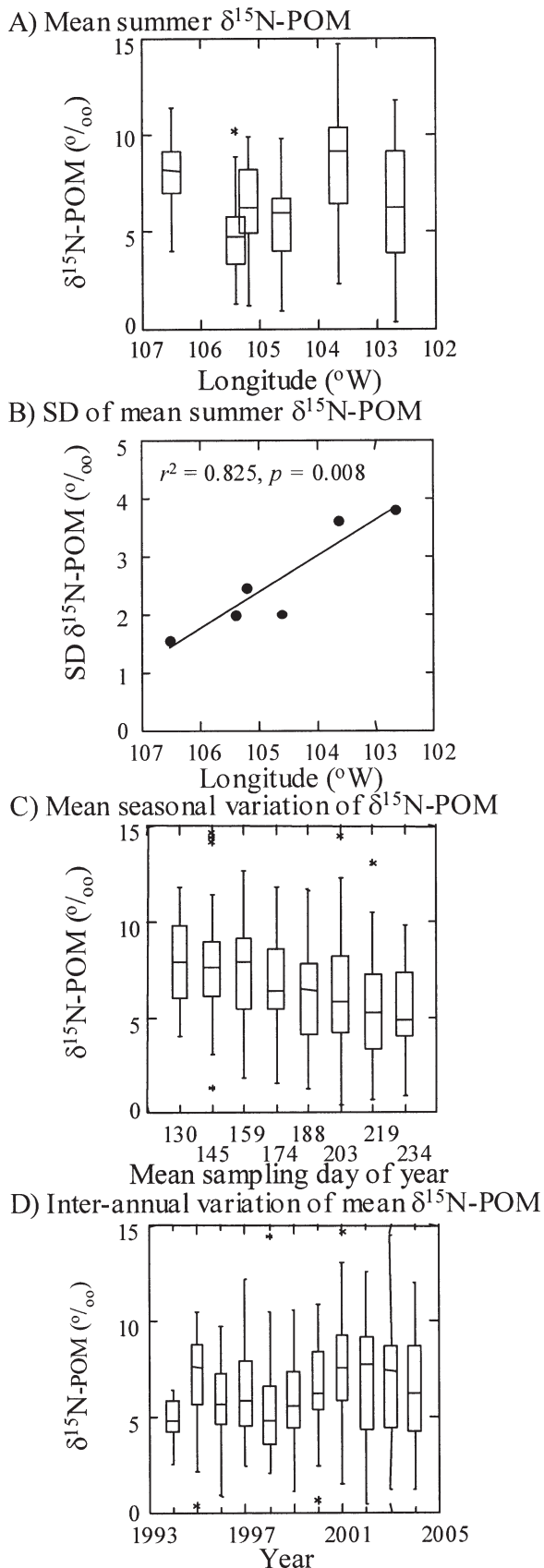


Fig. 2. (A) Spatial gradient of N isotopic signature of particulate organic matter in lakes of the Qu'Appelle Valley

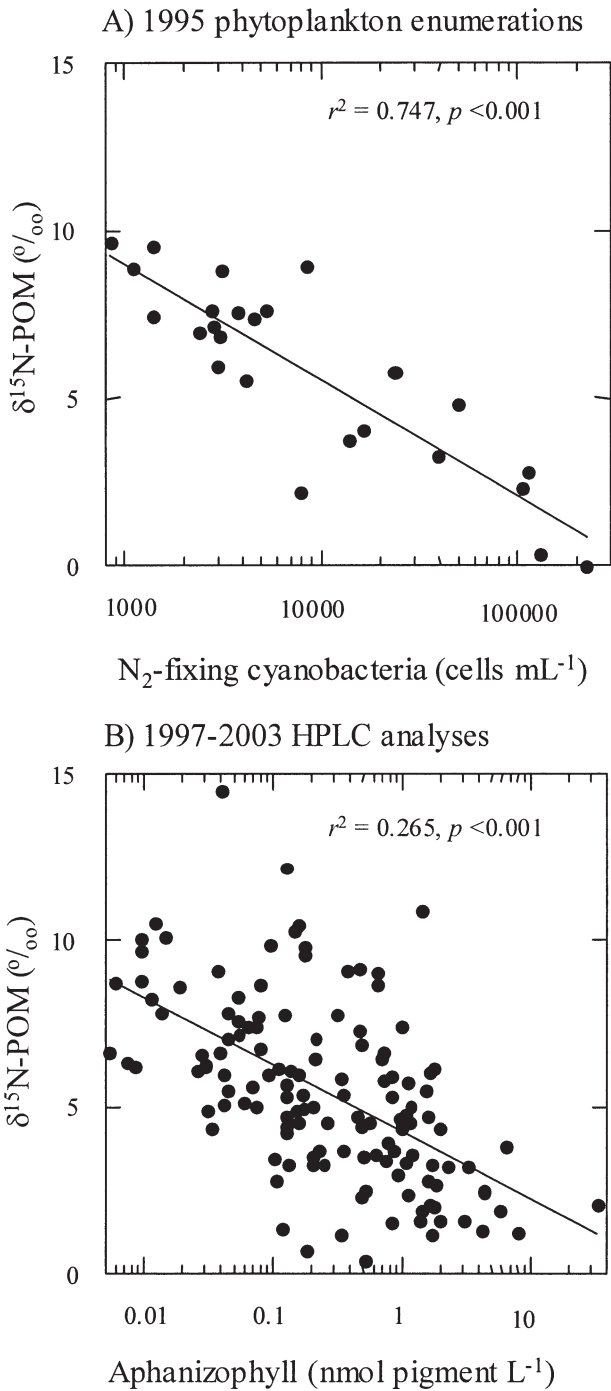


Fig. 3. Relationships between $\delta^{15}\text{N-POM}$ and water-column concentrations of (A) heterocystous N_2 -fixing cyanobacteria (1995 only) or (B) aphanizophyll (nmol pigment L^{-1}), a carotenoid biomarker of many potentially N_2 -fixing cyanobacteria (1997–2003) for DOY 130–234. Pigment concentrations measured from integrated samples using HPLC.

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catchment ($\delta^{15}\text{N-POM}$), (B) spatial gradient of standard deviation of $\delta^{15}\text{N-POM}$, and (C) seasonal (DOY 130–234) or (D) interannual (1994–2004) variability of mean $\delta^{15}\text{N-POM}$ for all study lakes. Asterisks as in Fig. 1.

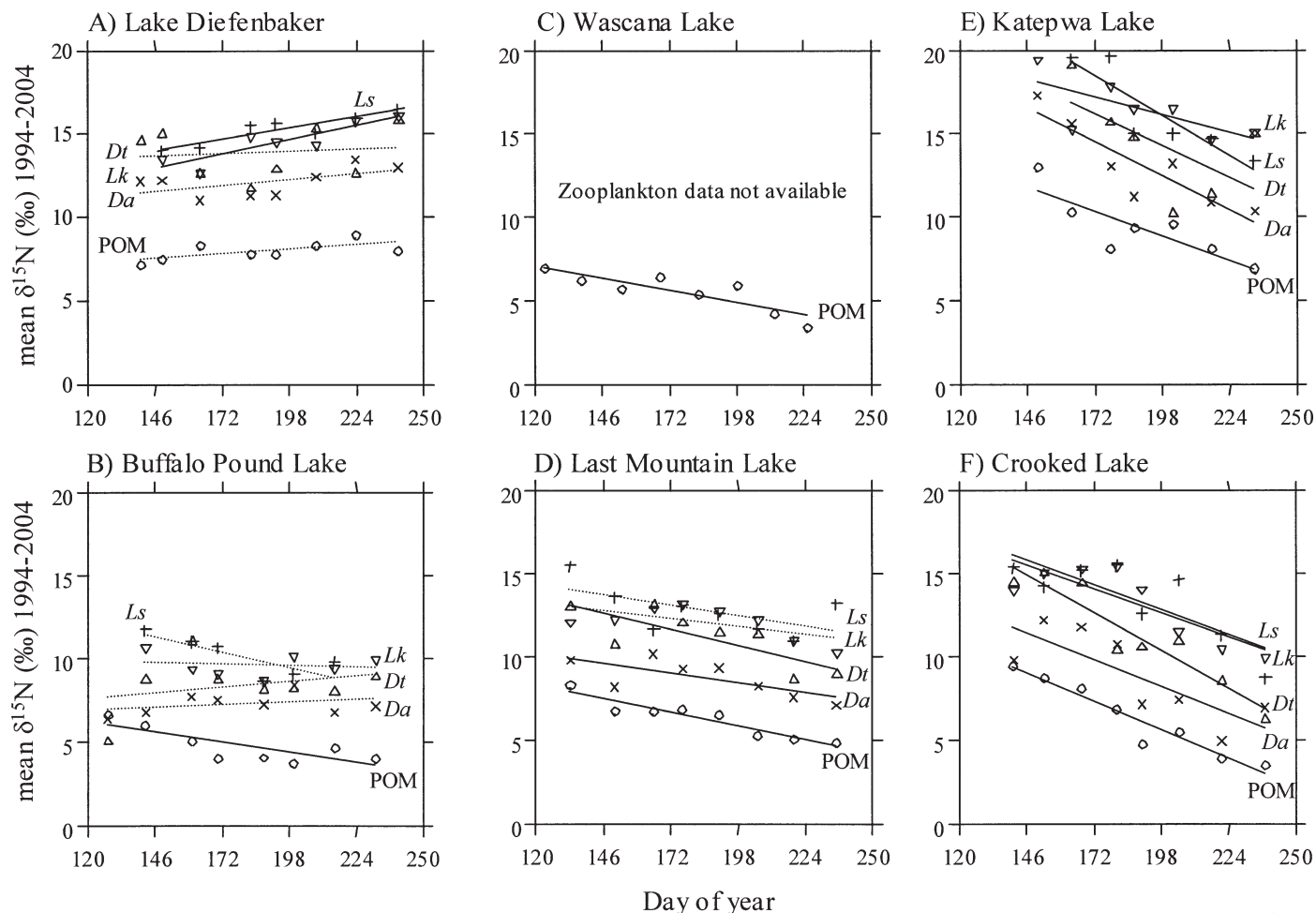


Fig. 4. Variation in mean (1994–2004) nitrogen isotopic composition ($\delta^{15}\text{N}$) of POM, *Daphnia* spp. (*D. galeata mendotae*, *D. pulicaria*), *Diacyclops thomasi* (*Dt*), *Leptodiptomus siciloides* (*Ls*), and *Leptodora kindtii* (*Lk*) in Qu'Appelle Valley lakes as a function of DOY. Solid lines indicate linear least-square regression models significant at the 0.05 level, dotted regressions are nonsignificant. Zooplankton data incomplete for Wascana Lake (not presented).

significant difference between the most enriched zooplankton species, including *D. thomasi* ($12.9 \pm 5\%$), *L. kindtii* ($13.4 \pm 4.3\%$), and *L. siciloides* ($13.7 \pm 3.1\%$), although there was some seasonal variation in the degree of enrichment, and hence inferred trophic position of species, among lakes (Fig. 4).

When analyzed by lake, strong spatial gradients were noted in the degree of seasonal variability in $\delta^{15}\text{N}$ -POM (Fig. 4). In all systems but Lake Diefenbaker, there was a significant ($p < 0.05$) decrease in mean $\delta^{15}\text{N}$ -POM during the sampling interval DOY 130–234. In addition, the slope of linear regressions describing these trends increased from headwater to downstream lakes (Fig. 4), thereby leading to strong spatial gradients in variability of $\delta^{15}\text{N}$ -POM (Fig. 2B). Similarly, slopes of linear regressions of $\delta^{15}\text{N}$ of zooplankton taxa versus sampling DOY were also spatially structured, with nonsignificant or positive slopes recorded in western Lakes Diefenbaker and Buffalo Pound (Fig. 4A,B), and strongly negative slopes in the two eastern-most lakes (Katepwa and Crooked; Fig. 4E,F). In contrast, rates of decline of the $\delta^{15}\text{N}$ signal of zooplankton from Last Mountain Lake, a midcatchment site, were more

modest and significant values were limited to lower levels of the food web (*Daphnia*, *Diacyclops*). Coherent temporal trends in $\delta^{15}\text{N}$ signatures of POM and all zooplankton species suggest that N associated with POM was rapidly and effectively transferred throughout the invertebrate food web.

Nitrogen budget—Mass balance estimates of net ecosystem N_2 fixation were sensitive to assumptions of the total initial pool of ecosystem N. For example, maximum estimates of the net quantity of nitrogen fixed (M_{fixedMax}) during the sampling period (DOY 130–234) varied from 0 Mg in Lake Diefenbaker to 1,207 Mg in Last Mountain Lake (Table 2), if fixed N was assumed to be incorporated into the total pool of dissolved and particulate N. In this case, the importance of fixed N to the overall N budget varied substantially among lakes (Table 2), expressed as a proportion of either initial standing stock on DOY 130 (0–201%) or of total N inputs during summer (0–77%). River inflow accounted for <30% of total N inputs at all sites except shallow and small Wascana Lake, whereas precipitation was a negligible source of nitrogen (<1%) at

Table 2. Mean N mass balance for lakes of the Qu'Appelle Valley drainage, calculated for DOY 130–234, 1994–2004.

	Diefenbaker	Buffalo Pound	Last Mountain	Wascana	Katepwa	Crooked
Particulate N stock on DOY 130 (Mg)	179	4	42	0.02	20	6
Total N stock on DOY 130 (Mg)	3,497	38	1,693	1	236	113
Total N stock change (DOY 240–DOY 130) (Mg)	0	+38	+105	+0.14	0	+12
N losses						
N sedimentation (Mg) (% of total N losses)	5,259 (89%)	364 (95%)	9,996 (99%)	3.4 (23%)	389 (80%)	119 (42%)
N outflow (Mg) (% of total N losses)	683 (11%)	18 (5%)	74 (1%)	12 (77%)	96 (20%)	162 (58%)
N sources						
N inflow (Mg) (% of total inputs)	1,172 (19%)	19 (5%)	39 (0.4%)	11 (70%)	85 (18%)	84 (29%)
N precipitation (Mg) (% of total inputs)	33 (0.6%)	2 (0.5%)	15 (0.2%)	0.03 (0.2%)	1.1 (0.2%)	1.0 (0.3%)
	Max	Max	Max	Max	Max	Max
	Min	Min	Min	Min	Min	Min
Mass of N fixed (Mg) (% of initial N stock) (% of total N inputs)	0 (0%) (0%)	27 (72%) (6.5%)	1,207 (71%) (12%)	0.7 (67%) (5%)	199 (84%) (41%)	227 (201%) (77%)
	0	2.7	30	0.02	16	11
	(0%)	(7.2%)	(1.8%)	(1.5%)	(7.0%)	(10%)
	(0%)	(0.7%)	(0.3%)	(0.1%)	(3.4%)	(3.8%)
Balance						
Other N sources (Mg) (% of total inputs)	4,737 (80%)	371 (88%)	8,913 (88%)	3.8 (25%)	199 (41%)	–19 (–6.5%)
	4,737	396	10,090	4.5	382	197
	(80%)	(94%)	(99%)	(30%)	(79%)	(67%)

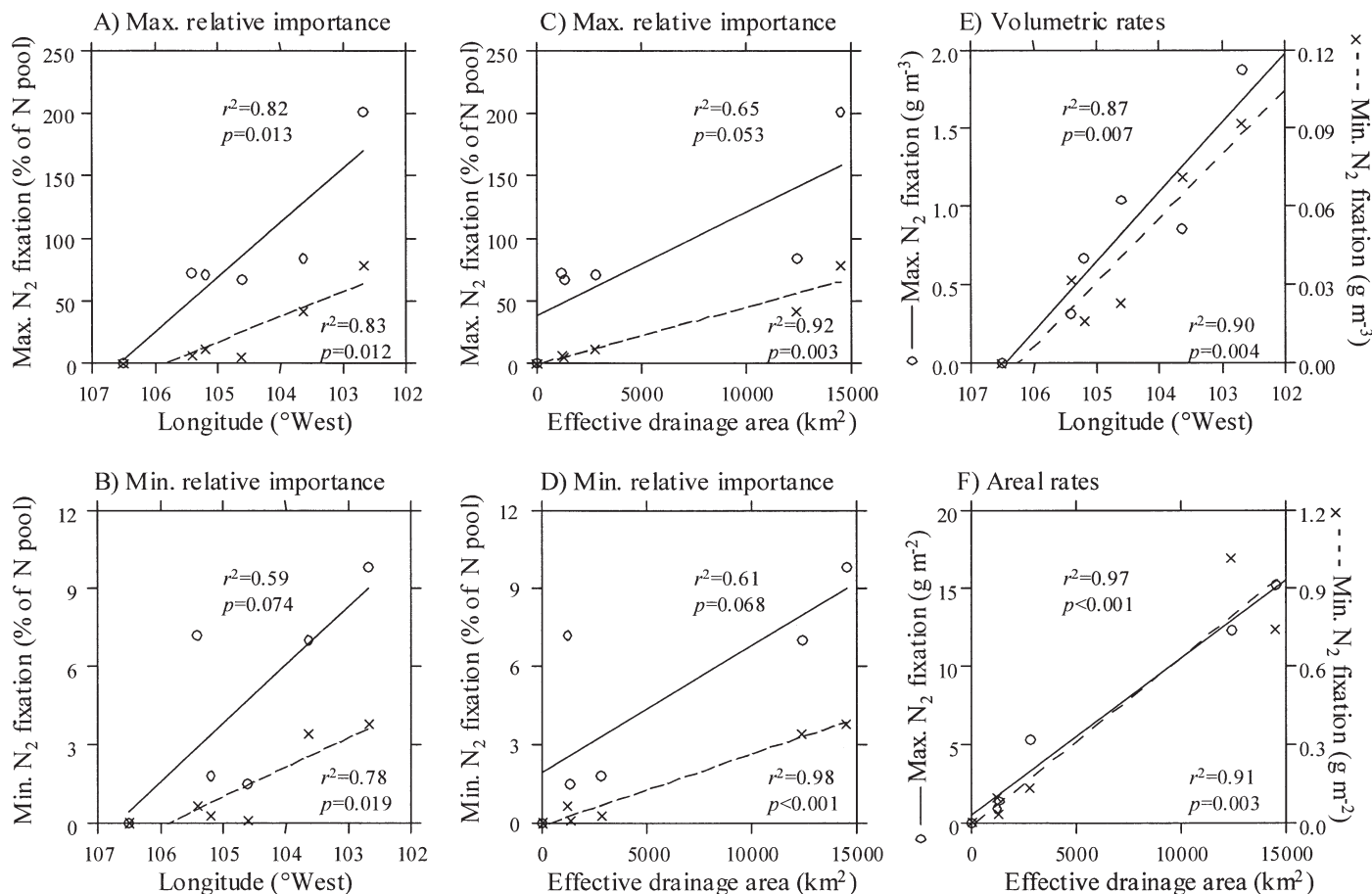


Fig. 5. Relationships between the relative importance of net N₂ fixation as a fraction of mean initial N standing stock on DOY 130 (solid line, circles) or mean total annual N input during DOY 130–234 (dashed line, crosses) and lake longitude (°W; left column) and effective drainage area (km²; middle column). Maximum estimates of net N₂ fixation in top row (A, C), whereas minimum estimates in bottom row (B, D). Maximum (solid line, circles) and minimum (dashed line, crosses) estimates of volumetric (E) and areal (F) rates of N₂ fixation are presented as a function of lake longitude and effective drainage area, respectively.

all lakes. Instead, inputs from other sources (e.g., unmonitored surface flow, ground water, internal loading, etc.) were >80% of total inputs in upstream lakes, but were less important in downstream basins (<80%). As well, sedimentation was the most significant output in relatively large, upstream Diefenbaker, Buffalo Pound, and Last Mountain lakes (>89% of outputs), but it was less important in smaller basins such as Wascana (23%) and Crooked lakes (42%) (Table 2).

Mass balances based on minimum estimates of total ecosystem N suggested that net ecosystem N₂ fixation (M_{fixedMin}) was only 0.3–8.0% of rates estimated using maximum estimates of N standing stock (Table 2). In this set of calculations, fixed N accounted for 0–10.0% of initial N standing stock but only 0–3.8% of total N inputs to lakes. As expected, the relative importance of other unmeasured N sources increased in these mass balances relative to those based on maximum estimates of ecosystem N, particularly for downstream Katepwa and Crooked lakes.

Nitrogen fixation exhibited strong spatial organization, whether expressed as a proportion of initial N stocks, as a proportion of total N inputs (Fig. 5A–D) or as volumetric (Fig. 5E) or areal (Fig. 5F) rates of N₂ fixation.

For example, when expressed as a proportion (%) of total inputs during the sampling period, fixed N was strongly and positively correlated with lake position in the landscape (as longitude), regardless of whether net N₂ fixation was calculated using maximum ($r^2 = 0.83$, $p = 0.012$; Fig. 5A) or minimum ($r^2 = 0.78$, $p = 0.019$; Fig. 5B) estimates of net N₂ fixation. However, because effective lake drainage area was also spatially structured (Fig. 1A), the relative importance of N₂ fixation also increased as a function of catchment area (Fig. 5C,D). Similarly, volumetric (g N fixed m⁻³) and areal (g N fixed m⁻²) estimates of N₂ fixation were strongly correlated to lake longitude ($r^2 > 0.87$, $p < 0.01$; Fig. 5E) and effective drainage area ($r^2 > 0.91$, $p < 0.003$; Fig. 5F), respectively, regardless of whether mass balances were based on M_{fixedMax} or M_{fixedMin} .

Because neither effective drainage nor longitudinal lake position are variables that biota react to directly, we also conducted a series of multiple linear regressions using limnological and catchment variables listed in the Material and methods section to identify the best predictors of the relative importance or quantity of N₂ fixation. These analyses excluded both longitude and effective drainage

area as predictors, but revealed that cloud cover was a positive correlate of the maximum ($r^2 = 0.81$, $p = 0.006$) and minimum ($r^2 = 0.76$, $p = 0.011$) volumetric rates of N fixation, while lake-bottom oxygen concentration was negatively correlated with both the maximum ($r^2 = 0.53$, $p = 0.062$) and minimum ($r^2 = 0.57$, $p = 0.049$) estimates of areal N fixation. No other environmental variables were correlated with any estimate of N fixation.

Discussion

Seasonal changes in stable isotopes of N and whole-lake N mass balances revealed that biologically fixed N is spatially structured and readily assimilated into aquatic food webs within lakes of the northern Great Plains. Specifically, both temporal variability in $\delta^{15}\text{N}$ -POM (Fig. 2B) and metrics of relative and absolute rates of N_2 fixation (Fig. 5) were strongly correlated with both lake position within the landscape (longitude) and effective catchment area, factors that were themselves correlated ($r^2 = 0.79$, $p = 0.012$; Fig. 1A). Furthermore, observations that temporal variability in $\delta^{15}\text{N}$ -POM was highly correlated to abundances of N_2 -fixing cyanobacteria (Fig. 3), as well as to the $\delta^{15}\text{N}$ signal of individual zooplankton species (Fig. 4) suggests that fixed N was rapidly assimilated by the aquatic food web, in spite of the potentially inedible nature of many cyanobacteria (e.g., Ghadouani et al. 2003). Unexpectedly, multiple regression analyses revealed few significant correlations of net ecosystem N_2 fixation with most commonly measured limnological variables (e.g., nutrient concentrations and ratios, algal biomass, ionic composition, temperature, stratification, etc.). Therefore, while the relative and absolute rates of net N_2 fixation exhibit high degrees of spatial structure, the mechanisms producing these landscape patterns remain difficult to identify.

Spatial variability in $\delta^{15}\text{N}$ -POM among lakes has been previously documented by several investigators and can arise from several processes. For example, spatial gradients of $\delta^{15}\text{N}$ in algae, invertebrates, and sediments have been used to identify enriched sources of ^{15}N arising from wastewater inputs (e.g., Savage and Elmgren 2004; Savage et al. 2004 and references therein), changes in salinity along estuary-to-ocean gradients (France 1994), and progressive increases in evaporation and possibly denitrification along a lake chain (Doi et al. 2004). However, these factors do not appear to have influenced the spatial patterns observed in this study. For example, although N-rich effluent from the city of Regina has degraded water quality in Pasqua and Echo lakes since about 1930 (Hall et al. 1999b), analysis of fossil pigments, diatoms, and chironomids reveals that effluent effects are rapidly attenuated and do not greatly influence the water chemistry in downstream Katepwa or other lakes (Hall et al. 1999b; Dixit et al. 2000; Quinlan et al. 2002). Similarly, while lake-water salinity and conductivity generally increase from west to east, neither are significant predictors of spatial patterns of N_2 fixation. Finally, although the headwater region is notably less humid than downstream areas, as indicated by lower cloud cover, this pattern does not correspond to a spatial

gradient of evaporative concentration, because headwater Lake Diefenbaker is a relatively fresh reservoir collecting mountain snowmelt water, Last Mountain Lake is subsaline (~ 0.6 g TDS L^{-1}) and all lakes have substantial surface inflows and outflows (Table 2). Instead, we show that seasonal and interannual temporal variability of $\delta^{15}\text{N}$ -POM arises from variations in the density of N_2 -fixing cyanobacteria (Fig. 3), such as is seen in other lakes (Gu et al. 1994; Yoshioka and Wada 1994; Rolff 2000).

Spatial gradients of water chemistry observed in Qu'Appelle Valley lakes are typical of many other lake chains, including prairie, boreal, arctic, and alpine sites (Soranno et al. 1999). In particular, conductivity, alkalinity, nutrient, and Chl *a* values usually increase from headwater to downstream lakes, whereas water-column transparency declines (Soranno et al. 1999; Quinlan et al. 2002). However, because spatial patterns of N and P may differ among catchments, reflecting site-specific variations in the relative rates of supply and retention of individual nutrients, we predict that spatial patterns of N_2 fixation may vary substantially among sites, depending on whether primarily N or P is retained by lake basins. In the case of Qu'Appelle Valley catchment, uniformly high availability of soil nutrients derived from quaternary glaciers and Holocene loess (Klassen 1989) and effective sequestration of N into sediments (Table 2), lead to conditions in which primarily P rather than N, is exported from individual lakes via rivers (Kenney 1990). This pattern leads to progressive declines in N supply and N : P ratios that favor stronger development of cyanobacterial populations (MacGregor et al. 2001; Havens et al. 2003; Tonno and Noges 2003) in more downstream lakes (Graham 1997; Patoine et al. 2005), and consequently greater potential for pelagic N_2 fixation (Table 2). We expect these patterns should also occur in catchments with either naturally high P supply (e.g., geological substrates derived from Pleistocene sea beds; lowland Europe), or in managed catchments in which P additions from agriculture greatly exceed soil P storage capacity, leading to low N : P supply ratios (Cooke and Prepas 1998; Cessna et al. 2001; Foy et al. 2002).

Mass balances constructed here suggest for the first time that N_2 fixation can be spatially structured, with negligible rates in headwater systems and up to 77% of total N ecosystem supply in downstream lakes, depending on mass-balance assumptions (Table 2). Regression analyses suggest that this landscape gradient arises from geographic variation in lakes' effective catchment area (Fig. 5) and presumably relates to site-specific variations in fluxes of ecologically relevant materials. Unfortunately, linear and multiple regression analyses showed relationships of N_2 fixation that were primarily nonsignificant with a comprehensive suite of commonly measured limnological variables related to nutrients, chemistry, hydrology, morphology, and catchment characteristics, all factors previously proposed as correlates of cyanobacterial abundance and metabolic activity (Howarth et al. 1988a; Levine and Schindler 1999; Downing et al. 2001). For example, volumetric estimates of N_2 fixation rates were directly correlated with cloud cover ($r^2 = 0.76$ – 0.81 , $p < 0.05$), contrary to expectations based on the physiological

requirements of high radiant energy to power N_2 fixation (Lewis and Levine 1984), whereas areal rates of N_2 fixation were positively correlated with deepwater anoxia ($r^2 = 0.53\text{--}0.57$, $p < 0.06$), such as seen elsewhere (Trimbee and Prepas 1988). However, both cloud cover and deepwater anoxia were substantially less effective at predicting N_2 fixation than was lake catchment area or landscape position (Fig. 5E,F) and were nonsignificant in multiple regressions that included either longitude or catchment area. Thus, while our ability to predict the importance and rate of atmospheric N_2 fixation based on landscape position is excellent, this analysis could not identify the precise mechanism by which such landscape patterns are generated.

The validity of our spatial analysis depends on the assumptions made to calculate whole-lake N mass balances. As with all such approaches, it is impractical or impossible to measure all potentially important N fluxes for sufficient duration to assure a representative estimate, particularly in climatically sensitive regions such as the northern Great Plains. Thus we made no attempt to construct year-specific mass balances, and instead focused on broad patterns evident at interannual or decadal time scales. Because our measurements are based on at least 5 yr of data, we believe that mean mass fluxes are accurately represented in our calculations.

Mass balances were based on three additional key assumptions. First, we assumed that N pools were mixed rapidly within each lake and that pelagic samples also reflected littoral and benthic N transformations. Consistent with this view, we note that all lakes are normally polymictic (except, occasionally, Katepwa Lake), have low transparency (low mean ratios Secchi : Z_{mean}), have small littoral zones (except Wascana Lake) and nearly flat bottoms (except for Last Mountain; Table 1), such that expected contributions of littoral N fluxes would be small.

Second, we assumed that temporal changes in $\delta^{15}\text{N}$ -POM arose from the balance between fixation of isotopically light N_2 ($\delta^{15}\text{N} \leq 0\text{‰}$) and N transformations that theoretically could increase $\delta^{15}\text{N}$ -POM over 10‰ (nitrification, denitrification, and ammonia volatilization; Peterson and Fry 1987). Consistent with this view, aerial deposition of N was trivial relative to other N inputs (Kochy and Wilson 2001; Table 2). Similarly, while N released from sediments through decomposition (i.e., other N sources) may have exhibited lower ^{15}N content than water column N, these releases should not exhibit strong spatial structure that would have produced landscape patterns of N fixation (Table 2). Finally, although selective uptake of ^{14}N - NH_3 can reduce $\delta^{15}\text{N}$ -POM signatures during periods of N sufficiency (Peterson and Fry 1987), this mechanism is unlikely to be important in Qu'Appelle lakes, sites that have exhibited summer N limitation and heterocystous cyanobacteria (Graham 1997; Patoine et al. 2005) during much of the 20th century (Hall et al. 1999b; Dixit et al. 2000). Thus although it appears that we have not missed a substantial input of isotopically light N, we recognize that comprehensive evaluation of this assumption will require direct independent measurement of all N transformations.

Finally, estimates of the mass of biologically fixed N were strongly dependent on whether fixed N was added to the total pool of dissolved and particulate N (M_{fixedMax}) or only that associated with algal POM (M_{fixedMin}), as well as the degree to which $\delta^{15}\text{N}$ -POM represented the isotopic signature of the total N pool (Fig. 5; Table 2). Although preliminary analyses confirm that $\delta^{15}\text{N}$ signatures of POM and total dissolved N are similar ($\pm 1.5\text{‰}$) at the start of the sample season in some Qu'Appelle lakes (P. R. Leavitt et al. unpubl.), precise estimation of the absolute quantity of N fixed requires compound-specific isotopic analyses of the main species of dissolved N (ammonia, nitrate, organic N), estimates of isotope composition of both algal and nonalgal POM pools, and quantification of the turnover rates of each pool. These data were not available for the 60 lake-years of this study. Instead, we estimated an upper and lower limit to net ecosystem N_2 fixation to quantify landscape patterns of N_2 fixation among lakes (Table 2). In this analysis, maximum estimates likely overestimated true net ecosystem N fixation because it is unlikely that particulate and dissolved components of ecosystem N were labeled with atmospheric N to the same extent. Similarly, our minimum estimate of the mass of fixed N was expected to greatly underestimate true biological fixation because this analysis does not account for growth, rapid turnover, and transfer of algal biomass to sinks such as zooplankton (Fig. 4) or sediments during the sampling period. However, while we recognize the potential shortcomings of the mass-balance approach, we also note that independent estimates of N_2 fixation based solely on direct enumeration of heterocyst abundances during 1995 and on physiological estimates of cell-specific rates of N_2 fixation produced the same spatial pattern and range of importance of N_2 fixation relative to initial standing stock of N as was calculated here using M_{fixedMax} (i.e., 0–169%; Patoine et al. 2005). Furthermore, the areal rates of N_2 fixation estimated by our maximum mass balances ($1\text{--}15 \text{ g m}^{-2} \text{ yr}^{-1}$) were very similar to those reported for other eutrophic lakes ($0.2\text{--}9.2 \text{ g m}^{-2} \text{ yr}^{-1}$) by Howarth et al. (1988b). Thus, while estimates of net ecosystem N fixation require refinement before comparison with other short-term metrics (e.g., acetylene reduction; Ferber et al. 2004), any such bias would be applied equally to each lake and should not interfere with the detection of spatial patterns (Fig. 5).

Despite the reported low palatability of N_2 -fixing cyanobacteria (de Bernardi and Giussani 1990; Ghadouani et al. 2003), mean seasonal trends of $\delta^{15}\text{N}$ signatures of POM and individual zooplankton species demonstrated that N_2 fixed by cyanobacteria was effectively assimilated by primary consumers and transferred throughout the food web of downstream lakes (Fig. 4). Similar patterns of $\delta^{15}\text{N}$ incorporation into primary (Gu et al. 1994) and secondary consumers have been recorded from other individual lakes (Rolff 2000; MacGregor et al. 2001). In addition, we suggest that this propagation of fixed N up the food chain may be a function of lake position within the landscape, being greatest in downstream lakes, irrespective of the lake's initial baseline isotopic signature (e.g., Katepwa vs. Crooked). At present, it is impossible to determine whether these patterns result from direct consumption of N_2 -fixing

cyanobacteria by *Daphnia* and subsequent trophic transfer, or whether N₂ fixed by prokaryotes is first released, then assimilated by more edible phytoplankton (MacGregor et al. 2001), although we note that gut analysis of all Qu'Appelle zooplankton taxa reveals the presence of pigments characteristic of colonial cyanobacteria (Graham 1997; M. D. Graham and P. R. Leavitt unpubl.).

In conclusion, the use of stable isotopes and whole-lake mass balances revealed that the relative and absolute rates of biological N₂ fixation varied predictably with lake position in the landscape, possibly because of spatially structured variation in effective catchment area. Further, this fixed N was rapidly incorporated into all members of the zooplankton community demonstrating that N derived from the atmosphere was essential for aquatic food-web nutrition. At present, we are conducting further investigations to evaluate whether these patterns arise from intense human activities throughout the catchment (e.g., agriculture), whether such spatial patterns precede the onset of ancestral European activities (e.g., Hall et al. 1999b), and whether similar patterns occur in other catchments (Bunting et al. 2005).

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