

Differential stimulation and suppression of phytoplankton growth by ammonium enrichment in eutrophic hardwater lakes over 16 years

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Abstract

Previous research suggests that fertilization of surface waters with chemically reduced nitrogen (N), including ammonium (NH₄⁺), may either enhance or suppress phytoplankton growth. To identify the factors influencing the net effect of NH₄⁺, we fertilized natural phytoplankton assemblages from two eutrophic hardwater lakes with growth-saturating concentrations of NH₄Cl in 241 incubation experiments conducted biweekly May–August during 1996–2011. Phytoplankton biomass (as chlorophyll *a*) was significantly ($p < 0.05$) altered in fertilized trials relative to controls after 72 h in 44.8% of experiments, with a marked rise in both spring suppression and summer stimulation of assemblages over 16 yr, as revealed by generalized additive models (GAMs). Binomial GAMs were used to compare contemporaneous changes in physico-chemical (temperature, Secchi depth, pH, nutrients; 19.5% deviance explained) and biological parameters (phytoplankton community composition; 40.0% deviance explained) to results from fertilization experiments. Models revealed that the likelihood of growth suppression by NH₄⁺ increased with abundance of diatoms, cryptophytes, and unicellular cyanobacteria, particularly when water temperatures and soluble reactive phosphorus (SRP) concentrations were low. In contrast, phytoplankton was often stimulated by NH₄⁺ when chlorophytes and non-N₂-fixing cyanobacteria were abundant, and temperatures and SRP concentrations were high. Progressive intensification of NH₄⁺ effects over 16 yr reflects changes in both spring (cooler water, increased diatoms and cryptophytes) and summer lake conditions (more chlorophytes, earlier cyanobacteria blooms), suggesting that the seasonal effects of NH₄⁺ will vary with future climate change and modes of N enrichment.

Since the commercialization of the Haber-Bosch process in the 1940s, the global pool of manufactured nitrogen (N) has increased nearly 20-fold (Glibert et al. 2006, 2014a), resulting in large increases in runoff and atmospheric deposition of reactive N (Nr) to both freshwaters and coastal marine ecosystems (Galloway et al. 2008; Howarth 2008; Beusen et al. 2016). The combined effects of near-exponential increases in use of N-based agricultural fertilizers and growth of storm- and waste-water

effluent discharge (Bernhardt et al. 2008) have resulted in a more than twofold increase in total N-loads entering downstream river basins in many parts of the world (Green et al. 2004; Howarth 2008). In turn, not only have increases in total N fluxes intensified eutrophication of many coastal (Rabalais et al. 2002; Howarth and Marino 2006) and freshwater systems (Leavitt et al. 2006; Bunting et al. 2007; Glibert et al. 2014a; Paerl et al. 2015), but there has been an increase in the proportion of chemically reduced forms N, including ammonium (NH₄⁺) and urea, relative to nitrate, NO₃[−] (Glibert et al. 2006, 2014a, 2016, Glibert 2017). With a growing global population and anticipated doubling of fertilizer N application (Glibert et al. 2006, 2014a), the global pool of Nr should double by 2050 (Galloway et al. 2008) resulting in increased pollution of surface waters with reduced N.

While an extensive body of literature has shown that increasing loads of N may promote eutrophication and the development of harmful algal blooms in marine systems (Glibert et al. 2006, 2014a; Howarth and Marino 2006; Zehr and Kudela 2011), the role of N in eutrophication of freshwater systems is less certain (Paerl et al. 2016; Schindler et al. 2016). In part, different

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viewpoints reflect observations that phytoplankton response to N varies with the chemical form of added N (Glibert et al. 2014a, 2016), composition of the algal assemblage (Donald et al. 2013), and limnological conditions at the time of fertilization (Harris et al. 2014; Hayes et al. 2015), including absolute concentration of N (Chen et al. 2009; Filstrup et al. 2018) or phosphorus (P) (Donald et al. 2011; Bogard et al. 2017). For example, while NH_4^+ is most energetically favorable for cellular growth (Turpin et al. 1985; Raven et al. 1992; Flores and Herrero 2005) and has long been considered to be the preferred form of N (Ludwig 1938; Harvey 1953; McCarthy 1981; Raven et al. 1992), in situ primary production may alternately be stimulated (Lomas and Glibert 1999a; Glibert et al. 2006, 2014b; Finlay et al. 2010) or suppressed by exposure to NH_4^+ (Dortch 1990; Flynn et al. 1997; Glibert et al. 2016). Understanding the environmental and community controls of the influence of NH_4^+ on primary production and phytoplankton composition is needed to make effective management decisions in both marine and freshwater ecosystems.

The extent to which NH_4^+ may stimulate or suppress phytoplankton growth may vary with ambient N concentration, phytoplankton community composition, and other environmental factors (Azov and Goldman 1982; Dugdale et al. 2007, 2013; Parker et al. 2012a; Glibert et al. 2016). For example, direct inhibition of phytoplankton growth by reduced N may arise because unionized NH_3 can disrupt electrochemical gradients and photophosphorylation at high pH (Hou et al. 2011). Lipid-soluble NH_3 is abundant in warm alkaline conditions (Trussell 1972) and can diffuse into the cytoplasm where it inhibits Photosystem II (PSII) by interacting with carboxylate groups coupled to the Mn_4CaO_5 cluster of the O_2 -evolving center (Britt et al. 1989; Hou et al. 2011; Tsuno et al. 2011). Although further research is needed, this pattern suggests that NH_3 inhibition should be paramount in lakes where pH and temperature are elevated. Alternately, NH_4^+ may suppress phytoplankton growth by repressing NO_3^- uptake and assimilation, in turn leading to imbalances in cellular redox and energy balance (Lomas and Glibert 1999a,b; Parker et al. 2012a; Glibert et al. 2016). Specifically, diatoms and some other algae use dissimilatory nitrate reduction (DNR) to dissipate excessive electron activity in conditions of high light and cool water, when cellular metabolism and photo-oxidative repair mechanisms may be temperature-limited (Lomas and Glibert 1999a,b). However, the DNR pathway may be suppressed by uptake of NH_4^+ , which favors suppression of NO_3^- transport across the cell membrane, decay of existing nitrate reductase (NR), and reduction of new NR production (Glibert et al. 2016). Once NR is repressed, electrochemical gradients are disrupted and phytoplankton growth may be reduced (Kobayashi et al. 2005; Kamp et al. 2011; Rosenwasser et al. 2014). Although NH_4^+ suppression of diatom growth is known mainly from marine ecosystems (Lomas and Glibert 1999a; Glibert et al. 2014b, 2016), the predominance of diatoms in lacustrine assemblages during spring and

fall suggests that freshwater phytoplankton communities may exhibit seasonal suppression by NH_4^+ pollution.

In vitro studies of individual taxa show that the threshold concentration for growth inhibition by NH_4^+ varies widely within and among major phytoplankton groups (reviewed in Collos and Harrison 2014; Glibert et al. 2016). In general, diatoms are most inhibited by addition of NH_4^+ , followed by cyanobacteria and dinoflagellates, whereas chlorophyte species were rarely suppressed by such amendments. Larger-scale marine studies have also recorded either repression of NO_3^- uptake or suppression of diatom growth by elevated concentrations of NH_4^+ (Wilkerson et al. 2006; Yoshiyama and Sharp 2006; Dugdale et al. 2007; Parker et al. 2012a,b; Xu et al. 2012), resulting in increased proportions of marine chlorophytes, cyanobacteria, and dinoflagellates under NH_4^+ -enriched conditions (reviewed in Glibert et al. 2016). Similarly, addition of NH_4^+ to warm eutrophic coastal and freshwaters favors growth of toxic non- N_2 -fixing cyanobacteria at the expense of diazotrophic taxa (McCarthy et al. 2009; Finlay et al. 2010; Donald et al. 2011; but see Dai et al. 2012; Shangguan et al. 2017a), in part because NH_4^+ uptake inhibits NtcA transcription promoter activity and suppresses formation of heterocytes (Herrero et al. 2001; Flores and Herrero 2005; Harris et al. 2014; Glibert et al. 2016). Together, these studies suggest that stimulation of phytoplankton growth by NH_4^+ should be most pronounced in eutrophic lakes during late summer when colonial cyanobacteria are most abundant (Paerl and Scott 2010).

To better understand the potential for differential effects of NH_4^+ in lakes, we quantified the response of natural phytoplankton assemblages to NH_4^+ amendment using 241 standard bioassay experiments conducted from May to August during a 16-yr period. Assemblages were obtained from two shallow solute-rich hardwater ecosystems characteristic of basins in the continental interior (Hammer 1986; Finlay et al. 2015). Although water security in this region of the northern Great Plains (NGP) is already at risk, these hardwater lakes and rivers are expected to receive more NH_4^+ pollution in the future due to continued urban growth and agricultural intensification (Vörösmarty et al. 2010). Consequently, the main objectives of this study were to quantify the net effect of excess NH_4^+ on the aggregate growth of natural phytoplankton assemblages, and to evaluate how responses to NH_4^+ may vary through time and among lakes due to variation in physico-chemical conditions and community composition. Finally, we sought to determine whether effects of NH_4^+ were more consistent with pH-, temperature-, or community composition-mediated mechanisms affecting aggregate phytoplankton growth.

Methods

Study area

The two study lakes, Buffalo Pound Lake and Wascana Lake, are located within the Qu'Appelle River basin, a catchment which drains 52,000 km^2 in southern Saskatchewan,

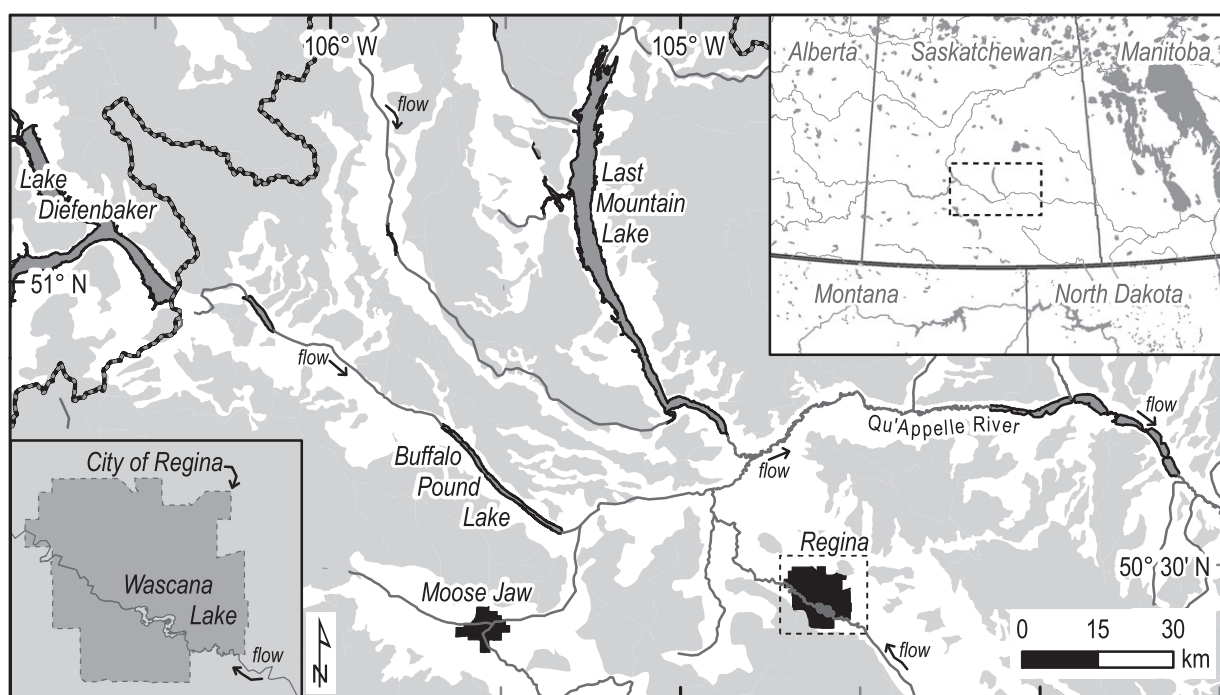


Fig. 1. Map of the Qu'Appelle River drainage basin (inset) including Buffalo Pound Lake and Wascana Lake, Saskatchewan, Canada. Buffalo Pound receives water from Lake Diefenbaker and drains to the east via the Qu'Appelle River, whereas Wascana Creek drains into Wascana Lake within the City of Regina (black) before reaching a confluence with the Qu'Appelle River. Heavy outline indicates maximum extent of drainage basin, while white and gray shading indicates contributing and noncontributing areas during the median flow year, respectively.

Canada (Fig. 1). Land use within the catchment is largely agricultural, with smaller areas of undisturbed grassland, surface waters, and urban centers (Hall et al. 1999a; Finlay et al. 2015). Regional climate is characterized as cool-summer humid continental (Köppen Dfb classification), with short summers (mean 19°C in July), cold winters (mean -16°C in January), and low-annual temperatures ($\sim 1^{\circ}\text{C}$) with high-seasonal variability (Leavitt et al. 2006). Regional mean temperatures have increased $\sim 2^{\circ}\text{C}$ since 1900, resulting in a 35-d decline in ice cover, mainly expressed as earlier dates of ice melting (Finlay et al. 2015). Spring snow melt accounts for 80% of annual surface runoff (Pham et al. 2009), leading to seasonally variable, but moderately low water residence times (< 0.7 yr) (Table 1). Both lakes are shallow and polymictic (McGowan et al. 2005a), with a highly eutrophic status arising from elevated nutrient influx from naturally fertile soils and regional agriculture (Patoine et al. 2006). Typical of hardwater lakes in the NGP, both Buffalo Pound and Wascana lakes are characterized by high summer pH (mean 8.9–9.0; maximum < 10.5) (Finlay et al. 2015); however, the basins exhibit contrasting mean summer mass ratios of total dissolved nitrogen (TDN) : soluble reactive phosphorus (SRP) of 29.9 and 5.8, respectively (Table 1).

Buffalo Pound Lake is a shallow natural waterbody that has been managed since the mid-1960s to supply water to the cities of Regina and Moose Jaw (Hall et al. 1999b). Beginning in 1967 and increasing at irregular intervals to the present,

Buffalo Pound has received surface flow from Lake Diefenbaker, a mesotrophic reservoir located west on the South Saskatchewan River (Fig. 1). In contrast, Wascana Lake was created by the impoundment of Wascana Creek in 1883, but was subsequently deepened to ~ 2 m in the 1930s and to 7.5 m in 2004 (Hughes 2004). Despite contrasting histories, the lakes exhibit similar patterns of plankton phenology (McGowan et al. 2005a,b; Dröscher et al. 2009; Vogt et al. 2011, 2018), with high-vernal densities of diatoms, cryptophytes, and copepods (*Diaptomus thomasi*, *Leptodiatomus siciloides*) generally giving way to a pronounced clearwater phase, characterized by abundant large-bodied *Daphnia* spp. (*D. pulicaria*, *D. galeata mendotae*, *D. magna*) during June, and regular summer blooms of both N_2 -fixing (*Anabaena*, *Aphanizomenon* spp.) and non- N_2 -fixing cyanobacteria (*Planktothrix*, *Microcystis* spp.) (McGowan et al. 2005a; Patoine et al. 2006; Donald et al. 2013).

Field methods

Both lakes were sampled biweekly between May and August of 1996–2011 as part of the Qu'Appelle Valley Long-term Ecological Research program (QU-LTER) (Vogt et al. 2018). Depth-integrated samples were collected by pooling 2.2-liter Van Dorn water bottle casts taken at 0.5-m intervals below the surface, and used for bioassay experiments and analysis of water chemistry, chlorophyll *a* (Chl *a*) content, and phytoplankton community composition (see below). Surface pH was

Table 1. Morphometric, chemical, and biological characteristics of the two study lakes. Data are mean values (SD, in parentheses) of measurements taken from May–August of 1996–2011.

Lake	Water				Secchi									
	Area (km ²)	Volume (10 ⁶ m ³)	residence (yr)	Z _{max} (m)	TDP (μg P L ⁻¹)	SRP (μg P L ⁻¹)	TDN (μg N L ⁻¹)	NO ₃ ⁻ (μg N L ⁻¹)	DOC (mg L ⁻¹)	TIC (mg L ⁻¹)	Conductivity (μS cm ⁻¹)	pH	depth (m)	Chl <i>a</i> (μg L ⁻¹)
Buffalo	29.1	87.5	0.7	4.3 (0.3)	29.0 (21.1)	16.3 (19.5)	488.6 (142.4)	69.5 (83.6)	7.5 (4.2)	32.1 (4.8)	477.0 (211.4)	8.9 (0.6)	1.2 (0.7)	32.5 (39.7)
Pound														
Wascana	0.5	0.7	0.15	3.4 (0.6)	325.4 (206.7)	254.5 (196.5)	1423.3 (668.2)	220.1 (333.9)	18.0 (7.7)	41.7 (12.3)	938.3 (418.9)	9.0 (0.7)	0.8 (0.5)	44.8 (44.5)

Chl *a*, chlorophyll *a*; DOC, dissolved organic carbon; SRP, soluble reactive phosphorus; TDN, total dissolved nitrogen; TDP, total dissolved phosphorus; TIC, total inorganic carbon; Z_{max} , maximum depth.

measured on site using a calibrated (three standard) handheld pH meter (accuracy ± 0.1 unit), while lake transparency (m) was measured using a 20-cm diameter Secchi disk. Temperature ($^{\circ}\text{C}$), conductivity ($\mu\text{S cm}^{-1}$), and oxygen profiles ($\text{mg O}_2 \text{ L}^{-1}$) were measured at 0.5-m intervals, using a YSI model 85 m or equivalent (Yellow Springs, Ohio, U.S.A.).

Laboratory methods

Depth-integrated water samples were filtered through a $0.45\text{-}\mu\text{m}$ pore membrane filter and analyzed at the University of Alberta Water Chemistry Laboratory for concentrations of SRP ($\mu\text{g P L}^{-1}$), total dissolved phosphorus (TDP, $\mu\text{g P L}^{-1}$), and TDN ($\mu\text{g N L}^{-1}$) (see Patoine et al. 2006; Finlay et al. 2015). Particulate organic matter (phytoplankton and detritus) was filtered onto GF/C glass-fiber filters (nominal pore size $1.2 \mu\text{m}$) and frozen (-10°C) until analysis for Chl *a* by standard trichromatic assays (Jeffrey and Humphrey 1975) and biomarker pigments by high-performance liquid chromatography (HPLC) (Leavitt and Hodgson 2001). Carotenoids, chlorophylls, and their derivatives were isolated and quantified using a Hewlett Packard model 1050 or 1100 HPLC system that had been calibrated with authentic standards (Leavitt et al. 2006). All HPLC pigment concentrations were expressed as $\text{nmol pigment L}^{-1}$ before calculation of pigment relative (%) abundance. HPLC analyses were restricted to taxonomically diagnostic pigments including fucoxanthin (siliceous algae), alloxanthin (cryptophytes), chlorophyll *b* (Chl *b*) (chlorophytes), echinenone (total cyanobacteria), myxoxanthophyll (colonial cyanobacteria), canthaxanthin (Nostocales cyanobacteria), aphanizophyll (N_2 -fixing cyanobacteria), and β -carotene (all phytoplankton). In addition, lutein (chlorophytes) and zeaxanthin (cyanobacteria) were inseparable on the HPLC system, and were combined as a measure of “bloom-forming taxa” (Leavitt and Hodgson 2001; Leavitt et al. 2006).

Ammonium amendment experiments

Nutrient enrichment experiments were conducted biweekly in each lake during May–August of 1996–2011. These 241 bioassays were used to estimate temporal variation in the potential effects of NH_4^+ on phytoplankton growth, measured as changes in Chl *a* content over 72 h (Finlay et al. 2010; Donald et al. 2011). Briefly, six acid-washed 250-mL bottles each received ca. 225 mL of $243\text{-}\mu\text{m}$ screened, depth-integrated water (see above). Triplicate bottles amended with 1 mL of $0.32 \text{ mol L}^{-1} \text{ NH}_4\text{Cl}$ (N treatment) or received no addition (control) to achieve a final NH_4^+ concentration (ca. 1.5 mM) similar to that arising from influx of tertiary-treated urban water (Waiser et al. 2011). Bottles were incubated in the laboratory for 72 h at ambient lake temperatures and under a 12 : 12 h light : dark regime with irradiance equivalent to that experienced at Secchi depth (Finlay et al. 2010; Donald et al. 2011). After incubation, phytoplankton were filtered onto GF/C filters and processed for estimates of Chl

a concentration using the trichromatic analyses detailed above. Phytoplankton response to NH_4^+ was recorded as absolute (treatment – control; $\mu\text{g Chl } a \text{ L}^{-1}$) or relative (%) changes in Chl *a* concentration in N treatments compared with control treatments to facilitate statistical analyses of time series (see below). Both HPLC and trichromatic estimates of phytoplankton abundance have previously shown to be highly and linearly correlated with those derived from direct microscopic enumeration in these study lakes (Leavitt and Hodgson 2001; Donald et al. 2013).

Numerical analyses

Generalized additive models (GAMs) (Wood 2006; Wood et al. 2016) were used to estimate long-term trends in Chl *a* response to fertilization with NH_4^+ , as well as temporal changes in the physico-chemical and phytoplankton community characteristics recorded *in situ* at time of phytoplankton collection. GAMs are a data-driven regression approach to the estimation of nonlinear, but not necessarily monotonic, relationships between covariates and response variable, and are routinely used to model environmental time series data (e.g., Monteith et al. 2014; Orr et al. 2015). The conditional distribution of the response in each GAM was assumed to be a gamma distribution for positive, continuous responses, and a Tweedie distribution for non-negative continuous responses (such as pigment concentrations). GAMs included marginal smooth terms of *day of year* (DoY) for the within-year (seasonal trend) and *year* for the between year (long-term trend) components. Additionally, a smooth interaction between these two components was estimated through the use of a tensor product smooth created from the two marginal smooths. In practical terms, this tensor product smooth allows for the seasonal trend in the response to vary smoothly through time within the long-term trend. Smoothness selection was performed using the residual maximum marginal likelihood (REML) method of Wood (2011), with penalties on both the null and range space of the smoothing matrices to perform variable selection in the models (Marra and Wood 2011).

Paired *t*-tests with pooled variance and Welch's approximation to the degrees of freedom were conducted on the results of each bioassay experiment to detect if Chl *a* content was increased or reduced significantly by NH_4^+ -amendments as compared with controls. In all tests, *p* values were adjusted to control the false discovery rate (FDR) due to multiple comparisons. Mann–Whitney *U* tests were used to evaluate the significance of differences in the initial abiotic and biotic conditions (temperature, pH, Secchi depth, TDN : SRP, SRP, and relative pigment abundance) between experiments in which growth of phytoplankton was either stimulated or suppressed significantly ($p < 0.05$) by added NH_4^+ . Spearman rank-order correlations were used to evaluate the presence of monotonic trends in select physico-chemical parameters.

Binomial GAMs with logit-link function were used to test relationships between selected abiotic (physico-chemical) and

biotic (phytoplankton abundance) covariates and the likelihood of suppression or stimulation in response to NH_4^+ fertilization. Binomial GAMs used only bioassay experiments (and associated environmental data) in which there was a statistically significant response of Chl *a* to added NH_4^+ . Abiotic covariates in the binomial GAMs included Secchi depth, water temperature, pH, TDN, and SRP. Biotic covariates included biomarker pigments from siliceous algae (mainly diatoms; fucoxanthin), cryptophytes (alloxanthin), chlorophytes (Chl *b*), total cyanobacteria (echinenone), Nostocales cyanobacteria (canthaxanthin), and the sum of bloom-forming chlorophytes and cyanobacteria (lutein-zeaxanthin). Other pigments (myxoxanthophyll, aphanizophyll, and β -carotene) were not included because they were either redundant with the selected biomarkers or exhibited inconsistent occurrence in the time series.

Separate binomial GAMs were run on sets of abiotic and biotic covariates, with predictor variables retained using the double-penalty method (Marra and Wood 2011). Each predictor was subject to a basis expansion to turn it into a smooth term, with 9 degrees of freedom (10–1 for the identifiability constraint on each smooth) for each variable. To avoid overfitting, GAMs were restricted to eight predictors (72 degrees of freedom + 1 for the intercept). Each model allowed for lake-specific effects of each covariate via factor-smooth-interactions, and those covariates that had statistically significant effects in one or both lakes were reserved. Finally, significant covariates identified from separate abiotic and biotic models were combined and used for a final binomial GAM to evaluate the potential interaction between limnological and biotic factors in predicting phytoplankton response to NH_4^+ . However, we note that the statistical significance of covariates included in this final model should be interpreted with care, as its test for significance does not account for the prior selection of covariates in abiotic or biotic GAMs.

Mann Whitney *U* tests were conducted using SYSTAT v. 13, whereas Spearman rank-order correlations were conducted in TIBCO Spotfire v. 6. All other analyses were conducted in R version 3.3.0 (R Core Team 2016) using the *mgcv* package v. 1.8-24 (Wood 2016).

Results

Limnological conditions

Surface-water pH increased during each summer and throughout the study period in both study lakes (Fig. 2a,b). Mean pH was similar in Buffalo Pound (9.0) and Wascana Lake (8.9), and increased by ~ 1 unit during summer in most years and both lakes. Analysis of fitted-response splines (Fig. 2c,d) and Spearman rank-order correlations revealed that water temperatures in early May declined ~ 2°C since 1996 in both lakes (Spearman $r_s = -0.37$, $p < 0.05$), but did not vary consistently at other times of the year. In all years, water transparency (as Secchi depth) was greatest in spring in

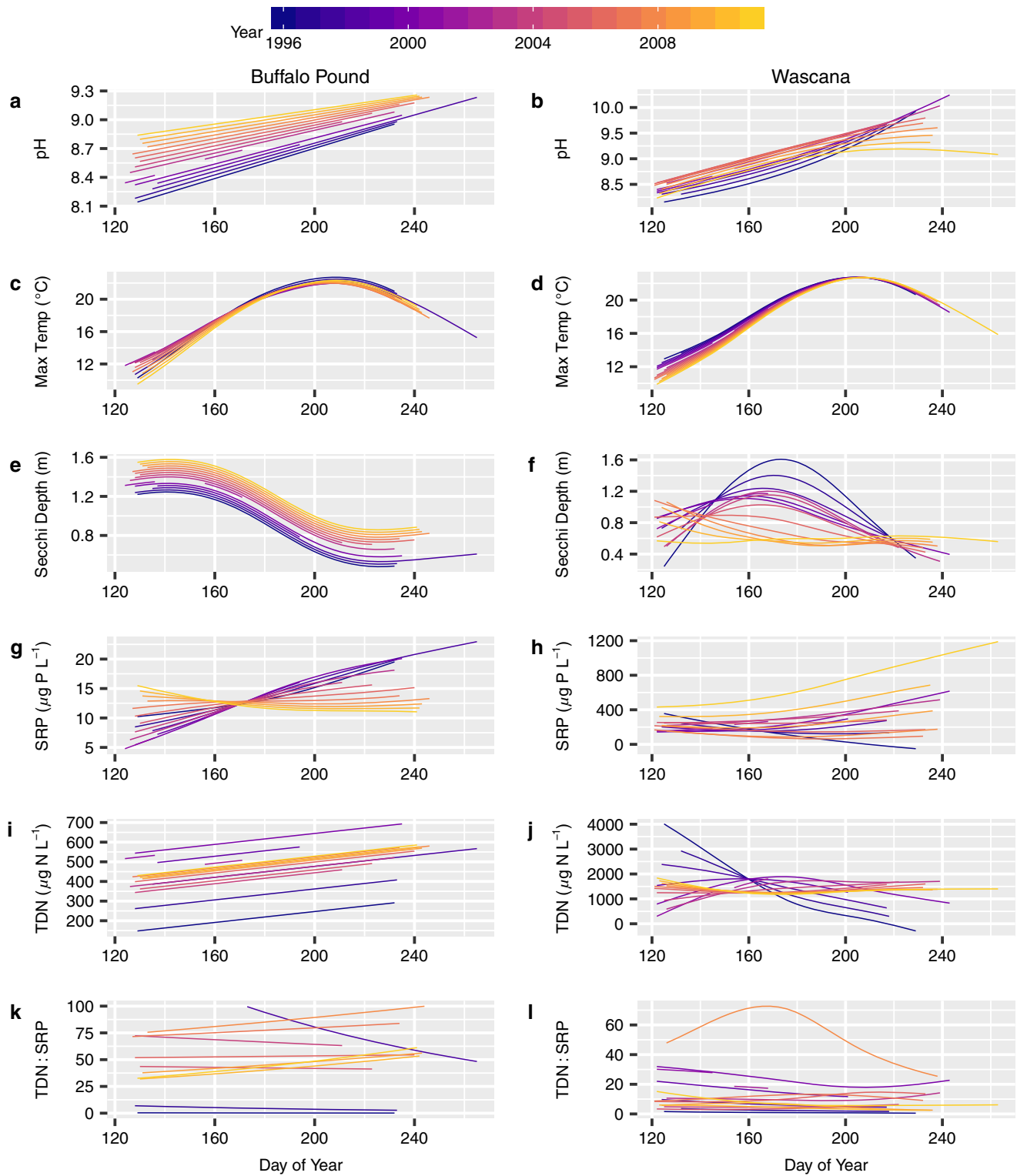


Fig. 2. Fitted curves showing seasonal trends in limnological characteristics in Buffalo Pound Lake (left) and Wascana Lake (right) between 1996 and 2011. Panels and adjusted variance explained (R^2 ; %) by mean trends in each lake include pH (**a**: 20%, **b**: 40%), maximum temperature ($^{\circ}\text{C}$) (**c**: 82%, **d**: 81%), Secchi depth (m) (**e**: 18%, **f**: 28%), SRP ($\mu\text{g P L}^{-1}$) (**g**: 4%, **h**: 60%), TDN ($\mu\text{g N L}^{-1}$) (**i**: 20%, **j**: 17%), and TDN : SRP (**k**: 42%, **l**: 55%). Note difference in y-axis scales between lakes. x-axis shows DoY between spring and fall. Time series are shaded from dark blue (1996) to orange (2011).

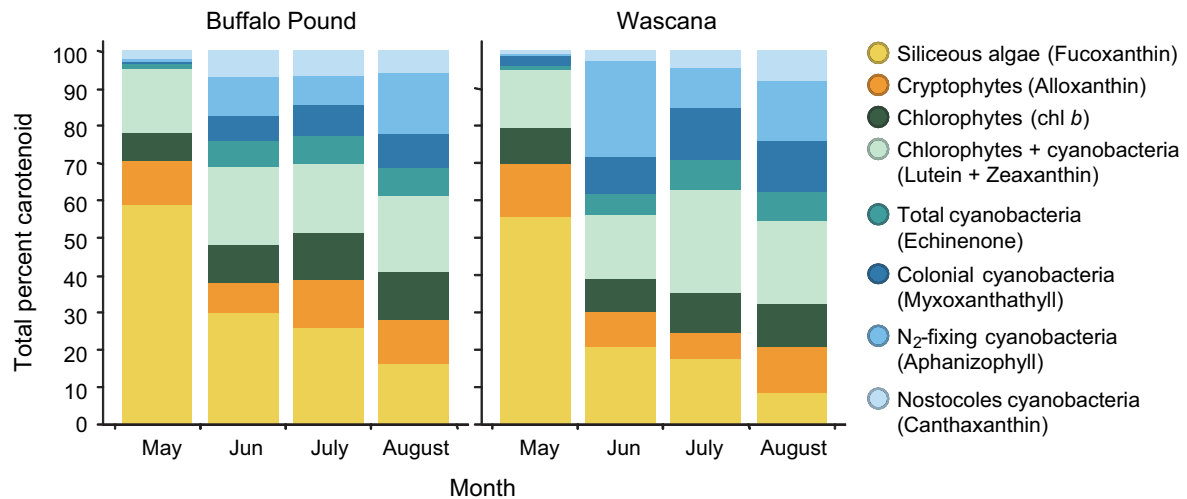


Fig. 3. Mean monthly changes in relative (%) pigment abundance in Buffalo Pound (left) and Wascana lakes (right) during 1996–2011.

Buffalo Pound (Fig. 2e), but changed through time in Wascana Lake, with a pronounced clearwater phase during June in 1996 (Dröscher et al. 2009), which moved earlier toward spring and diminished in intensity by 2011 (Fig. 2f).

Temporal trends in nutrient concentrations were markedly different in the two study lakes (Fig. 2). Mean (\pm SD) SRP concentrations were 15-fold lower Buffalo Pound ($16.3 \pm 19.5 \mu\text{g P L}^{-1}$) than in Wascana Lake ($254.4 \pm 196.5 \mu\text{g P L}^{-1}$), with differences among seasons declining over the 16 yr in Buffalo Pound (Fig. 2g) but not in Wascana Lake (Fig. 2h). In contrast, TDN concentrations in Buffalo Pound exhibited a monotonic increase through the summer and little variation among years (Fig. 2i), whereas Wascana Lake exhibited strong seasonal declines in TDN during the 1990s and less seasonality thereafter (Fig. 2j). In general, nitrate was the predominant form of inorganic N (see Bogard et al. 2012), with concentrations which were fivefold to 10-fold lower (Table 1) than thresholds associated with suppression of phytoplankton growth (Chen et al. 2009; Filstrup et al. 2018). Due to contrasting trends in individual nutrients, mass ratios of TDN : SRP varied substantially through time and among lakes. In Buffalo Pound, TDN : SRP mass ratios (56.6 ± 57.8) varied by an order of magnitude over the 16-yr period (Fig. 2k), whereas in Wascana Lake ratios exhibited less variation among seasons (13.2 ± 26.7) and little directional change during the monitoring period (Fig. 2l).

Phytoplankton phenology

On average, Buffalo Pound and Wascana lakes both exhibited similar patterns of seasonal phytoplankton ontogeny typical of shallow eutrophic lakes (Fig. 3). In both cases, spring phytoplankton assemblages composed mainly of diatoms (fucoxanthin) and cryptophytes (alloxanthin) were replaced in late-summer by communities composed mainly of chlorophytes (Chl *b*, lutein-zeaxanthin), colonial cyanobacteria

(myxoxanthophyll, canthaxanthin) and during August, diazotrophic cyanobacteria (aphanizophyll) (Fig. 3). However, despite these similarities, analysis of fitted splines for individual biomarker pigments showed that the patterns of seasonal abundance of phytoplankton groups changed over the course of the 16-yr study (Fig. 4). For example, although annual patterns of total algal abundance (as Chl *a*; Fig. 4a) have been generally similar in Buffalo Pound since 1997, the abundance of spring siliceous algae (largely diatoms) has declined $\sim 50\%$ in recent years (fucoxanthin; Fig. 4b), as have those of cryptophytes in summer (alloxanthin; Fig. 4c), while mid-summer abundances of chlorophytes (Chl *b*; Fig. 4h) and potentially N_2 -fixing cyanobacteria (aphanizophyll; Fig. 4g) have increased. In Wascana Lake, siliceous algae (Fig. 4b) and cryptophytes (Fig. 4c) have increased throughout the open water season, particularly during spring. Chlorophytes have also become more abundant in the spring in Wascana Lake (Fig. 4h), while total cyanobacteria (echinenone; Fig. 4d), colonial forms (myxoxanthophyll; Fig. 4e), and potentially N_2 -fixing taxa (canthaxanthin and aphanizophyll; Fig. 4f,g) have shifted seasonality, occurring earlier in the summer and at increasing magnitudes in recent years. Finally, changes in seasonal and temporal concentrations of ubiquitous β -carotene (Fig. 4j) were very similar to those of trichromatic Chl *a* in both lakes (Fig. 4a).

Ammonium amendment experiments

Phytoplankton responses (as Chl *a*) to fertilization with NH_4^+ ranged from a 2691% increase (mean stimulation = $188.1\% \pm 365.8\%$) to a 160% suppression (mean suppression = $54.5\% \pm 25.7\%$). A significant increase in mean phytoplankton abundance relative to control trials was observed in 55 of 241 experiments (FDR-adjusted $p < 0.05$), whereas abundance declined significantly in 53 experiments (FDR-adjusted $p < 0.05$). Overall, the frequency of stimulation of algal

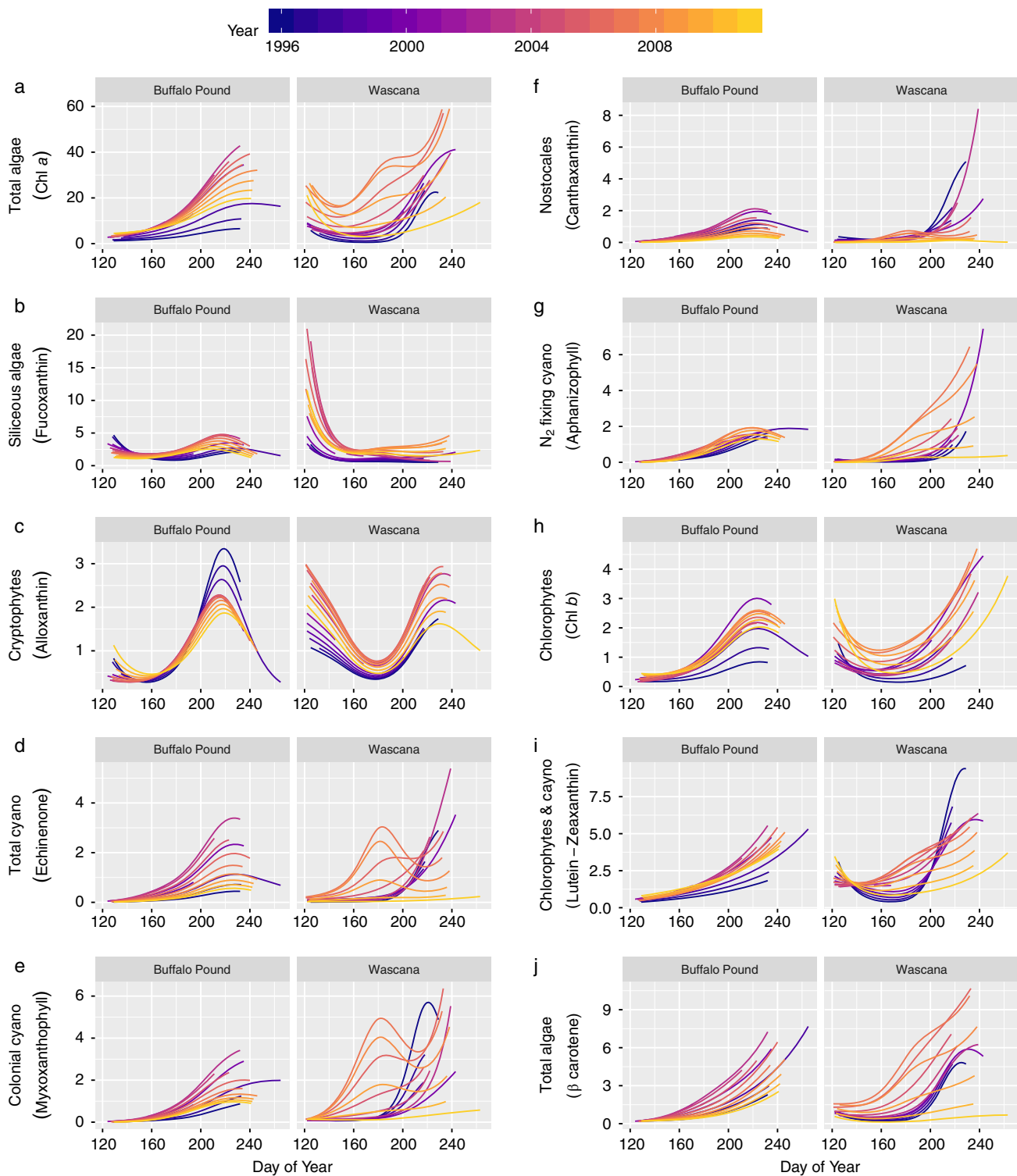


Fig. 4. Fitted response plots showing seasonal trends in chlorophyll and carotenoid pigment concentrations between 1996 and 2011 in Wascana Lake and Buffalo Pound lake. Pigments and % deviance explained by mean trends are presented for both lakes, including; **(a)** Chl *a* (all phytoplankton) (62%, 63%, respectively), **(b)** fucoxanthin (siliceous algae, mainly diatoms) (26%, 42%), **(c)** alloxanthin (cryptophytes) (49%, 32%), **(d)** echinenone (total cyanobacteria) (59%, 65%), **(e)** myxoxanthophyll (colonial cyanobacteria) (54%, 59%), **(f)** canthaxanthin (Nostocales cyanobacteria) (58%, 65%), **(g)** aphanizophyll (N_2 -fixing cyanobacteria) (43%, 45%), **(h)** Chl *b* (chlorophytes) (58%, 54%), **(i)** lutein + zeaxanthin (chlorophytes and cyanobacteria) (52%, 43%), and **(j)** β -carotene (all phytoplankton) (63%, 59%). All pigments are quantified using HPLC in $\text{nmol pigment L}^{-1}$, except trichromatic determinations of Chl *a* ($\mu\text{g Chl L}^{-1}$). x-axis denotes DoY from 01 May (DoY 121) to 31 August (DoY 243).

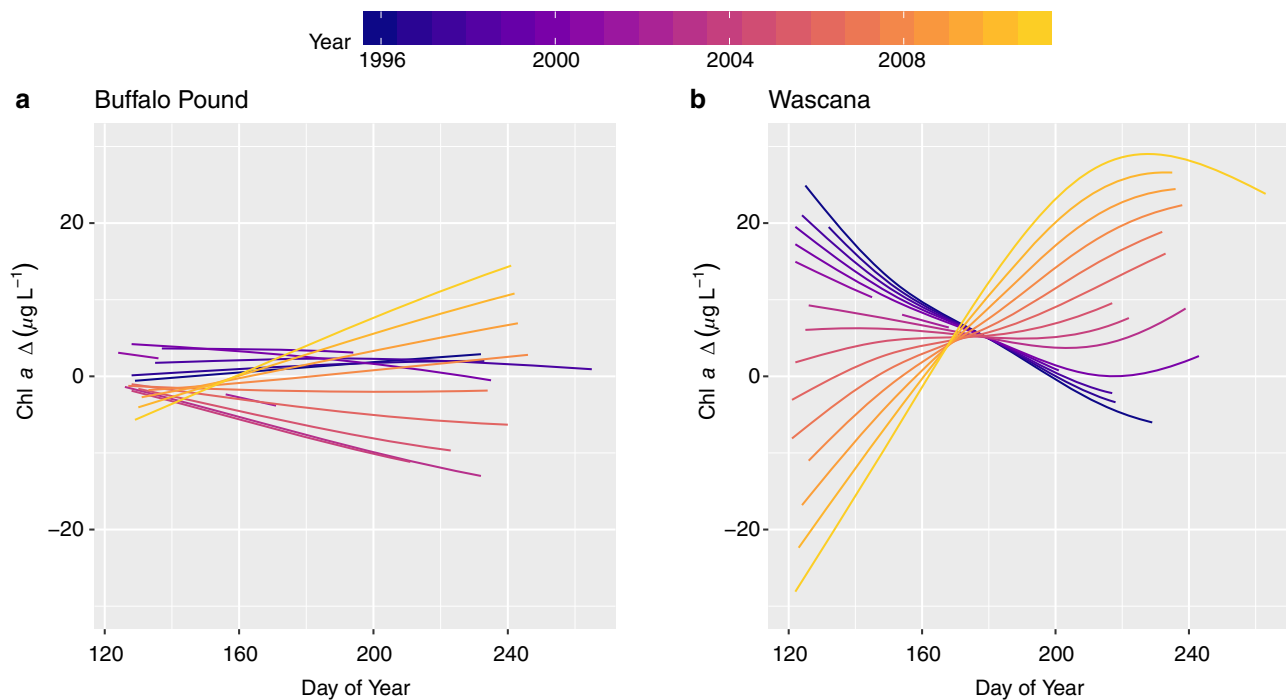


Fig. 5. Fitted curves of changes in Chl *a* concentration ($\mu\text{g L}^{-1}$) in trials amended with NH_4^+ relative to control in bioassay experiments conducted at Buffalo Pound (**a**) and Wascana (**b**) lakes during the open water seasons of 1996 (blue; darkest lines) to 2011 (orange; lightest lines). x-axis shows date in DoY between spring and fall. Percent deviance explained by mean trends was 26% for Buffalo Pound, and 20% for Wascana Lake.

abundance by NH_4^+ was similar among months (May = 11, June = 16, July = 14, August = 12), whereas phytoplankton suppression was recorded most frequently during experiments conducted in May (21), with decreasing occurrences in June (16), July (12), and August (4).

Analysis of GAM-fitted splines showed that the magnitude of seasonal phytoplankton response (as Chl *a*) to NH_4^+ amendment increased during the 16-yr study period (Fig. 5). During the first 5 yr of experiments, addition of NH_4^+ mainly increased phytoplankton abundance, particularly during spring in Wascana Lake (Fig. 5b). However, in both lakes, stimulation of growth by NH_4^+ shifted to a progressively later date during summer, while growth suppression intensified during spring. In general, the magnitude of response to added NH_4^+ was always greater in Wascana Lake (Fig. 5), where ratios of TDN : SRP were consistently lower than those of Buffalo Pound and SRP was abundant (Table 1; Fig. 2).

Predictors of phytoplankton response to NH_4^+ addition

Three separate binomial logit GAMs were used to identify how the likelihood of stimulation or suppression of phytoplankton growth by added NH_4^+ varied as a function of ambient limnological conditions at the time of the experiment. These models included only abiotic factors (Fig. 6), only initial phytoplankton composition (Fig. 7), or significant predictors from both categories (Supporting Information

Fig. S1). The abiotic model explained 19.5% of the deviance in likelihood of significant NH_4^+ effects (Fig. 6), with concentrations of SRP (both lakes) and water temperature (Wascana only) being retained as significant ($p < 0.05$) predictors (Table 2). Specifically, the likelihood of growth inhibition was greatest when water was cool and SRP levels were low, while significant stimulation by NH_4^+ was more likely in warm nutrient-rich waters, although statistically significant effects of temperature were restricted to Wascana Lake (Fig. 6; Table 2).

Binomial logit GAMs parameterized using only phytoplankton pigments retained all six biomarkers (Fig. 7; Table 3), while explaining 40.0% of deviance in the likelihood of significant community response to added NH_4^+ . In this case, the likelihood of growth suppression increased with the concentration of pigments from cryptophytes (alloxanthin; both lakes), siliceous algae (fucoxanthin; Wascana lake), and total cyanobacteria (echinenone; both lakes), while the likelihood of stimulation increased with the abundance of chlorophytes (Chl *b*; Wascana) and blooming-forming taxa (lutein-zeaxanthin; Buffalo Pound), although the magnitude and significance of effects was usually greater in Wascana Lake (Fig. 7; Table 3). Unlike other phytoplankton, effects of NH_4^+ enrichment on Nostocales cyanobacteria (canthaxanthin) were inconsistent among lakes, with elevated pigment concentrations being associated with a higher and more variable likelihood

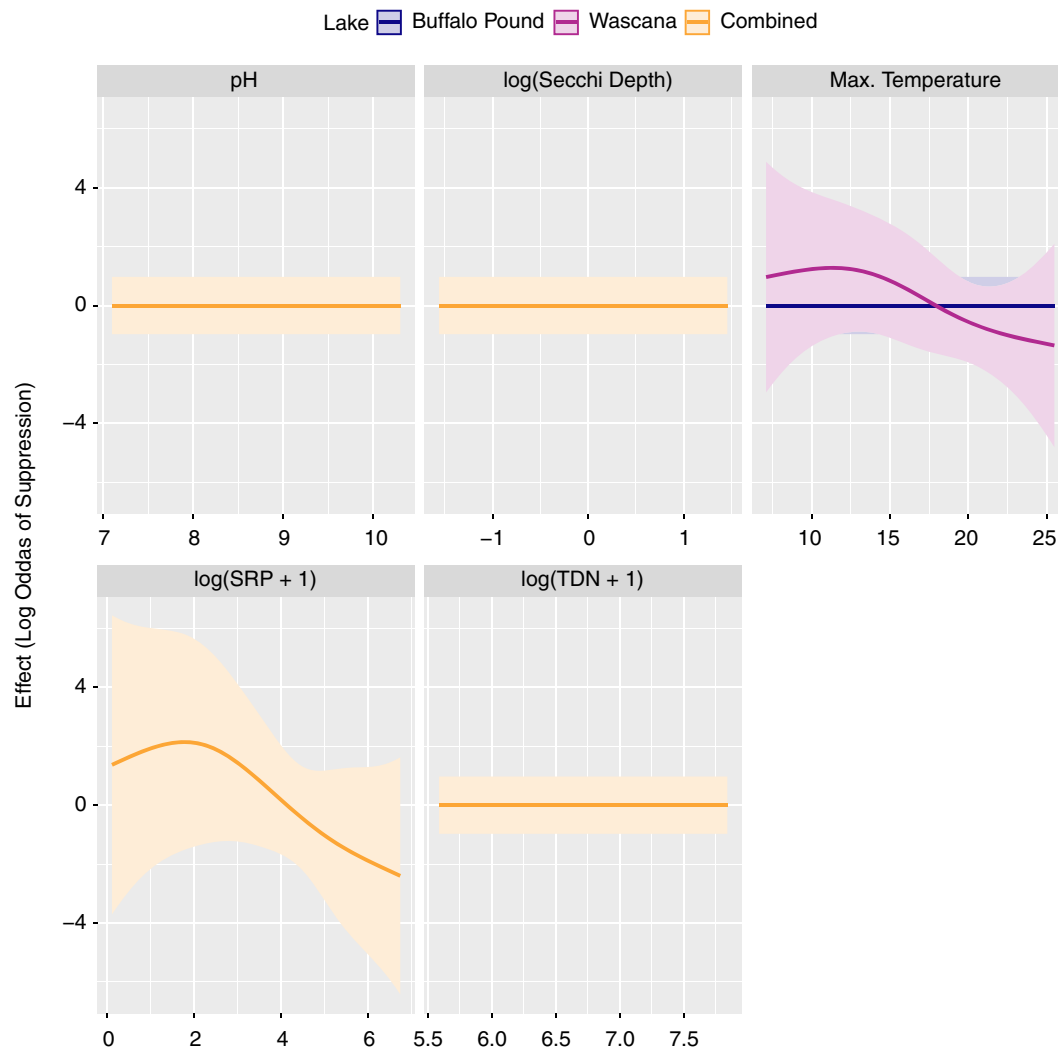


Fig. 6. Partial plots of significant common (i.e., both study lakes) and lake-specific binomial GAM covariates (abiotic predictors). The y-axis denotes the log (odds of suppression) by NH_4^+ , with increased likelihood of suppression above the origin ($y > 0$), and increased likelihood of stimulation as values decrease below the origin ($y < 0$). Coloured shading represents the 95% confidence interval around the fit. Abbreviations include BP (Buffalo Pound Lake), W (Wascana Lake), and Secchi (Secchi depth). Units are in m (Secchi depth), $^{\circ}\text{C}$ (temperature), $\mu\text{g P L}^{-1}$ (SRP), and $\mu\text{g N L}^{-1}$ (TDN).

Table 2. Summary of abiotic model output and significant predictors of the dichotomous response of phytoplankton abundance to NH_4^+ amendment ($p < 0.05$ in bold). Units are in $^{\circ}\text{C}$ (temperature), $\mu\text{g P L}^{-1}$ (SRP), and $\mu\text{g N L}^{-1}$ (TDN).

Variable	Lake(s)	Effective degrees of freedom	Reference degrees of freedom	χ^2	<i>p</i> value
pH	Buffalo Pound	2.320e-06	4	0.0	0.579
pH	Wascana	2.162e-05	4	0.0	0.461
log(Secchi depth)	Buffalo Pound	9.842e-06	4	0.0	0.634
log(Secchi depth)	Wascana	2.176e-05	4	0.0	0.658
Temperature	Buffalo Pound	1.233e-05	4	0.0	0.644
Temperature	Wascana	1.548e+00	4	8.4	0.003
log1p(SRP)	Both	1.819e+00	4	7.0	0.014
log1p(TDN)	Buffalo Pound	05.793e-06	4	0.0	1.000
log1p(TDN)	Wascana	3.535e-06	4	0.0	0.850

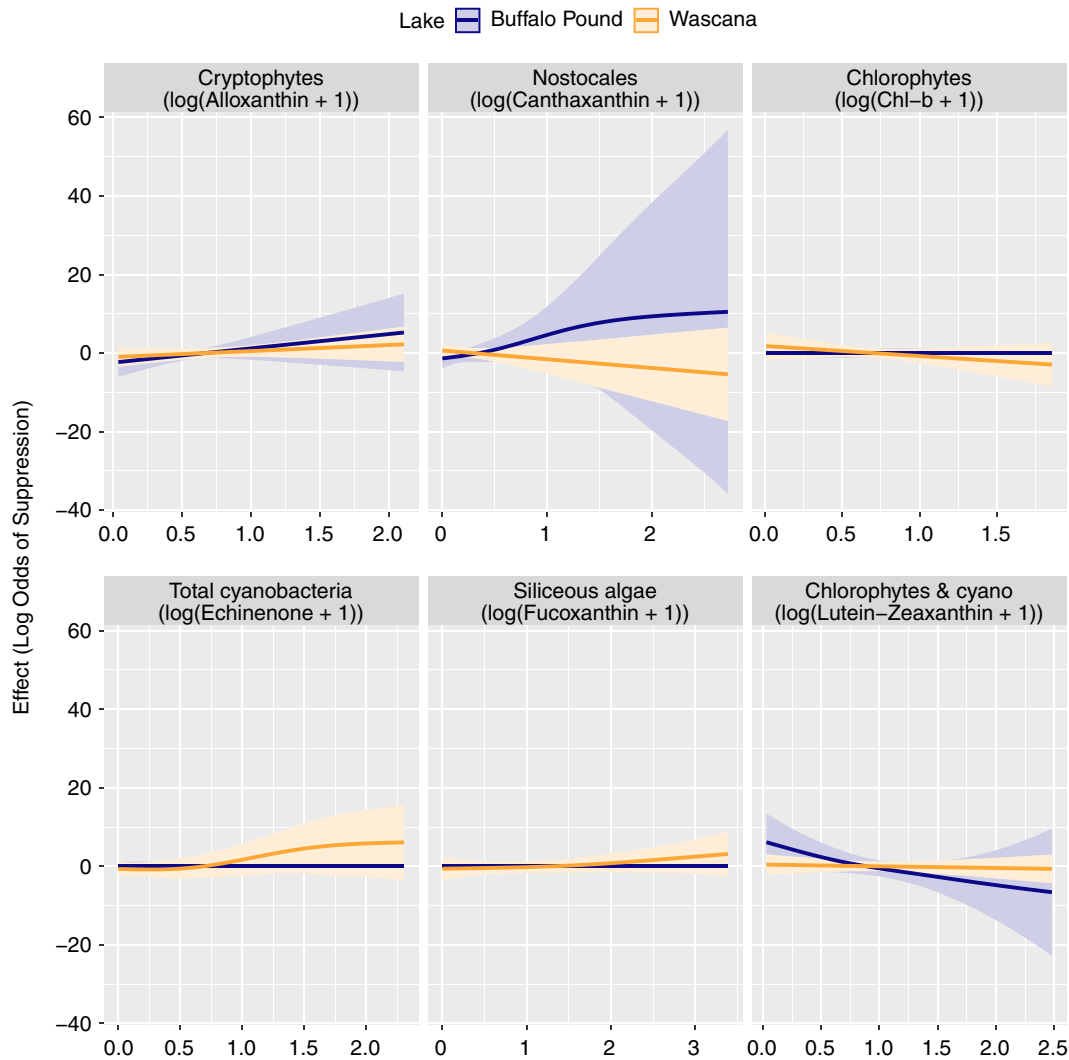


Fig. 7. Partial plots of significant lake-specific binomial GAM covariates (biomarker predictors). The y-axis denotes the log (odds of suppression) by NH_4^+ , with increased likelihood of suppression above the origin ($y > 0$), and increased likelihood of stimulation as values decrease below the origin ($y < 0$). Coloured shading represents the 95% confidence interval around the fit. Lakes are abbreviated as BP (Buffalo Pound) and W (Wascana), and all units are in $\text{nmol pigment L}^{-1}$.

of significant growth inhibition by NH_4^+ in Buffalo Pound Lake, but more likely growth enhancement by NH_4^+ in Wascana Lake (Fig. 7). Over both lakes, Nostocales cyanobacteria (as canthaxanthin) were significantly more abundant during suppressed experiments, while non- N_2 -fixing cyanobacteria (as myxoxanthophyll) are more abundant in experiments where algal growth was stimulated by NH_4^+ (Table 4).

The binomial logit GAM parameterized with significant factors from both the individual abiotic and biotic GAMs explained 47.4% of the deviance in the likelihood of significant community response to added NH_4^+ (Supporting Information Fig. S1). Model analysis showed that the likelihood of suppression increased with cryptophyte and total cyanobacteria abundance in both lakes, and was predicted by low temperatures in Wascana Lake, but not in Buffalo Pound (Supporting

Information Table S1, Fig. S1). In contrast, the likelihood of NH_4^+ -stimulation of phytoplankton growth increased with abundance of chlorophytes (as Chl *b*, in Wascana), the sum of chlorophytes and cyanobacteria (as lutein-zeaxanthin, in Buffalo Pound), and warm temperatures (in Wascana alone). In addition, effects of initial SRP concentration on algal abundance were marginally significant ($p < 0.10$) in both lakes (Supporting Information Table S1), with NH_4^+ suppression most likely to occur when SRP was low, and NH_4^+ stimulation most likely to occur when SRP concentrations were high ($> 50 \mu\text{g P L}^{-1}$) (Supporting Information Fig. S1).

Discussion

Synthesis of physiological, field, and theoretical studies suggests that fertilization of surface waters with NH_4^+ can either

Table 3. Summary of biotic model output and significant pigment biomarker predictors of the dichotomous response of phytoplankton abundance to NH_4^+ amendment ($p < 0.05$ in bold). All units are in nmol L^{-1} .

Variable	Lake	Effective degrees of freedom	Reference degrees of freedom	χ^2	p value
$\log(\text{fucoxanthin} + 1)$	Buffalo Pound	2.803e-05	4	0.000	0.455
$\log(\text{fucoxanthin} + 1)$	Wascana	1.520e+00	4	5.048	0.029
$\log(\text{alloxanthin} + 1)$	Buffalo Pound	8.735e-01	4	4.753	0.016
$\log(\text{alloxanthin} + 1)$	Wascana	8.132e-01	4	3.587	0.029
$\log(\text{Chl } b + 1)$	Buffalo Pound	9.454e-05	4	0.000	0.317
$\log(\text{Chl } b + 1)$	Wascana	8.570e-01	4	4.305	0.014
$\log([\text{lutein} + \text{zeaxanthin}] + 1)$	Buffalo Pound	1.825e+00	4	11.133	<0.000
$\log([\text{lutein} + \text{zeaxanthin}] + 1)$	Wascana	3.483e-01	4	0.547	0.191
$\log(\text{canthaxanthin} + 1)$	Buffalo Pound	1.331e+00	4	6.295	0.010
$\log(\text{canthaxanthin} + 1)$	Wascana	8.226e-01	4	3.360	0.026
$\log(\text{echinenone} + 1)$	Buffalo Pound	1.243e-05	4	0.000	0.493
$\log(\text{echinenone} + 1)$	Wascana	1.908e+00	4	8.146	0.005

enhance or suppress phytoplankton growth depending on species composition and physiological status (Lomas and Glibert 1999b; Donald et al. 2013; Collos and Harrison 2014), as well as environmental conditions including pH (Azov and Goldman 1982; Drath et al. 2008), light and temperature (Lomas and Glibert 1999a; Glibert et al. 2016), and ambient nutrient availability (Donald et al. 2011). Analysis of 241 fertilization experiments conducted in two eutrophic lakes over 16 yr provided little support for the hypothesis that high pH and warm water combine to inhibit phytoplankton growth through NH_3 effects on photosynthesis (Azov and Goldman 1982; Hou et al. 2011; Tsuno et al. 2011). Instead, analysis with binomial logit GAMs showed that phytoplankton growth was more likely to be inhibited under cool vernal conditions with abundant cryptophytes (both lakes) and diatoms

(Wascana only) (Fig. 7) and low dissolved P content (Fig. 6) congruent with the energy-balance hypothesis of Lomas and Glibert (1999a) and Glibert et al. (2016), while both chlorophytes and non- N_2 -fixing cyanobacteria (Fig. 7) benefitted from summer amendment with NH_4^+ as seen elsewhere (Donald et al. 2011; Dolman et al. 2012; Paerl et al. 2016). Unexpectedly, the magnitude of both spring suppression and summer stimulation increased over 16 yr (Fig. 5), reflecting cooling spring temperatures (Finlay et al. 2015; Betts et al. 2016) and larger vernal blooms of NH_4^+ -sensitive cryptophytes and, in Wascana Lake, diatoms (Fig. 4), as well as earlier and more intense blooms of chlorophytes and non- N_2 -fixing cyanobacteria (McGowan et al. 2005b) which prefer NH_4^+ (Collos and Harrison 2014; Glibert et al. 2016). Together these observations suggest that the seasonal effectiveness of

Table 4. Summary statistics from Mann–Whitney U tests on pH (all dates), TDN : SRP (all dates), and relative abundances of select cyanobacterial pigment biomarkers (July and August; i.e., during period of peak cyanobacterial abundance) between stimulated and suppressed experiments. Statistically significant values denoted in bold ($p < 0.05$) or italics ($p < 0.10$).

Pigment	p value	Mann–Whitney U test statistic	χ^2 approximation	df	Group	Count	Rank sum
pH	0.57	1494.50	0.324	1	Stimulated	53	2925.50
					Suppressed	53	2745.50
TDN : SRP	0.023	509.00	5.186	1	Stimulated	34	1104.00
					Suppressed	43	1899.00
Canthaxanthin	<0.001	67.50	13.258	1	Stimulated	26	418.00
					Suppressed	14	484.50
Aphanizophyll	0.440	155.00	0.596	1	Stimulated	26	506.00
					Suppressed	14	314.00
<i>Myxoxanthophyll</i>	<i>0.065</i>	137.00	3.397	1	Stimulated	26	488.00
					Suppressed	14	415.00
Echinenone	<0.001	86.50	9.907	1	Stimulated	26	437.50
					Suppressed	14	465.50

nutrient management techniques on NH_4^+ pollution may vary with future climate warming.

Physico-chemical predictors of phytoplankton suppression by NH_4^+

Previous physiological research hypothesized that exposure to NH_4^+ at $\text{pH} > 9$ may reduce phytoplankton growth because its unionized form, NH_3 , is abundant under alkaline conditions (Trussell 1972) and may inhibit photosystem II (Azov and Goldman 1982; Britt et al. 1989; Boussac et al. 1990) or accumulate in the cell and cause other metabolic damage (Markou et al. 2016). However, although pH routinely exceeds 9 (Finlay et al. 2015) and mid-summer temperatures were up to 25°C in both lakes (Fig. 2c,d), phytoplankton abundance as Chl *a* was stimulated, not suppressed, by NH_4^+ amendment, particularly during the most recent 10 yr (Fig. 5). Furthermore, there was no significant difference ($p > 0.05$) between pH in experiments in which NH_4^+ suppressed phytoplankton growth and those where it enhanced their abundance (Table 4). Due to the fact that phytoplankton community composition was quantified using biomarker pigments (Leavitt and Hodgson 2001), it is not possible to evaluate whether species replacements occurred at high pH in response to NH_4^+ amendment (e.g., Donald et al. 2013). However, because phytoplankton biomass and gross community composition changes rapidly (< 4 d) in response to NH_4^+ fertilization under field conditions in both freshwater and marine water (Finlay et al. 2010; Donald et al. 2011; Glibert et al. 2014b; Shangguan et al. 2017b), it is reasonable to conclude that exposure to elevated pH alone was insufficient to suppress natural phytoplankton assemblages in these trials.

Binomial logit GAMs using abiotic variables alone (Fig. 6; Table 2), or a combination of significant abiotic and biotic parameters (Supporting Information Fig. S1, Table S1), showed that temperature and P concentration were important physico-chemical predictors of NH_4^+ effects on natural phytoplankton assemblages, particularly in Wascana Lake. These findings are consistent with observations from coastal marine ecosystems (Lomas and Glibert 1999a; Parker et al. 2012a; Dugdale et al. 2013; Glibert et al. 2014b, 2016) and in vitro physiological studies (Long et al. 1994; Lomas and Glibert 1999b; Glibert et al. 2016) that document heightened NH_4^+ suppression in cool illuminated waters. Normally, uptake and metabolism of NO_3^- is effective in cool waters due to low temperature optima of relevant enzymes (Gao et al. 1983; Kristiansen 1983; Lomas and Glibert 1999a,b), thereby allowing DNR to function as a dissipatory mechanism which buffers the flow of electrons and protects the chloroplast's electron transport chain from over-reduction (Glibert et al. 2016). However, when the metabolism of NO_3^- is repressed by elevated ambient and cellular concentrations of NH_4^+ , cells in higher light fields may maintain their redox state mainly through photorespiration, resulting in increased energetic costs and reduced growth (Raven 2011). Increased photorespiration when grown

on NH_4^+ is well documented for diatoms (Parker and Armbrust 2005; Allen et al. 2006; Shi et al. 2015) as well as higher plants (Britto and Kronzucker 2002). These effects may be particularly pronounced for cool-water diatoms and cryptophytes compared with chlorophytes and colonial cyanobacteria, as the latter groups may have alternative mechanisms for maintaining cellular energy balance, including higher rates of Mehler activity and use of different accessory pigments (Litchman 2000; Schwaderer et al. 2011; Glibert et al. 2016).

Community predictors of phytoplankton suppression by NH_4^+

Comparison of independent GAMs run with biotic (Fig. 7) and abiotic parameters (Fig. 6) showed that variation in phytoplankton community composition explained the largest proportion of the deviance ($\sim 40\%$) in net response to NH_4^+ (Supporting Information Fig. S1, Table S1; Tables 2–3). In general, the degree of growth suppression increased with the abundance of diatoms (as fucoxanthin), cryptophytes (as alloxanthin), and possibly unicellular cyanobacteria (as echinenone; see below). Both diatoms and cryptophytes are common during spring in the Qu'Appelle study lakes (McGowan et al. 2005b; Vogt et al. 2011; Donald et al. 2013) and other eutrophic freshwaters (Reynolds 1984; Sommer et al. 1986; Lathrop and Carpenter 1992), particularly in Wascana Lake (Fig. 4b,c). We infer that the siliceous algal biomarker fucoxanthin represents mainly diatoms in this study because previous microscopic enumeration demonstrates that other fucoxanthin-containing taxa (chrysophytes, some dinoflagellates) are rare during spring (Patoine et al. 2006; Finlay et al. 2010; Donald et al. 2013). Similarly, we infer that the increased likelihood of growth suppression with total cyanobacteria (as echinenone), but not colonial cyanobacteria (as myxoxanthophyll or aphanizophyll), suggests that the unicellular cyanobacteria alone (not the colonial forms) were suppressed by addition of NH_4^+ (Table 4). Such picocyanobacteria are ubiquitous in eutrophic lake ecosystems and are often abundant in spring (Mózes et al. 2006; Cai and Kong 2013).

Growth suppression by NH_4^+ is best understood for diatoms (Glibert et al. 2016), taxa known to prefer NO_3^- over NH_4^+ as an N source (Lomas and Glibert 1999b; Domingues et al. 2011; Donald et al. 2011). Diatoms are well adapted to use NO_3^- and exhibit an easily induced NR (Blomqvist et al. 1994), higher density of NO_3^- uptake transporters (Glibert et al. 2016), a capacity to store NO_3^- in internal vacuoles (Lomas and Glibert 2000), and the ability to respire NO_3^- under dark or anoxic conditions (Kamp et al. 2011). DNR of cellular NO_3^- is a particularly important mechanism reducing photo-inhibition of growth (Zhang et al. 2012), as diatoms have unusually efficient light-harvesting mechanisms that are susceptible to oxidative damage and reduced cell growth at low temperatures where enzymatic repair is slowed (Litchman 2000; Schwaderer et al. 2011). In particular, under spring-like

conditions of cool water and rapidly rising irradiance, exposure to NH_4^+ both reduces NO_3^- uptake and DNR activity resulting in photo-inhibition of growth (reviewed in Glibert et al. 2016).

While literature on cryptophyte response to NH_4^+ is limited (Donald et al. 2013; Collos and Harrison 2014), results of this study suggest that this phytoplankton group is also inhibited by NH_4^+ in cool surface waters (Figs. 4c, 7). Laboratory studies suggest that thresholds for NH_4^+ toxicity in cryptophytes can be similar to those of diatoms (Collos and Harrison 2014), while field experiments suggest that cryptophytes and diatoms are equally suppressed by excess NH_4^+ and stimulated by fertilization with NO_3^- (Donald et al. 2013). Given that both algal groups are common in spring (McGowan et al. 2005a,b; Dröscher et al. 2009), and that cryptophytes are also adapted to exploit low-light environments in eutrophic lakes (Arvola et al. 1991; Gervais 1998), we suggest that cryptophytes may also use DNR reduce photo-inhibition under vernal conditions. However, additional research is required to confirm this hypothesis.

Unicellular cyanobacteria (as echinenone, see above) may have also exhibited suppression by NH_4^+ , at least in Wascana Lake (Fig. 7). By virtue of their small radius, unicellular cyanobacteria may experience more cellular damage when exposed to high-energy irradiance than do large cells or colonial taxa (Garcia-Pichel 1994). Further, exposure to NH_4^+ may hinder photoprotective mechanisms in these taxa (Dai et al. 2008; Collos and Harrison 2014), particularly under low-ambient temperatures (Schwaderer et al. 2011; Collos and Harrison 2014; Kovács et al. 2016). Although the presence of canthaxanthin from Nostocales cyanobacteria was a marginal predictor of growth inhibition in Buffalo Pound Lake (Fig. 7), this relationship was highly variable (wide confidence intervals), and even opposite (stimulation) in Wascana Lake experiments. Such high variability may arise between sites because Nostocales are facultative N_2 -fixers, whose growth can be inhibited by addition of NH_4^+ (Herrero et al. 2001; Flores and Herrero 2005; Dai et al. 2008; Donald et al. 2013). Further resolution of the mechanisms underlying differential response of cyanobacteria to added NH_4^+ will require more complete microscopic or molecular identification of species' responses to N fertilization.

Physico-chemical predictors of phytoplankton stimulation by NH_4^+

Analysis of fertilization experiments using binomial logit GAMs suggests that warm temperatures can enhance growth stimulation by NH_4^+ in some lakes (Fig. 6) similar to findings elsewhere (Dai et al. 2012; Kosten et al. 2012; Beaulieu et al. 2013). In general, temperature optima for cyanobacteria and chlorophytes range from 25°C to 35°C (Lürding et al. 2013), similar to values observed during summer (Fig. 2c, d) when addition of NH_4^+ stimulated phytoplankton growth (Fig. 5). At these temperatures, the susceptibility of

phytoplankton to photoinhibition also declines (Edwards et al. 2016), reflecting more effective enzymatic repair of photo-damage (Roos and Vincent 1998) and carbon fixation by Rubisco at high temperatures. In addition, enzymes for NH_4^+ assimilation exhibit high temperature optima relative to those for NO_3^- reduction and assimilation (Lomas and Glibert 1999a,b). Although the GAM parameterized only with abiotic parameters (Fig. 6) suggests that the likelihood of stimulation by NH_4^+ increases significantly under P-rich conditions ($> 50 \mu\text{g SRP L}^{-1}$; Table 2), P effects were only marginally significant ($p < 0.10$) in the GAM parameterized with both biomarker and abiotic factors (Supporting Information Fig. S1, Table S1). In general, the threshold for P influence observed in these microcosms was consistent with that identified from both month-long mesocosm experiments (Finlay et al. 2010; Bogard et al. 2017), suggesting that growth stimulation during summer is most likely to occur in P-rich lakes (Donald et al. 2011).

Community predictors of phytoplankton stimulation by NH_4^+

Growth enhancement by NH_4^+ was greatest when phytoplankton communities exhibited a high abundance of chlorophytes (Chl *b*, lutein-zeaxanthin) (Fig. 7), taxa that can outcompete other taxa for chemically reduced N species when light is sufficient (Jensen et al. 1994). In particular, chlorophytes exhibit rapid and diverse mechanisms of N uptake (Fernandez and Galvan 2007), as well as elevated glutamine synthetase and glutamate dehydrogenase activities, that allows them to rapidly convert excess NH_4^+ into amino acids (Collos and Harrison 2014). Comparison of binomial logit GAM results for individual prokaryotic biomarkers (e.g., canthaxanthin vs. echinenone) also suggested that cyanobacterial functional groups (unicellular, colonial, N_2 fixing) exhibited differential sensitivity to added NH_4^+ (Fig. 7; Supporting Information Fig. S1). For example, potentially diazotrophic Nostocales cyanobacteria (as canthaxanthin) were significantly more abundant during suppressed experiments, while non- N_2 -fixing cyanobacteria (as myxoxanthophyll) are more abundant in experiments where algal growth was stimulated by NH_4^+ (Table 4). These findings are consistent with analysis of changes in species composition in large P-rich mesocosms showing that fertilization with NH_4^+ selectively increases the abundance of colonial non- N_2 -fixing cyanobacteria such as *Microcystis* and *Planktothrix* spp. at the expense of other species (Donald et al. 2011; Beaulieu et al. 2013), particularly when SRP concentrations are high ($> 50 \mu\text{g P L}^{-1}$, Fig. 6), TDN : SRP mass ratios are low (< 20) and surface water is $> 22^\circ\text{C}$ (Donald et al. 2011; Dolman et al. 2012; Kosten et al. 2012). These cyanobacteria exhibit high-temperature optima (Carey et al. 2012; Paerl and Paul 2012) and often have a competitive advantage under P-rich conditions, due to superior NH_4^+ -uptake kinetics (Blomqvist et al. 1994; Lee et al. 2015; Yang et al. 2017).

Ontogeny of seasonal response to NH_4^+

Atmospheric and lake warming over the past few decades (Adrian et al. 2009; O'Reilly et al. 2015) have resulted in changes to phytoplankton phenology, with earlier and larger blooms across a range of freshwater and marine ecosystems (Thackeray et al. 2008; Adrian et al. 2009; de Senerpont Domis et al. 2013). Here, we find that the magnitude of vernal suppression and summer stimulation of natural phytoplankton assemblages has increased during the last decade of study, concomitant with pronounced climatic and limnological changes (Finlay et al. 2015; Vogt et al. 2018). Specifically, timing of enhanced suppression of phytoplankton by NH_4^+ during experiments coincides with the onset of cooler waters (Fig. 2c,d), higher transparency (Fig. 2e,f), and higher in situ biomass of cryptophytes and diatoms during spring (Fig. 4b,c). Warmer air temperatures during late winter, but cooler conditions in spring (Betts et al. 2016), can result in earlier ice melt dates (Finlay et al. 2015), but prolonged mixing of cool spring waters (Dröscher et al. 2009). In turn, such vernal mixing favors diatoms and flagellates, taxa adapted to low irradiance or high turbulence, but which are more susceptible to suppression by NH_4^+ (Table 3, Fig. 7; Supporting Information Figs. S1). In contrast, timing of increased summer growth stimulation by NH_4^+ (Fig. 5) was concomitant with elevated abundance of chlorophytes (Fig. 4h,i), low densities of light-sensitive diatoms (Fig. 4b), and earlier blooms of some colonial cyanobacteria (Fig. 4f), all patterns which are consistent with advancing phytoplankton phenology (reviewed in Adrian et al. 2009) and with the general stimulation of primary production in the study lakes by elevated temperature (Vogt et al. 2018).

Conclusions

Continued urban growth (Wigginton et al. 2016) and the intensification of agricultural use of chemically reduced forms of N fertilizer (Glibert et al. 2006) are expected to nearly double the availability of reactive N over the next 30 yr (Millennium Ecosystem Assessment 2005), resulting in increased fertilization of freshwater and marine ecosystems with NH_4^+ (Rabalais et al. 2002; Leavitt et al. 2006; Howarth 2008; Beusen et al. 2016). Effective management of these fertilized ecosystems requires improved information on the unique and interactive roles of N during eutrophication (Glibert et al. 2006, 2014a; Paerl et al. 2016; Schindler et al. 2016).

In this study, we conclude that the net effect of NH_4^+ on natural phytoplankton assemblages depended on the community composition in the receiving waterbody, as well as the physico-chemical conditions at the time of NH_4^+ influx, although we recognize that half of experiments showed little response to amendments. Overall, evidence from GAMs suggests that NH_4^+ pollution is more likely to suppress lake production during spring, when low light adapted phytoplankton

(diatoms, cryptophytes, possibly pico-cyanobacteria) predominate in cool illuminated waters, such as seen in coastal marine ecosystems (Lomas and Glibert 1999a; Hall et al. 2005; Dugdale et al. 2013; Parker et al. 2012a,b). In contrast, we find that assemblages with abundant chlorophytes and possibly non- N_2 -fixing cyanobacteria are more likely to exhibit growth stimulation by added NH_4^+ , particularly in warm, P-rich waters (Donald et al. 2011; Dolman et al. 2012). Although we recognize that it can be difficult to extrapolate to whole-ecosystems from in vitro studies, our findings on N stimulation are consistent with results of short-term nutrient enrichment studies (e.g., Berg et al. 1997; Glibert et al. 2014b; Yang et al. 2017; Shangguan et al. 2017a), month-long mesocosm experiments (Finlay et al. 2010; Donald et al. 2011), long-term monitoring (Vogt et al. 2011; Dai et al. 2012), mass-balance studies (Leavitt et al. 2006; Patoine et al. 2006), and paleolimnology (Leavitt et al. 2006; Patoine and Leavitt 2006) all of which identify unique effects of N in P-rich ecosystems. Further, the novel observation that the timing and intensity of phytoplankton response to NH_4^+ is apparently changing in response to climatic variability during the past 20 yr underscores that management strategies in the future will have to account for a complex interaction of global warming, nutrient pollution, and the unique effects of different chemical forms of N (Glibert 2017). In addition, further research is needed to determine whether stimulation by NH_4^+ is common only to P-rich lakes (Donald et al. 2011), and to better identify the factors which may prevent phytoplankton from responding to NH_4^+ enrichment (e.g., grazing, micronutrients, light, etc.).

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Conflict of Interest

None declared.

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