

1Differential stimulation and suppression of phytoplankton growth by ammonium

2enrichment in eutrophic hardwater lakes over 16 years

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17Running head: Differential phytoplankton response to NH₄⁺ enrichment

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20Abstract

21Previous research suggests that fertilization of surface waters with chemically-reduced nitrogen
22(N), including ammonium (NH_4^+), may either enhance or suppress phytoplankton growth. To
23identify the factors influencing the net effect of NH_4^+ , we fertilized natural phytoplankton
24assemblages from two eutrophic hardwater lakes with growth-saturating concentrations of
25 NH_4Cl in 241 incubation experiments conducted biweekly May-August during 1996– 2011.
26Phytoplankton biomass (as Chl *a*) was significantly ($p < 0.05$) altered in fertilized trials relative
27to controls after 72 hr in 44.8% of experiments, with a marked rise in both spring suppression
28and summer stimulation of assemblages over 16 years, as revealed by generalized additive
29models (GAMs). Binomial GAMs were used to compare contemporaneous changes in physico-
30chemical (temperature, Secchi depth, pH, nutrients; 19.5% deviance explained) and biological
31parameters (phytoplankton community composition; 40.0% deviance explained) to results from
32fertilization experiments. Models revealed that that the likelihood of growth suppression by
33 NH_4^+ increased with abundance of diatoms, cryptophytes and unicellular cyanobacteria,
34particularly when waters temperatures and SRP concentrations were low. In contrast,
35phytoplankton was often stimulated by NH_4^+ when chlorophytes and non- N_2 -fixing cyanobacteria
36were abundant, and temperatures and SRP concentrations were high. Progressive intensification
37of NH_4^+ effects over 16 years reflects changes in both spring (cooler water, increased diatoms
38and cryptophytes) and summer lake conditions (more chlorophytes, earlier cyanobacteria
39blooms), suggesting that the seasonal effects of NH_4^+ will vary with future climate change and
40modes of N enrichment.

41Introduction

42Since the commercialization of the Haber-Bosch process in the 1940s, the global pool of
43manufactured nitrogen (N) has increased nearly 20-fold (Glibert et al. 2006, 2014a), resulting in
44large increases in runoff and atmospheric deposition of reactive N (Nr) to both freshwaters and
45coastal marine ecosystems (Howarth 2008; Galloway et al. 2008; Beusen et al. 2016). The
46combined effects of near-exponential increases in use of N-based agricultural fertilizers and
47growth of storm- and waste-water effluent discharge (Bernhardt et al. 2008) have resulted in a
48more than two-fold increase in total N-loads entering downstream river basins in many parts of
49the world (Green et al. 2004; Howarth 2008). In turn, not only have increases in total N fluxes
50intensified eutrophication of many coastal (Rabalais et al. 2002; Howarth and Marino 2006) and
51freshwater systems (Leavitt et al. 2006; Bunting et al. 2007; Glibert et al. 2014a; Paerl et al.
522015), but there has been an increase in the proportion of chemically-reduced forms N, including
53ammonium (NH_4^+) and urea, relative to nitrate, NO_3^- (Glibert et al. 2006, 2014a, 2016; Glibert
542017). With a growing global population and anticipated doubling of fertilizer N application
55(Glibert et al. 2006, 2014a), the global pool of Nr should double by 2050 (Galloway et al. 2008)
56resulting in increased pollution of surface waters with reduced N.

57 While an extensive body of literature has shown that increasing loads of N may promote
58eutrophication and the development of harmful algal blooms (HABs) in marine systems (Glibert
59et al. 2006, 2014a; Howarth and Marino 2006; Zehr and Kudela 2011), the role of N in
60eutrophication of freshwater systems is less certain (Schindler et al. 2016; Paerl et al. 2016). In
61part, different viewpoints reflect observations that phytoplankton response to N varies with the
62chemical form of added N (Glibert et al. 2014a, 2016), composition of the algal assemblage
63(Donald et al. 2013), and limnological conditions at the time of fertilization (Harris et al. 2014;

64 Hayes et al. 2015), including absolute concentration of N (Chen et al. 2009; Filstrup et al 2018)
65 or phosphorus (P) (Donald et al. 2011; Bogard et al. 2017). For example, while NH_4^+ is most
66 energetically favourable for cellular growth (Turpin et al. 1985; Raven et al. 1992; Flores and
67 Herrero 2005) and has long been considered to be the preferred form of N (Ludwig 1938; Harvey
68 1953; McCarthy 1981; Raven et al. 1992), in situ primary production may alternately be
69 stimulated (Lomas and Glibert 1999a; Finlay et al. 2010; Glibert et al. 2006, 2014b) or
70 suppressed by exposure to NH_4^+ (Dortch 1990; Flynn et al. 1997; Glibert et al. 2016).
71 Understanding the environmental and community controls of the influence of NH_4^+ on primary
72 production and phytoplankton composition is needed to make effective management decisions in
73 both marine and freshwater ecosystems.

74 The extent to which NH_4^+ may stimulate or suppress phytoplankton growth may vary with
75 ambient N concentration, phytoplankton community composition, and other environmental
76 factors (Azov and Goldman 1982; Dugdale et al. 2007, 2013; Parker et al. 2012a; Glibert et al.
77 2016). For example, direct inhibition of phytoplankton growth by reduced N may arise because
78 un-ionized NH_3 can disrupt electrochemical gradients and photophosphorylation at high pH (Hou
79 et al. 2011). Lipid-soluble NH_3 is abundant in warm alkaline conditions (Trussell 1972) and can
80 diffuse into the cytoplasm where it inhibits Photosystem II (PSII) by interacting with carboxylate
81 groups coupled to the Mn_4CaO_5 cluster of the O_2 -evolving center (Britt et al. 1989; Tsuno et al.
82 2011; Hou et al. 2011). Although further research is needed, this pattern suggests that NH_3
83 inhibition should be paramount in lakes where pH and temperature are elevated. Alternately,
84 NH_4^+ may suppress phytoplankton growth by repressing NO_3^- uptake and assimilation, in turn
85 leading to imbalances in cellular redox and energy balance (Lomas and Glibert 1999a, b; Parker
86 et al. 2012a; Glibert et al. 2016). Specifically, diatoms and some other algae use dissimilatory

87nitrate reduction (DNR) to dissipate excessive electron activity in conditions of high light and
88cool water, when cellular metabolism and photo-oxidative repair mechanisms may be
89temperature-limited (Lomas and Glibert 1999a, b). However, the DNR pathway may be
90suppressed by uptake of NH_4^+ , which favours suppression of NO_3^- transport across the cell
91membrane, decay of existing nitrate reductase (NR), and reduction of new NR production
92(Glibert et al. 2016). Once NR is repressed, electrochemical gradients are disrupted and
93phytoplankton growth may be reduced (Kobayashi et al. 2005; Kamp et al. 2011; Rossenwasser
94et al. 2014). Although NH_4^+ suppression of diatom growth is known mainly from marine
95ecosystems (Lomas and Glibert 1999a; Glibert et al. 2014b, 2016), the predominance of diatoms
96in lacustrine assemblages during spring and fall suggests that freshwater phytoplankton
97communities may exhibit seasonal suppression by NH_4^+ pollution.

98 In vitro studies of individual taxa show that the threshold concentration for growth
99inhibition by NH_4^+ varies widely within and among major phytoplankton groups (reviewed in
100Collos and Harrison 2014; Glibert et al. 2016). In general, diatoms are most inhibited by
101addition of NH_4^+ , followed by cyanobacteria and dinoflagellates, whereas chlorophyte species
102were rarely suppressed by such amendments. Larger-scale marine studies have also recorded
103either repression of NO_3^- uptake or suppression of diatom growth by elevated concentrations of
104 NH_4^+ (Yoshiyama and Sharp 2006; Wilkerson et al. 2006; Dugdale et al. 2007; Xu et al. 2012;
105Parker et al. 2012a, b), resulting in increased proportions of marine chlorophytes, cyanobacteria,
106and dinoflagellates under NH_4^+ -enriched conditions (reviewed in Glibert et al. 2016). Similarly,
107addition of NH_4^+ to warm eutrophic coastal and freshwaters favours growth of toxic non- N_2 -
108fixing cyanobacteria at the expense of diazotrophic taxa (McCarthy et al. 2009; Finlay et al.
1092010; Donald et al. 2011; but see Dai et al. 2012; Shangguan et al. 2017a), in part because NH_4^+

110uptake inhibits NtcA transcription promoter activity and suppresses formation of heterocysts
111(Herrero et al. 2001; Flores and Herrero 2005; Harris et al. 2014; Glibert et al. 2016). Together,
112these studies suggest that stimulation of phytoplankton growth by NH₄⁺ should be most
113pronounced in eutrophic lakes during late summer when colonial cyanobacteria are most
114abundant (Paerl and Scott 2010).

115 To better understand the potential for differential effects of NH₄⁺ in lakes, we quantified
116the response of natural phytoplankton assemblages to NH₄⁺ amendment using 241 standard
117bioassay experiments conducted from May to August during a 16-year period. Assemblages
118were obtained from two shallow solute-rich hardwater ecosystems characteristic of basins in the
119continental interior (Hammer 1986; Finlay et al. 2015). Although water security in this region of
120the northern Great Plains is already at risk, these hardwater lakes and rivers are expected to
121receive more NH₄⁺ pollution in the future due to continued urban growth and agricultural
122intensification (Vörösmarty et al. 2010). Consequently, the main objectives of this study were to
123quantify the net effect of excess NH₄⁺ on the aggregate growth of natural phytoplankton
124assemblages, and to evaluate how responses to NH₄⁺ may vary through time and among lakes
125due to variation in physico-chemical conditions and community composition. Finally, we sought
126to determine whether effects of NH₄⁺ were more consistent with pH-, temperature- or community
127composition-mediated mechanisms affecting aggregate phytoplankton growth.

128**Methods**

129*Study area*

130 The two study lakes, Buffalo Pound Lake and Wascana Lake, are located within the
131Qu'Appelle River basin, a catchment which drains 52,000 km² in southern Saskatchewan,

132Canada (Figure 1). Land use within the catchment is largely agricultural, with smaller areas of
133undisturbed grassland, surface waters and urban centers (Hall et al. 1999a; Finlay et al. 2015).

134Regional climate is characterized as cool-summer humid continental (Köppen Dfb
135classification), with short summers (mean 19°C in July), cold winters (mean -16°C in January),
136and low annual temperatures (~1°C) with high seasonal variability (Leavitt et al. 2006).

137Regional mean temperatures have increased ~2°C since 1900, resulting in a 35-day decline in ice
138cover, mainly expressed as earlier dates of ice melting (Finlay et al. 2015). Spring snow melt
139accounts for 80% of annual surface runoff (Pham et al. 2009), leading to seasonally variable, but
140moderately-low water residence times (< 0.7 yr) (Table 1). Both lakes are shallow and
141polymictic (McGowan et al. 2005a), with a highly eutrophic status arising from elevated nutrient
142influx from naturally-fertile soils and regional agriculture (Patoine et al. 2006). Typical of
143hardwater lakes in the northern Great Plains (NGP), both Buffalo Pound and Wascana lakes are
144characterized by high summer pH (mean 8.9-9.0; maximum < 10.5) (Finlay et al. 2015);
145however, the basins exhibit contrasting mean summer mass ratios of total dissolved nitrogen
146(TDN): soluble reactive phosphorus (SRP) of 29.9 and 5.8, respectively (Table 1).

147 Buffalo Pound Lake is a shallow natural water body that has been managed since the
148mid-1960s to supply water to the cities of Regina and Moose Jaw (Hall et al. 1999b). Beginning
149in 1967 and increasing at irregular intervals to the present, Buffalo Pound has received surface
150flow from Lake Diefenbaker, a mesotrophic reservoir located west on the South Saskatchewan
151River (Figure 1). In contrast, Wascana Lake was created by the impoundment of Wascana Creek
152in 1883, but was subsequently deepened to ~2 m in the 1930s and to 7.5 m in 2004 (Hughes
1532004). Despite contrasting histories, the lakes exhibit similar patterns of plankton phenology
154(McGowan et al. 2005a, b; Dröscher et al. 2009; Vogt et al. 2011, 2018), with high vernal

155densities of diatoms, cryptophytes and copepods (*Diaptomus thomasi*, *Leptodiaptomus*
156*siciloides*) giving way to a pronounced clearwater phase, characterized by abundant large-bodied
157*Daphnia* spp. (*D. pulicaria*, *D. galeata mendotae*, *D. magna*) during June, and regular summer
158blooms of both N₂-fixing (*Anabaena*, *Aphanizomenon* spp.) and non-N₂-fixing cyanobacteria
159(*Planktothrix*, *Microcystis* spp.) (McGowan et al. 2005a; Patoine et al. 2006; Donald et al. 2013).

160Field methods

161 Both lakes were sampled biweekly between May and August of 1996– 2011 as part of the
162Qu'Appelle Valley Long-term Ecological Research program (QU-LTER) (Vogt et al. 2018).
163Depth-integrated samples were collected by pooling 2.2-L Van Dorn water bottle casts taken at
1640.5-m intervals below the surface, and used for bioassay experiments and analysis of water
165chemistry, chlorophyll *a* (Chl *a*) content, and phytoplankton community composition (see
166below). Surface pH was measured on site using a calibrated (three standard) handheld pH meter
167(accuracy ± 0.1 unit), while lake transparency (m) was measured using a 20-cm diameter Secchi
168disk. Temperature (°C), conductivity (μS cm⁻¹), and oxygen profiles (mg O₂ L⁻¹) were measured
169at 0.5-m intervals, using a YSI model 85 meter or equivalent (Yellow Springs, Ohio, USA).

170Laboratory methods

171 Depth-integrated water samples were filtered through a 0.45-μm pore membrane filter
172and analyzed at the University of Alberta Water Chemistry Laboratory for concentrations of SRP
173(μg P L⁻¹), total dissolved phosphorus (TDP, μg P L⁻¹), and TDN (μg N L⁻¹) (see Patoine et al.
1742006; Finlay et al. 2015). Particulate organic matter (phytoplankton and detritus) was filtered
175onto GF/C glass-fiber filters (nominal pore size 1.2 μm) and frozen (-10°C) until analysis for Chl
176*a* by standard trichromatic assays (Jeffrey and Humphrey 1975) and biomarker pigments by high

177 performance liquid chromatography (HPLC) (Leavitt and Hodgson 2001). Carotenoids,
178 chlorophylls and their derivatives were isolated and quantified using a Hewlett Packard model
179 1050 or 1100 HPLC system that had been calibrated with authentic standards (Leavitt et al.
180 2006). All HPLC pigment concentrations were expressed as nmoles pigment L⁻¹ before
181 calculation of pigment relative (%) abundance. HPLC analyses were restricted to taxonomically-
182 diagnostic pigments including fucoxanthin (siliceous algae), alloxanthin (cryptophytes), Chl *b*
183 (chlorophytes), echinenone (total cyanobacteria), myxoxanthophyll (colonial cyanobacteria),
184 canthaxanthin (Nostocales cyanobacteria), aphanizophyll (N₂-fixing cyanobacteria), and β-
185 carotene (all phytoplankton). In addition, lutein (chlorophytes) and zeaxanthin (cyanobacteria)
186 were inseparable on the HPLC system, and were combined as a measure of ‘bloom-forming
187 taxa’ (Leavitt and Hodgson 2001; Leavitt et al. 2006).

188 *Ammonium amendment experiments*

189 Nutrient enrichment experiments were conducted biweekly in each lake during May-
190 August of 1996– 2011. These 241 bioassays were used to estimate temporal variation in the
191 potential effects of NH₄⁺ on phytoplankton growth, measured as changes in Chl *a* content over 72
192 h (Finlay et al. 2010; Donald et al. 2011). Briefly, six acid-washed 250-mL bottles each received
193 ca. 225 mL of 243-μm screened, depth-integrated water (see above). Triplicate bottles amended
194 with 1 mL of 0.32 mole L⁻¹ NH₄Cl (N treatment) or received addition (control) to achieve a final
195 NH₄⁺ concentration (ca. 1.5 mM) similar to that arising from influx of tertiary-treated urban
196 waterwater (Waiser et al. 2011). Bottles were incubated in the laboratory for 72 hours at ambient
197 lake temperatures and under a 12 h : 12 h light : dark regime with irradiance equivalent to that
198 experienced at Secchi depth (Finlay et al. 2010; Donald et al. 2011). After incubation,
199 phytoplankton were filtered onto GF/C filters and processed for estimates of Chl *a* concentration

200using the trichromatic analyses detailed above. Phytoplankton response to NH_4^+ was recorded as
201absolute (treatment – control; $\mu\text{g Chl } a \text{ L}^{-1}$) or relative (%) changes in Chl *a* concentration in N
202treatments compared with control treatments to facilitate statistical analyses of time series (see
203below). Both HPLC and trichromatic estimates of phytoplankton abundance have previously
204shown to be highly and linearly correlated with those derived from direct microscopic
205enumeration in these study lakes (Leavitt and Hodgson 2001; Donald et al. 2013).

206*Numerical analyses*

207 Generalized additive models (GAMs) (Wood 2006; Wood et al. 2016) were used to
208estimate long-term trends in Chl *a* response to fertilization with NH_4^+ , as well as temporal
209changes in the physico-chemical and phytoplankton community characteristics recorded in situ at
210time of phytoplankton collection. GAMs are a data-driven regression approach to the estimation
211of non-linear, but not necessarily monotonic, relationships between covariates and response
212variable, and are routinely used to model environmental time series data (e.g. Monteith et al
2132014; Orr et al 2015). The conditional distribution of the response in each GAM was assumed to
214be a gamma distribution for positive, continuous responses, and a Tweedie distribution for non-
215negative continuous responses (such as pigment concentrations). GAMs included marginal
216smooth terms of *day of year* (DoY) for the within-year (seasonal trend) and *year* for the between
217year (long-term trend) components. Additionally, a smooth interaction between these two
218components was estimated through the use of a tensor product smooth created from the two
219marginal smooths. In practical terms, this tensor product smooth allows for the seasonal trend in
220the response to vary smoothly through time within the long-term trend. Smoothness selection
221was performed using the residual maximum marginal likelihood (REML) method of Wood

222(2011), with penalties on both the null and range space of the smoothing matrices to perform
223variable selection in the models (Marra and Wood 2011).

224 Paired *t* tests with pooled variance and Welch's approximation to the degrees of freedom,
225were conducted on the results of each bioassay experiment to detect if Chl *a* content was
226increased or reduced significantly by NH₄⁺-amendments as compared with controls. In all tests,
227228Mann-Whitney U tests were used to evaluate the significance of differences in the initial abiotic
229and biotic conditions (temperature, pH, Secchi depth, TDN:SRP, SRP, relative pigment
230abundance) between experiments in which growth of phytoplankton was either stimulated or
231suppressed significantly (*p* < 0.05) by added NH₄⁺. Spearman rank-order correlations were used
232to evaluate the presence of monotonic trends in select physico-chemical parameters.

233 Binomial GAMs with logit-link function were used to test relationships between selected
234abiotic (physico-chemical) and biotic (phytoplankton abundance) covariates and the likelihood of
235suppression or stimulation in response to NH₄⁺ fertilization. Binomial GAMs used only bioassay
236experiments (and associated environmental data) in which there was a statistically-significant
237response of Chl *a* to added NH₄⁺. Abiotic covariates in the binomial GAMs included Secchi
238depth, water temperature, pH, TDN, and SRP. Biotic covariates included biomarker pigments
239from siliceous algae (mainly diatoms; fucoxanthin), cryptophytes (alloxanthin), chlorophytes
240(Chl *b*), total cyanobacteria (echinenone), Nostocales cyanobacteria (canthaxanthin), and the sum
241of bloom-forming chlorophytes and cyanobacteria (lutein-zeaxanthin). Other pigments
242(myxoxanthophyll, aphanizophyll, β-carotene) were not included because they were either
243redundant with the selected biomarkers or exhibited inconsistent occurrence in the time series.

244 Separate binomial GAMs were run on sets of abiotic and biotic covariates, with predictor
245variables retained using the double-penalty method (Marra and Wood 2011). Each predictor was
246subject to a basis expansion to turn it into a smooth term, with 9 degrees of freedom (10 - 1 for
247the identifiability constraint on each smooth) for each variable. To avoid over-fitting, GAMs
248were restricted to 8 predictors (72 degrees of freedom + 1 for the intercept). Each model allowed
249for lake-specific effects of each covariate via factor-smooth-interactions, and those covariates
250that had statistically significant effects in one or both lakes were reserved. Finally, significant
251covariates identified from separate abiotic and biotic models were combined and used for a final
252binomial GAM to evaluate the potential interaction between limnological and biotic factors in
253predicting phytoplankton response to NH_4^+ . However, we note that the statistical significance of
254covariates included in this final model should be interpreted with care, as its test for significance
255does not account for the prior selection of covariates in abiotic or biotic GAMs.

256 Mann Whitney U tests were conducted using SYSTAT v. 13, whereas Spearman rank-
257order correlations were conducted in TIBCO Spotfire v. 6. All other analyses were conducted in
258R version 3.3.0 (R Core Team 2016) using the *mgcv* package v. 1.8-15 (Wood 2016).

259Results

260Limnological conditions

261 Surface water pH increased during each summer and throughout the study period in both
262study lakes (Figure 2a, b). Mean pH was similar in Buffalo Pound (9.0) and Wascana Lake
263(8.9), and increased by ~1 unit during summer in most years and both lakes. Analysis of fitted-
264response splines (Figure 2c, d) and Spearman rank-order correlations revealed that water
265temperatures in early May declined $\sim 2^\circ\text{C}$ since 1996 in both lakes (Spearman $r_s = -0.37$, $p <$

2660.05), but did not vary consistently at other times of the year. In all years, water transparency (as
267Secchi depth) was greatest in spring in Buffalo Pound (Figure 2e), but changed through time in
268Wascana Lake, with a pronounced clearwater phase during June in 1996 (Dröscher et al. 2009),
269which moved earlier towards spring and diminished in intensity by 2011 (Figure 2f).

270 Temporal trends in nutrient concentrations were markedly different in the two study lakes
271(Figure 2). Mean (\pm SD) SRP concentrations were 15-fold lower Buffalo Pound ($16.3 \pm 19.5 \mu\text{g}$
272P L $^{-1}$) than in Wascana Lake ($254.4 \pm 196.5 \mu\text{g P L}^{-1}$), with differences among seasons declining
273over the 16 years in Buffalo Pound (Figure 2g) but not in Wascana Lake (Figure 2h). In contrast,
274TDN concentrations in Buffalo Pound exhibited a monotonic increase through the summer and
275little variation among years (Figure 2i), whereas Wascana Lake exhibited strong seasonal
276declines in TDN during the 1990s and less seasonality thereafter (Figure 2j). In general, nitrate
277was the predominant form of inorganic N (see Bogard et al. 2012), with concentrations which
278were five- to 10-fold lower (Table 1) than thresholds associated with suppression of
279phytoplankton growth (Chen et al. 2009; Filstrup et al. 2018). Due to contrasting trends in
280individual nutrients, mass ratios of TDN:SRP varied substantially through time and among lakes.
281In Buffalo Pound, TDN:SRP mass ratios (56.6 ± 57.8) varied by an order of magnitude over the
28216-year period (Figure 2k), whereas in Wascana Lake ratios exhibited less variation among
283seasons (13.2 ± 26.7) and little directional change during the monitoring period (Figure 2l).

284*Phytoplankton phenology*

285 On average, Buffalo Pound and Wascana lakes both exhibited similar patterns of seasonal
286phytoplankton ontogeny typical of shallow eutrophic lakes (Figure 3). In both cases, spring
287phytoplankton assemblages composed mainly of diatoms (fucoxanthin) and cryptophytes

288(alloxanthin) were replaced in late-summer by communities composed mainly of chlorophytes
289(Chl *b*, lutein-zeaxanthin), colonial cyanobacteria (myxoxanthophyll, canthaxanthin) and, during
290August, diazotrophic cyanobacteria (aphanizophyll) (Figure 3). However, despite these
291similarities, analysis of fitted splines for individual biomarker pigments showed that the patterns
292of seasonal abundance of phytoplankton groups changed over the course of the 16-year study
293(Figure 4). For example, although annual patterns of total algal abundance (as Chl *a*; Figure 4a)
294have been generally similar in Buffalo Pound since 1997, the abundance of spring siliceous algae
295(largely diatoms) has declined ~50% in recent years (fucoxanthin; Figure 4b), as have those of
296cryptophytes in summer (alloxanthin; Figure 4c), while mid-summer abundances of chlorophytes
297(Chl *b*; Figure 4h) and potentially N₂-fixing cyanobacteria (aphanizophyll; Figure 4g) have
298increased. In Wascana Lake, siliceous algae (Figure 4b) and cryptophytes (Figure 4c) have
299increased throughout the open water season, particularly during spring. Chlorophytes have also
300become more abundant in the spring in Wascana Lake (Figure 4h), while total cyanobacteria
301(echinenone; Figure 4d), colonial forms (myxoxanthophyll; Figure 4e) and potentially N₂-fixing
302taxa (canthaxanthin and aphanizophyll; Figure 4f, g) have shifted seasonality, occurring earlier in
303the summer and at increasing magnitudes in recent years. Finally, changes in seasonal and
304temporal concentrations of ubiquitous β-carotene (Figure 4j) were very similar to those of
305trichromatic Chl *a* in both lakes (Figure 4a).

306*Ammonium amendment experiments*

307 Phytoplankton responses (as Chl *a*) to fertilization with NH₄⁺ ranged from a 2691%
308increase (mean stimulation = 188.1 ± 365.8%) to a 160% suppression (mean suppression = 54.5
309± 25.7%). A significant increase in mean phytoplankton abundance relative to control trials was
310observed in 55 of 241 experiments (FDR-adjusted *p* < 0.05), whereas abundance declined

311significantly in 53 experiments (FDR-adjusted $p < 0.05$). Overall, the frequency of stimulation
312of algal abundance by NH_4^+ was similar among months (May = 11, June = 16, July = 14, August
313= 12), whereas phytoplankton suppression was recorded most frequently during experiments
314conducted in May (21), with decreasing occurrences in June (16), July (12) and August (4).

315 Analysis of GAM-fitted splines showed that the magnitude of seasonal phytoplankton
316response (as Chl *a*) to NH_4^+ amendment increased during the 16-year study period (Figure 5).
317During the first five years of experiments, addition of NH_4^+ mainly increased phytoplankton
318abundance, particularly during spring in Wascana Lake (Figure 5b). However, in both lakes,
319stimulation of growth by NH_4^+ shifted to a progressively later date during summer, while growth
320suppression intensified during spring. In general, the magnitude of response to added NH_4^+ was
321always greater in Wascana Lake (Figure 5), where ratios of TDN:SRP were consistently lower
322than those of Buffalo Pound and SRP was abundant (Table 1, Figure 2).

323*Predictors of phytoplankton response to NH_4^+ addition*

324 Three separate binomial logit GAMs were used to identify how the likelihood of
325stimulation or suppression of phytoplankton growth by added NH_4^+ varied as a function of
326ambient limnological conditions at the time of the experiment. These models included only
327abiotic factors (Figure 6), only initial phytoplankton composition (Figure 7), or significant
328predictors from both categories (Supplementary Information Figure S1). The abiotic model
329explained 19.5% of the deviance in likelihood of significant NH_4^+ effects (Figure 6), with
330concentrations of SRP (both lakes) and water temperature (Wascana only) being retained as
331significant ($p < 0.05$) predictors (Table 2). Specifically, the likelihood of growth inhibition was
332greatest when water was cool and SRP levels were low, while significant stimulation by NH_4^+

333 was more likely in warm nutrient rich waters, although statistically-significant effects of
334 temperature were restricted to Wascana Lake (Figure 6, Table 2).

335 Binomial logit GAMs parameterized using only phytoplankton pigments retained all six
336 biomarkers (Figure 7, Table 3), while explaining 40.0% of deviance in the likelihood of
337 significant community response to added NH_4^+ . In this case, the likelihood of growth
338 suppression increased with the concentration of pigments from cryptophytes (alloxanthin; both
339 lakes), siliceous algae (fucoxanthin; Wascana L.), and total cyanobacteria (echinenone; both
340 lakes), while the likelihood of stimulation increased with the abundance of chlorophytes (Chl *b*;
341 Wascana) and blooming-forming taxa (lutein-zeaxanthin; Buffalo Pound), although the
342 magnitude and significance of effects was usually greater in Wascana Lake (Figure 7, Table 3).
343 Unlike other phytoplankton, effects of NH_4^+ enrichment on Nostocales cyanobacteria
344 (canthaxanthin) were inconsistent among lakes, with elevated pigment concentrations being
345 associated with a higher and more variable likelihood of significant growth inhibition by NH_4^+ in
346 Buffalo Pound Lake, but more likely growth enhancement by NH_4^+ in Wascana Lake (Figure 7).
347 Over both lakes, Nostocales cyanobacteria (as canthaxanthin) were significantly more abundant
348 during suppressed experiments, while non- N_2 -fixing cyanobacteria (as myxoxanthophyll) are
349 more abundant in experiments where algal growth was stimulated by NH_4^+ (Table 4).

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

356NH₄⁺-stimulation of phytoplankton growth increased with abundance of chlorophytes (as Chl *b*,
357in Wascana), the sum of chlorophytes and cyanobacteria (as lutein-zeaxanthin, in Buffalo
358Pound), and warm temperatures (in Wascana alone). In addition, effects of initial SRP
359concentration on algal abundance were marginally significant (*p* < 0.10) in both lakes
360(Supplementary Information Table S1), with NH₄⁺ suppression most likely to occur when SRP
361was low, and NH₄⁺ stimulation most likely to occur when SRP concentrations were high (> 50 µg
362P L⁻¹) (Supplementary Information Figure S1).

363Discussion

364 Synthesis of physiological, field and theoretical studies suggests that fertilization of
365surface waters with NH₄⁺ can either enhance or suppress phytoplankton growth depending on
366species composition and physiological status (Lomas and Glibert 1999b; Donald et al. 2013;
367Collos and Harrison 2014), as well as environmental conditions including pH (Azov and
368Goldman 1982; Drath et al. 2008), light and temperature (Lomas and Glibert 1999a; Glibert et al.
3692016), and ambient nutrient availability (Donald et al. 2011). Analysis of 241 fertilization
370experiments conducted in two eutrophic lakes over 16 years provided little support for the
371hypothesis that high pH and warm water combine to inhibit phytoplankton growth through NH₃
372effects on photosynthesis (Azov and Goldman 1982; Tsuno et al. 2011; Hou et al. 2011).
373Instead, analysis with binomial logit GAMs showed that phytoplankton growth was more likely
374to be inhibited under cool vernal conditions with abundant cryptophytes (both lakes) and diatoms
375(Wascana only) (Figure 7) and low dissolved P content (Figure 6) congruent with the energy-
376balance hypothesis of Lomas and Glibert (1999a) and Glibert et al. (2016), while both
377chlorophytes and non-N₂-fixing cyanobacteria (Figure 7) benefitted from summer amendment
378with NH₄⁺ as seen elsewhere (Donald et al. 2011; Dolman et al. 2012; Paerl et al. 2016).

379 Unexpectedly, the magnitude of both spring suppression and summer stimulation increased over
380 16 years (Figure 5), reflecting cooling spring temperatures (Finlay et al. 2015; Betts et al. 2016)
381 and larger vernal blooms of NH_4^+ -sensitive cryptophytes and, in Wascana Lake, diatoms (Figure
382 24), as well as earlier and more intense blooms of chlorophytes and non- N_2 -fixing cyanobacteria
383 (McGowan et al. 2005b) which prefer NH_4^+ (Collos and Harrison 2014; Glibert et al. 2016).
384 Together these observations suggest that the seasonal effectiveness of nutrient management
385 techniques NH_4^+ pollution may vary with future climate warming.

386 *Physico-chemical predictors of phytoplankton suppression by NH_4^+*

387 Previous physiological research hypothesized that exposure to NH_4^+ at $\text{pH} > 9$ may
388 reduce phytoplankton growth because its unionized form, NH_3 , is abundant under alkaline
389 conditions (Trussell 1972) and may inhibit photosystem II (Azov and Goldman 1982; Britt et al.
390 1989; Boussac et al. 1990) or accumulate in the cell and cause other metabolic damage (Markou
391 et al. 2006). However, although pH routinely exceeds 9 (Finlay et al. 2015) and mid-summer
392 temperatures were up to 25°C in both lakes (Figure 2c, d), phytoplankton abundance as Chl *a*
393 was stimulated, not suppressed, by NH_4^+ amendment, particularly during the most recent 10
394 years (Figure 5). Furthermore, there was no significant difference ($p > 0.05$) between pH in
395 experiments in which NH_4^+ suppressed phytoplankton growth and those where it enhanced their
396 abundance (Table 4). Due to the fact that phytoplankton community composition was quantified
397 using biomarker pigments (Leavitt and Hodgson 2001), it is not possible to evaluate whether
398 species replacements occurred at high pH in response to NH_4^+ amendment (e.g., Donald et al.
399 2013). However, because phytoplankton biomass and gross community composition changes
400 rapidly (<4 days) in response to NH_4^+ fertilization under field conditions in both fresh and marine
401 waters (Finlay et al. 2010; Donald et al. 2011; Glibert et al. 2014b; Shangguan et al. 2017b), it is

402 reasonable to conclude that exposure to elevated pH alone was insufficient to suppress natural
403 phytoplankton assemblages in these trials.

404 Binomial logit GAMs using abiotic variables alone (Figure 6, Table 2), or a combination
405 of significant abiotic and biotic parameters (Supplementary Information Figure S1, Table S1),
406 showed that temperature and P concentration were important physico-chemical predictors of
407 NH₄⁺ effects on natural phytoplankton assemblages, particularly in Wascana Lake. These
408 findings are consistent with observations from coastal marine ecosystems (Lomas and Glibert
409 1999a; Parker et al. 2012a; Dugdale et al. 2013; Glibert et al. 2014b, 2016) and in vitro
410 physiological studies (Long et al. 1994; Lomas and Glibert 1999b; Glibert et al. 2016) that
411 document heightened NH₄⁺ suppression in cool illuminated waters. Normally, uptake and
412 metabolism of NO₃⁻ is effective in cool waters due low temperature optima of relevant enzymes
413 (Kristiansen 1983; Gao et al. 1983; Lomas and Glibert 1999a, b), thereby allowing DNR to
414 function as a dissipatory mechanism which buffers the flow of electrons and protects the
415 chloroplast's electron transport chain from over-reduction (Glibert et al. 2016). However, when
416 the metabolism of NO₃⁻ is repressed by elevated ambient and cellular concentrations of NH₄⁺,
417 cells in higher light fields may maintain their redox state mainly through photorespiration,
418 resulting in increased energetic costs and reduced growth (Raven 2011). Increased
419 photorespiration when grown on NH₄⁺ is well documented for diatoms (Parker and Armbrust
420 2005; Allen et al. 2006; Shi et al. 2015) as well as higher plants (Britto and Kunzucker 2002).
421 These effects may be particularly pronounced for cool-water diatoms and cryptophytes compared
422 with chlorophytes and colonial cyanobacteria, as the latter groups may have alternative
423 mechanisms for maintaining cellular energy balance, including higher rates of Mehler activity and
424 use of different accessory pigments (Litchman 2000; Schwaderer et al. 2011; Glibert et al. 2016).

425 *Community predictors of phytoplankton suppression by NH₄⁺*

426 Comparison of independent GAMs run with biotic (Figure 7) and abiotic parameters
427 (Figure 6) showed that variation in phytoplankton community composition explained the largest
428 proportion of the deviance (~40%) in net response to NH₄⁺ (Supplementary Information Figure
429 S1, Table S1; Tables 2, 3). In general, the degree of growth suppression increased with the
430 abundance of diatoms (as fucoxanthin), cryptophytes (as alloxanthin), and possibly unicellular
431 cyanobacteria (as echinenone; see below). Both diatoms and cryptophytes are common during
432 spring in the Qu'Appelle study lakes (McGowan et al. 2005b; Vogt et al. 2011; Donald et al.
433 2013) and other eutrophic freshwaters (Reynolds 1984; Sommer et al. 1986; Lathrop and
434 Carpenter 1992), particularly in Wascana Lake (Figure 4b, c). We infer that the siliceous algal
435 biomarker fucoxanthin represents mainly diatoms in this study because previous microscopic
436 enumeration demonstrates that other fucoxanthin-containing taxa (chrysophytes, some
437 dinoflagellates) are rare during spring (Patoine et al. 2006; Finlay et al. 2010; Donald et al.
438 2013). Similarly, we infer that the increased likelihood of growth suppression with total
439 cyanobacteria (as echinenone), but not colonial cyanobacteria (as myxoxanthophyll or
440 aphanizophyll), suggests that the unicellular cyanobacteria alone (not the colonial forms) were
441 suppressed by addition of NH₄⁺ (Table 4). Such pico-cyanobacteria are ubiquitous in eutrophic
442 lake ecosystems and are often abundant in spring (Mózes et al. 2006; Cai and Kong 2013).

443 Growth suppression by NH₄⁺ is best understood for diatoms (Glibert et al 2016), taxa
444 known to prefer NO₃⁻ over NH₄⁺ as an N source (Lomas and Glibert 1999b; Domingues et al.
445 2011; Donald et al. 2011). Diatoms are well adapted to use NO₃⁻ and exhibit an easily-induced
446 nitrate reductase (Blomqvist et al. 1994), higher density of NO₃⁻ uptake transporters (Glibert et
447 al. 2016), a capacity to store NO₃⁻ in internal vacuoles (Lomas and Glibert 2000), and the ability

448to respire NO_3^- under dark or anoxic conditions (Kamp et al. 2011). DNR of cellular NO_3^- is a
449particularly important mechanism reducing photo-inhibition of growth (Zhang et al. 2012), as
450diatoms have unusually efficient light-harvesting mechanisms that are susceptible to oxidative
451damage and reduced cell growth at low temperatures where enzymatic repair is slowed
452(Litchman 2000; Schwaderer et al. 2011). In particular, under spring-like conditions of cool
453water and rapidly-rising irradiance, exposure to NH_4^+ both reduces NO_3^- uptake and DNR
454activity resulting in photo-inhibition of growth (reviewed in Glibert et al. 2016).

455 While literature on cryptophyte response to NH_4^+ is limited (Donald et al. 2013; Collos
456and Harrison 2014), results of this study suggest that this phytoplankton group is also inhibited
457by NH_4^+ in cool surface waters (Figures 3c-f, 6). Laboratory studies suggest that thresholds for
458 NH_4^+ toxicity in cryptophytes can be similar to those of diatoms (Collos and Harrison 2014),
459while field experiments suggest that cryptophytes and diatoms are equally suppressed by excess
460 NH_4^+ and stimulated by fertilization with NO_3^- (Donald et al. 2013). Given that both algal groups
461are common in spring (McGowan et al. 2005a, b; Dröscher et al. 2009), and that cryptophytes are
462also adapted to exploit low-light environments in eutrophic lakes (Arvola et al. 1991; Gervais
4631998), we suggest that cryptophytes may also use DNR reduce photo-inhibition under vernal
464conditions. However, additional research is required to confirm this hypothesis.

465 Unicellular cyanobacteria (as echinenone, see above) may have also exhibited
466suppression by NH_4^+ , at least in Wascana Lake (Figure 7). By virtue of their small radius,
467unicellular cyanobacteria may experience more cellular damage when exposed to high energy
468irradiance than do large cells or colonial taxa (Garcia-Pichel 1994). Further, exposure to NH_4^+
469may hinder photoprotective mechanisms in these taxa (Dai et al. 2008; Collos and Harrison
4702014), particularly under low ambient temperatures (Schwaderer et al. 2011; Collos and Harrison

4712014; Kovács et al. 2016). Although the presence of canthaxanthin from Nostocales
472cyanobacteria was a marginal predictor of growth inhibition in Buffalo Pound Lake (Figure 7),
473this relationship was highly variable (wide confidence intervals), and even opposite (stimulation)
474in Wascana Lake experiments (Supplementary Information Figure S2). Such high variability
475may arise between sites because Nostocales are facultative N₂-fixers, whose growth can be
476inhibited by addition of NH₄⁺ (Herrero et al. 2001; Flores and Herrero 2005; Dai et al. 2008;
477Donald et al. 2013). Further resolution of the mechanisms underlying differential response of
478cyanobacteria to added NH₄⁺ will require more complete microscopic or molecular identification
479of species' responses to N fertilization.

480 *Physico-chemical predictors of phytoplankton stimulation by NH₄⁺*

481 Analysis of fertilization experiments using binomial logit GAMs suggests that warm
482temperatures can enhance growth stimulation by NH₄⁺ in some lakes (Figure 6) similar to
483findings elsewhere (Kosten et al. 2012; Dai et al. 2012; Beaulieu et al. 2013). In general,
484temperature optima for cyanobacteria and chlorophytes range from 25 to 35°C (Lürling et al.
4852013), similar to values observed during summer (Figure 2c, d) when addition of NH₄⁺
486stimulated phytoplankton growth (Figure 5). At these temperatures, the susceptibility of
487phytoplankton to photoinhibition also declines (Edwards et al. 2016), reflecting more effective
488enzymatic repair of photo-damage (Roos and Vincent 1998) and carbon fixation by Rubisco at
489high temperatures. In addition, enzymes for NH₄⁺ assimilation exhibit high temperature optima
490relative to those for NO₃⁻ reduction and assimilation (Lomas and Glibert 1999a, b). Although the
491GAM parameterized only with abiotic parameters (Figure 6) suggests that the likelihood of
492stimulation by NH₄⁺ increases significantly under P-rich conditions (>50 µg SRP L⁻¹; Table 2), P
493effects were only marginally significant ($p < 0.10$) in the GAM parameterized with both

494biomarker and abiotic factors (Supplementary Information Figure S1, Table S1). In general, the
495threshold for P influence observed in these microcosms was consistent with that identified from
496both month-long mesocosm experiments (Finlay et al. 2010; Bogard et al. 2017), suggesting that
497growth stimulation during summer is most likely to occur in P-rich lakes (Donald et al. 2011).

498*Community predictors of phytoplankton stimulation by NH₄⁺*

499 Growth enhancement by NH₄⁺ was greatest when phytoplankton communities exhibited a
500high abundance of chlorophytes (Chl *b*, lutein-zeaxanthin) (Figure 6), taxa that can outcompete
501other taxa for chemically-reduced N species when light is sufficient (Jensen et al. 1994). In
502particular, chlorophytes exhibit rapid and diverse mechanisms of N uptake (Fernandez and
503Galvan 2007), as well as elevated glutamine synthetase and glutamate dehydrogenase activities,
504that allows them to rapidly convert excess NH₄⁺ into amino acids (Collos and Harrison 2014).
505Comparison of binomial logit GAM results for individual prokaryotic biomarkers (e.g.,
506canthaxanthin vs. echineneone) also suggested that cyanobacterial functional groups (unicellular,
507colonial, N₂ fixing) exhibited differential sensitivity to added NH₄⁺ (Figure 7; Supplementary
508Information Figure S1). For example, potentially-diazotrophic Nostocales cyanobacteria (as
509canthaxanthin) were significantly more abundant during suppressed experiments, non-N₂-fixing
510cyanobacteria (as myxoxanthophyll) are more abundant in experiments where algal growth was
511stimulated by NH₄⁺ (Table 4). These findings are consistent with analysis of changes in species
512composition in large P-rich mesocosms showing that fertilization with NH₄⁺ selectively increases
513the abundance of colonial non-N₂-fixing cyanobacteria such as *Microcystis* and *Planktothrix* spp.
514at the expense of other species (Donald et al 2011; Beaulieu et al. 2013), particularly when SRP
515concentrations are high (> 50 µg P/L, Figure 6), TDN:SRP mass ratios are low (< 20, Table 5)
516and surface water is > 22°C (Donald et al. 2011; Dolman et al. 2012; Kosten et al. 2012). These

517cyanobacteria exhibit high temperature optima (Carey et al. 2012; Paerl and Paul 2012) and often
518have a competitive advantage under P-rich conditions, due to superior NH_4^+ -uptake kinetics
519(Bломqvist et al. 1994; Lee et al. 2015; Yang et al. 2017).

520*Ontogeny of seasonal response to NH_4^+*

521 Atmospheric and lake warming over the past few decades (Adrian et al. 2009; O'Reilly et
522al. 2015) have resulted in changes to phytoplankton phenology, with earlier and larger blooms
523across a range of freshwater and marine ecosystems (Thackeray et al. 2008; Adrian et al. 2009;
524De Senerpont Domis et al. 2013). Here we find that the magnitude of vernal suppression and
525summer stimulation of natural phytoplankton assemblages has increased during the last decade
526of study, concomitant with pronounced climatic and limnological changes (Finlay et al. 2015;
527Vogt et al. 2018). Specifically, timing of enhanced suppression of phytoplankton by NH_4^+
528during experiments coincides with the onset of cooler waters (Figure 2c,d), higher transparency
529(Figure 2e,f), and higher *in situ* biomass of cryptophytes and diatoms during spring (Figure 4b,
530c). Warmer air temperatures during late winter, but cooler conditions in spring (Betts et al.
5312016), can result in earlier ice melt dates (Finlay et al. 2015), but prolonged mixing of cool
532spring waters (Dröscher et al. 2009). In turn, such vernal mixing favours diatoms and flagellates,
533taxa adapted to low irradiance or high turbulence, but which are more susceptible to suppression
534by NH_4^+ (Table 3, Figure 7; Supplementary Information Figures S1). In contrast, timing of
535increased summer growth stimulation by NH_4^+ (Figure 5) was concomitant with elevated
536abundance of chlorophytes (Figure 4h, i), low densities of light-sensitive diatoms (Figure 4b),
537and earlier blooms of some colonial cyanobacteria (Figure 4f), all patterns which are consistent
538with advancing phytoplankton phenology (reviewed in Adrian et al. 2009) and with the general
539stimulation of primary production in the study lakes by elevated temperature (Vogt et al. 2018).

540Conclusions

541 Continued urban growth (Wigginton et al. 2016) and the intensification of agricultural
542use of chemically-reduced forms of N fertilizer (Glibert et al. 2006) are expected to nearly
543double the availability of reactive N over the next 30 years (Millennium Ecosystem Assessment
5442005), resulting in increased fertilization of freshwater and marine ecosystems with NH_4^+
545(Rabalais et al. 2002; Howarth 2008; Leavitt et al. 2006, Beusen et al. 2016). Effective
546management of these fertilized ecosystems requires improved information on the unique and
547interactive roles of N during eutrophication (Glibert et al. 2006, 2014a; Schindler et al. 2016;
548Paerl et al. 2016).

549 In this study, we conclude that the net effect of NH_4^+ on natural phytoplankton
550assemblages depended on the community composition in the receiving water body, as well as
551the physico-chemical conditions at the time of NH_4^+ influx, although we recognize that half of
552experiments showed little response to amendments. Overall, evidence from GAMs suggests that
553 NH_4^+ pollution is more likely to suppress lake production during spring, when low light adapted
554phytoplankton (diatoms, cryptophytes, possibly pico-cyanobacteria) predominate in cool
555illuminated waters, such as seen in coastal marine ecosystems (Lomas and Glibert 1999a; Hall et
556al. 2005; Dugdale et al. 2012, 2013; Parker et al. 2012a, b). In contrast, we find that assemblages
557with abundant chlorophytes and possibly non- N_2 -fixing cyanobacteria are more likely to exhibit
558growth stimulation by added NH_4^+ , particularly in warm, P-rich waters (Donald et al. 2011;
559Dolman et al. 2012). Although we recognize that it can be difficult to extrapolate to whole-
560ecosystems from *in vitro* studies, our findings on N stimulation are consistent with results of
561short-term nutrient enrichment studies (e.g., Berg et al. 1997, Glibert et al. 2014b, Shangguan et
562al. 2017a; Yang et al. 2017), month-long mesocosm experiments (Finlay et al. 2010; Donald et

563al. 2011), long-term monitoring (Dai et al. 2012; Vogt et al. 2011), mass-balance studies (Leavitt
564et al. 2006; Patoine et al. 2006) and paleolimnology (Patoine and Leavitt 2006; Leavitt et al.
5652006) all of which identify unique effects of N in P-rich ecosystems. Further, the novel
566observation that the timing and intensity of phytoplankton response to NH₄⁺ is apparently
567changing in response to climatic variability during the past 20 years underscores that
568management strategies in the future will have to account for a complex interaction of global
569warming, nutrient pollution, and the unique effects of different chemical forms of N (Glibert
5702017). In addition, further research is needed to determine whether stimulation by NH₄⁺ is
571common only to P-rich lakes (Donald et al. 2011), and to better identify the factors which may
572prevent phytoplankton from responding to NH₄⁺ enrichment (e.g., grazing, micronutrients, light,
573etc.).

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584

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927Table 1. Morphometric, chemical, and biological characteristics of the two study lakes. Data are mean values (standard deviation, in
 928parentheses) of measurements taken between May - August of 1996 - 2011. Abbreviations represent maximum depth (Z_{\max}), total
 929dissolved- and soluble reactive phosphorus (TDP, SRP), total dissolved nitrogen (TDN), dissolved organic and total inorganic carbon
 930(DOC, TIC), and chlorophyll *a* (Chl *a*).

Lake	Area (km ²)	Volume (10 ⁶ m ³)	Water residence (yr)	Z_{\max} (m)	TDP ($\mu\text{g P L}^{-1}$)	SRP ($\mu\text{g P L}^{-1}$)	TDN ($\mu\text{g N L}^{-1}$)	NO_3^- ($\mu\text{g N L}^{-1}$)	DOC (mg L ⁻¹)	TIC (mg L ⁻¹)	Conductivity ($\mu\text{S cm}^{-1}$)	pH	Secchi depth (m)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)
Buffalo Pound	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)
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)

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

Variable	Lake(s)	Effective degrees of freedom	Reference degrees of freedom	χ^2	p-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

933

934Table 3. Summary of biotic model output and significant pigment biomarker predictors of the dichotomous response of phytoplankton

935abundance 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(fucoxanthin+1)	Buffalo Pound	2.803e-05	4	0.000	0.455
log(fucoxanthin+1)	Wascana	1.520e+00	4	5.048	0.029
log(alloxanthin+1)	Buffalo Pound	8.735e-01	4	4.753	0.016
log(alloxanthin +1)	Wascana	8.132e-01	4	3.587	0.029
log(Chl b+1)	Buffalo Pound	9.454e-05	4	0.000	0.317
log(Chl b +1)	Wascana	8.570e-01	4	4.305	0.014
log((lutein + zeaxanthin) +1)	Buffalo Pound	1.825e+00	4	11.133	<0.000
log((lutein + zeaxanthin) +1)	Wascana	3.483e-01	4	0.547	0.191
log(canthaxanthin+1)	Buffalo Pound	1.331e+00	4	6.295	0.010
log(canthaxanthin+1)	Wascana	8.226e-01	4	3.360	0.026
log(echinone+1)	Buffalo Pound	1.243e-05	4	0.000	0.493
log(echinone +1)	Wascana	1.908e+00	4	8.146	0.005

936

937Table 4. Summary statistics from Mann-Whitney U tests on pH (all dates), TDN:SRP (all dates), and relative abundances of select
 938cyanobacterial pigment biomarkers (July and August; i.e., during period of peak cyanobacterial abundance) between stimulated and
 939suppressed experiments. Statistically significant values denoted in bold ($p < 0.05$) or italics ($p < 0.10$).

Pigment	p-value	Mann-Whitney U test statistic	Chi-square 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>	0.065	137.00	3.397	1	Stimulated	26	488.00
					Suppressed	14	415.00
Echinone	<0.001	86.50	9.907	1	Stimulated	26	437.50
					Suppressed	14	465.50

940

941Figure Legends

942Figure 1. Map of the Qu'Appelle River drainage basin (inset) including Buffalo Pound Lake and
943Wascana Lake, Saskatchewan, Canada. Buffalo Pound receives water from Lake Diefenbaker
944and drains to the east via the Qu'Appelle River, whereas Wascana Creek drains into Wascana
945Lake within the City of Regina (black) before reaching a confluence with the Qu'Appelle River.
946Heavy outline indicates maximum extent of drainage basin, while white and grey shading
947indicates contributing and non-contributing areas during the median flow year, respectively.

948Figure 2. Fitted curves showing seasonal trends in limnological characteristics in Buffalo Pound
949Lake (left) and Wascana Lake (right) between 1996 – 2011. Panels and adjusted variance
950explained (R^2 ; %) by mean trends in each lake include pH (a 20%, b 40%), maximum
951temperature ($^{\circ}\text{C}$) (c 82% , d 81%), Secchi depth (m) (e 18%, f 28%), soluble reactive
952phosphorus (SRP; $\mu\text{g P L}^{-1}$) (g 4%, h 60%), total dissolved nitrogen (TDN; $\mu\text{g N L}^{-1}$) (i 20%, j
95317%), and TDN:SRP (k 42% , l 55%). Note difference in y-axis scales between lakes. X-axis
954shows day of year (DoY) between spring and fall. Time series are shaded from dark blue (1996)
955to orange (2011).

956Figure 3. Mean monthly changes in relative (%) pigment abundance in Buffalo Pound (left) and
957Wascana lakes (right) during 1996 - 2011.

958Figure 4. Fitted response plots showing seasonal trends in chlorophyll and carotenoid pigment
959concentrations between 1996 - 2011 in Wascana Lake and Buffalo Pound lake. Pigments and %
960deviance explained by mean trends are presented for both lakes, including; a) chlorophyll *a* (all
961phytoplankton) (62%, 63%, respectively), b) fucoxanthin (siliceous algae, mainly diatoms)
962(26%, 42%), c) alloxanthin (cryptophytes) (49%, 32%), d) echinenone (total cyanobacteria)

963(59%, 65%), e) myxoxanthophyll (colonial cyanobacteria) (54%, 59%), f) canthaxanthin
964(Nostocales cyanobacteria) (58%, 65%) , g) aphanizophyll (N_2 -fixing cyanobacteria) (43%,
96545%), h) chlorophyll b (chlorophytes) (58%, 54%), i) lutein + zeaxanthin (chlorophytes and
966cyanobacteria) (52%, 43%), and j) β -carotene (all phytoplankton) (63%, 59%). All pigments are
967quantified using HPLC in nmoles pigment L⁻¹, except trichromatic determinations of Chl a (μ g
968Chl L⁻¹). X-axis denotes day of year (DoY) from 01 May (DoY 121) to 31 August (DoY 243).

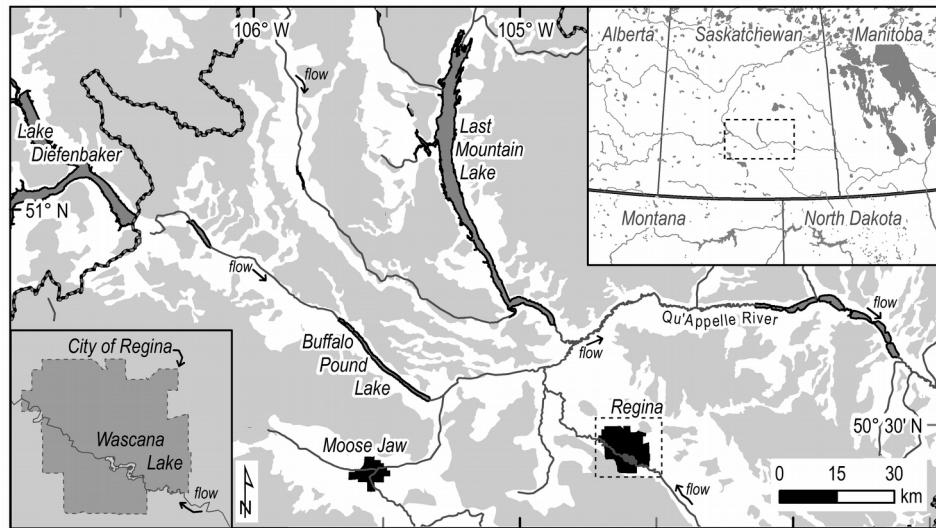
969Figure 5. Fitted curves of changes in Chl a concentration (μ g L⁻¹) in trials amended with NH₄⁺
970relative to control in bioassay experiments conducted at Buffalo Pound (a) and Wascana (b)
971lakes during the open water seasons of 1996 (blue; darkest lines) to 2011 (orange; lightest lines).
972X-axis shows date in day of year (DoY) between spring and fall. Percent deviance explained by
973mean trends was 26% for Buffalo Pound, and 20% for Wascana Lake.

974Figure 6. Partial plots of significant common (i.e., both study lakes) and lake-specific binomial
975GAM covariates (abiotic predictors). The y-axis denotes the log (odds of suppression) by NH₄⁺,
976with increased likelihood of suppression above the origin ($y > 0$), and increased likelihood of
977stimulation as values decrease below the origin ($y < 0$). Grey shading represent the 95%
978confidence interval around the fit. Abbreviations include BP (Buffalo Pound Lake), W
979(Wascana Lake), and Secchi (Secchi depth). Units are in m (Secchi depth), °C (temperature), μ g
980P/L⁻¹ (SRP), and μ g N/L⁻¹ (TDN).

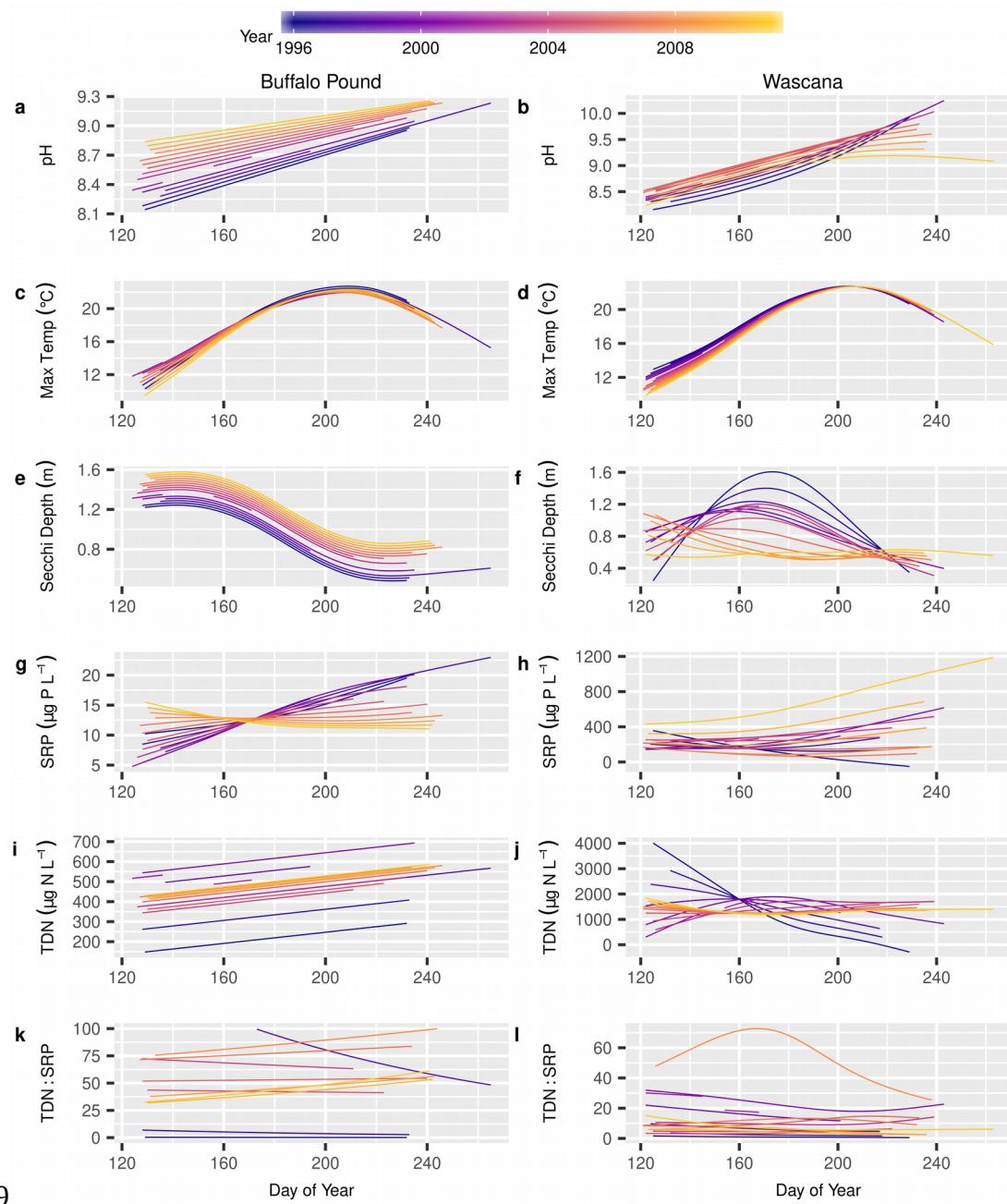
981 Figure 7. Partial plots of significant lake-specific binomial GAM covariates (biomarker
982predictors). The y-axis denotes the log (odds of suppression) by NH₄⁺, with increased likelihood
983of suppression above the origin ($y > 0$), and increased likelihood of stimulation as values
984decrease below the origin ($y < 0$). Grey shading represent the 95% confidence interval around

985the fit. Lakes are abbreviated as BP (Buffalo Pound) and W (Wascana), and all units are in
986nmoles pigment L⁻¹.

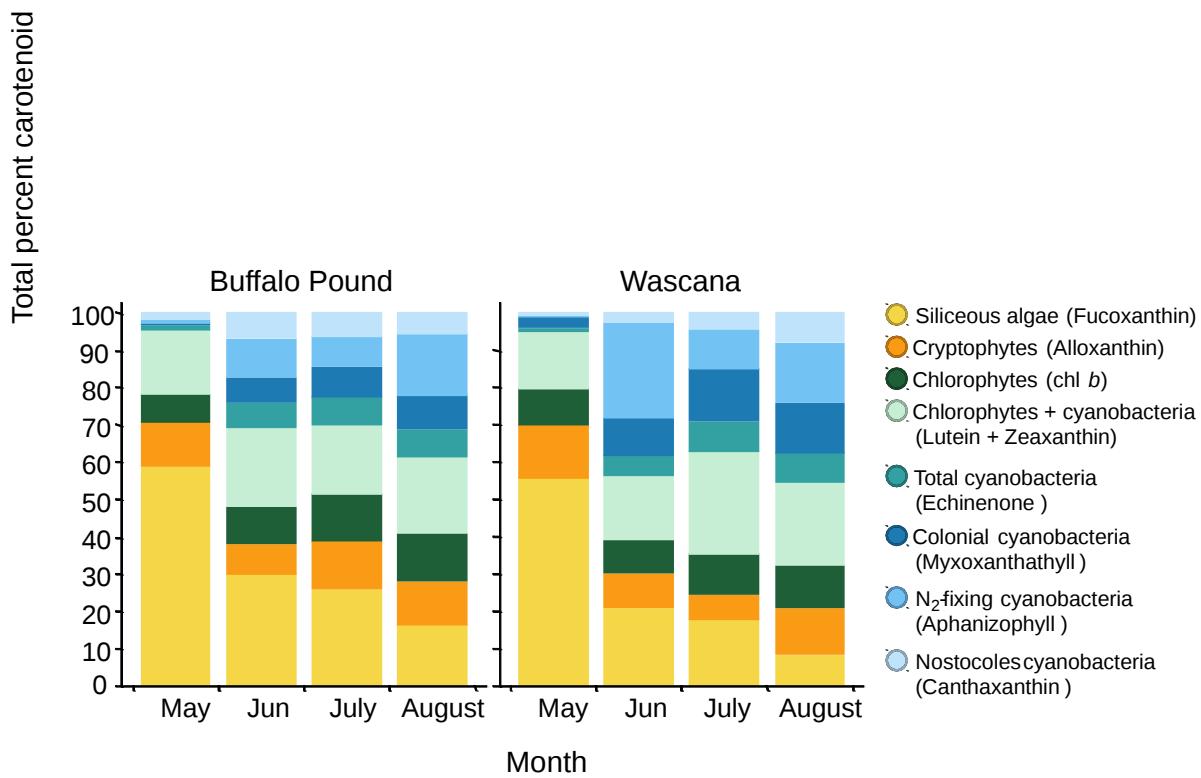
987Fig. 1



988 Fig. 2

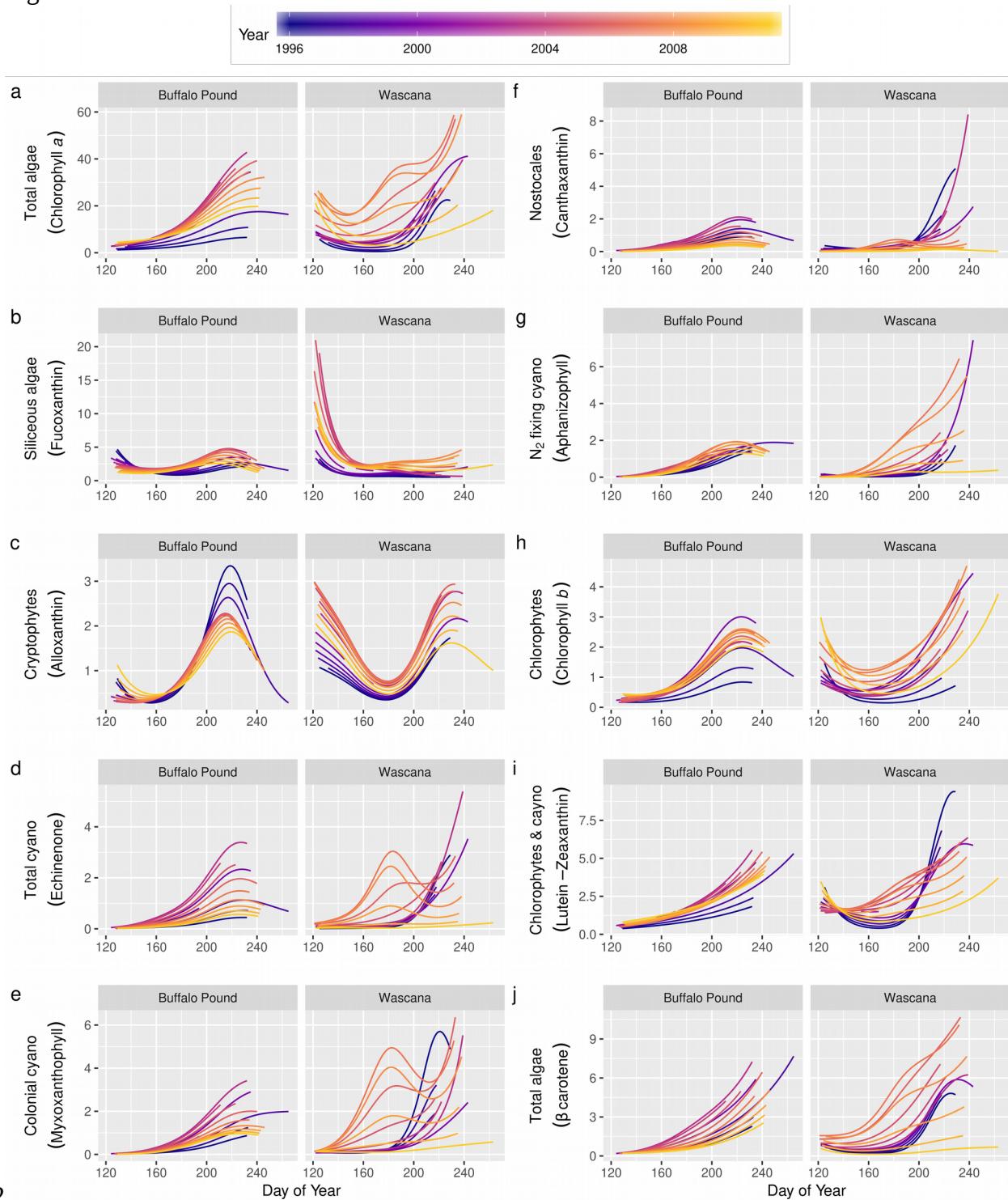


989



990Fig. 3

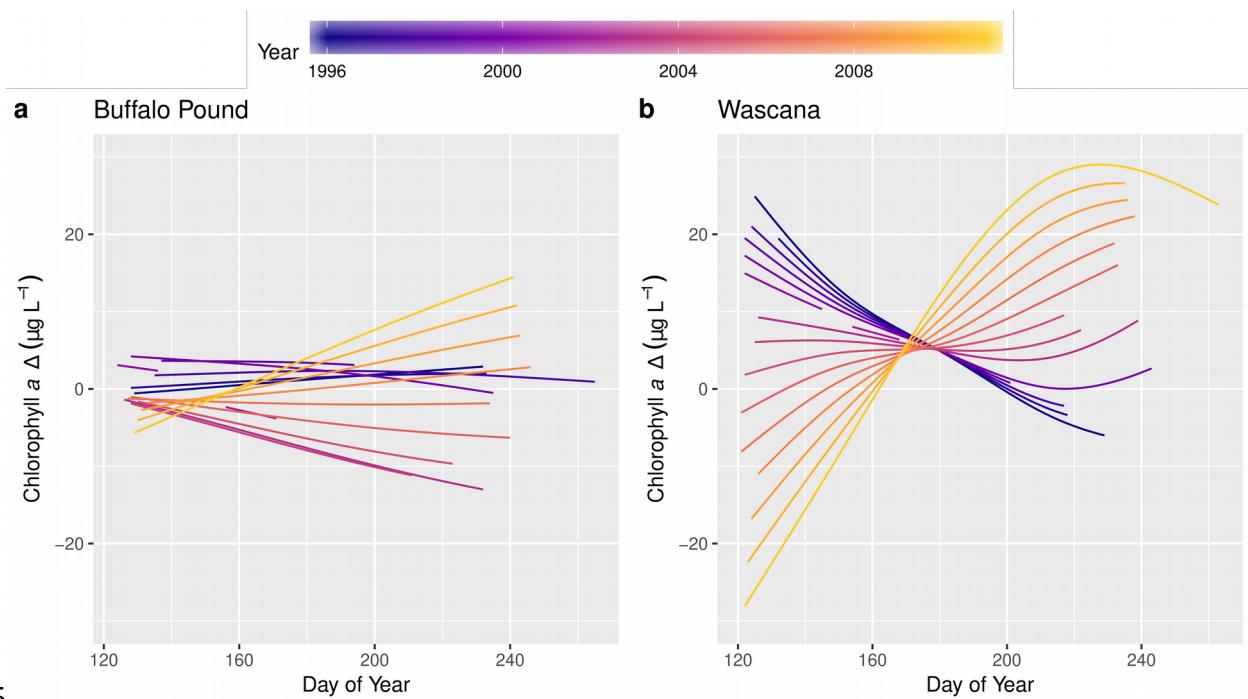
991Fig. 4.



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994 Fig. 5



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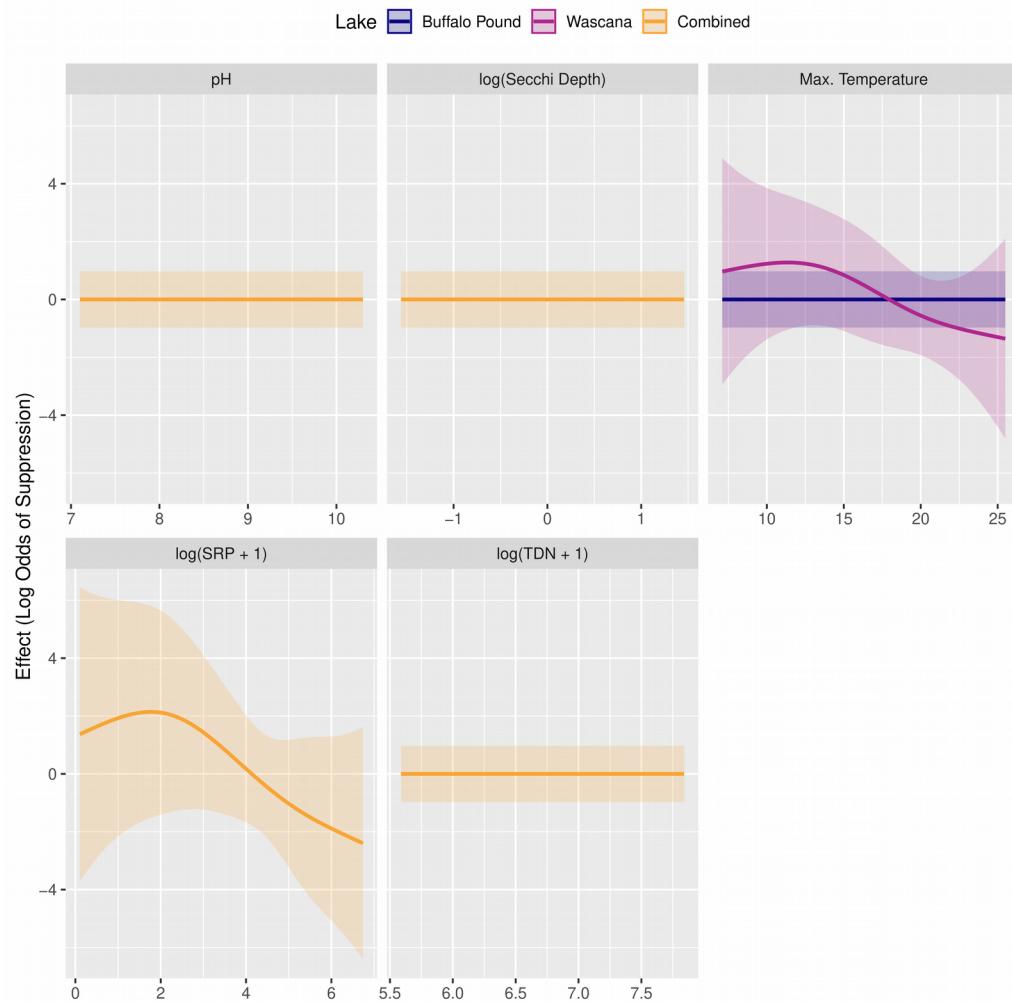
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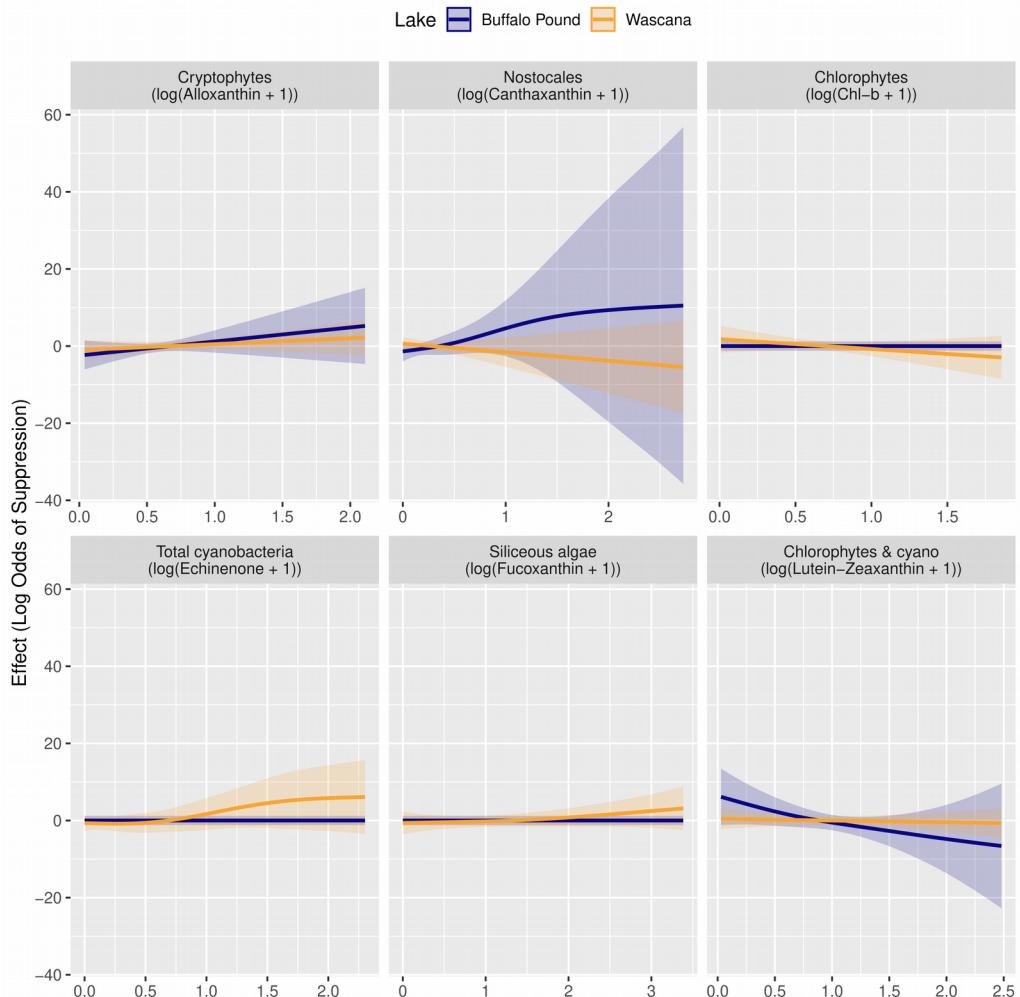
996Fig. 6.

997

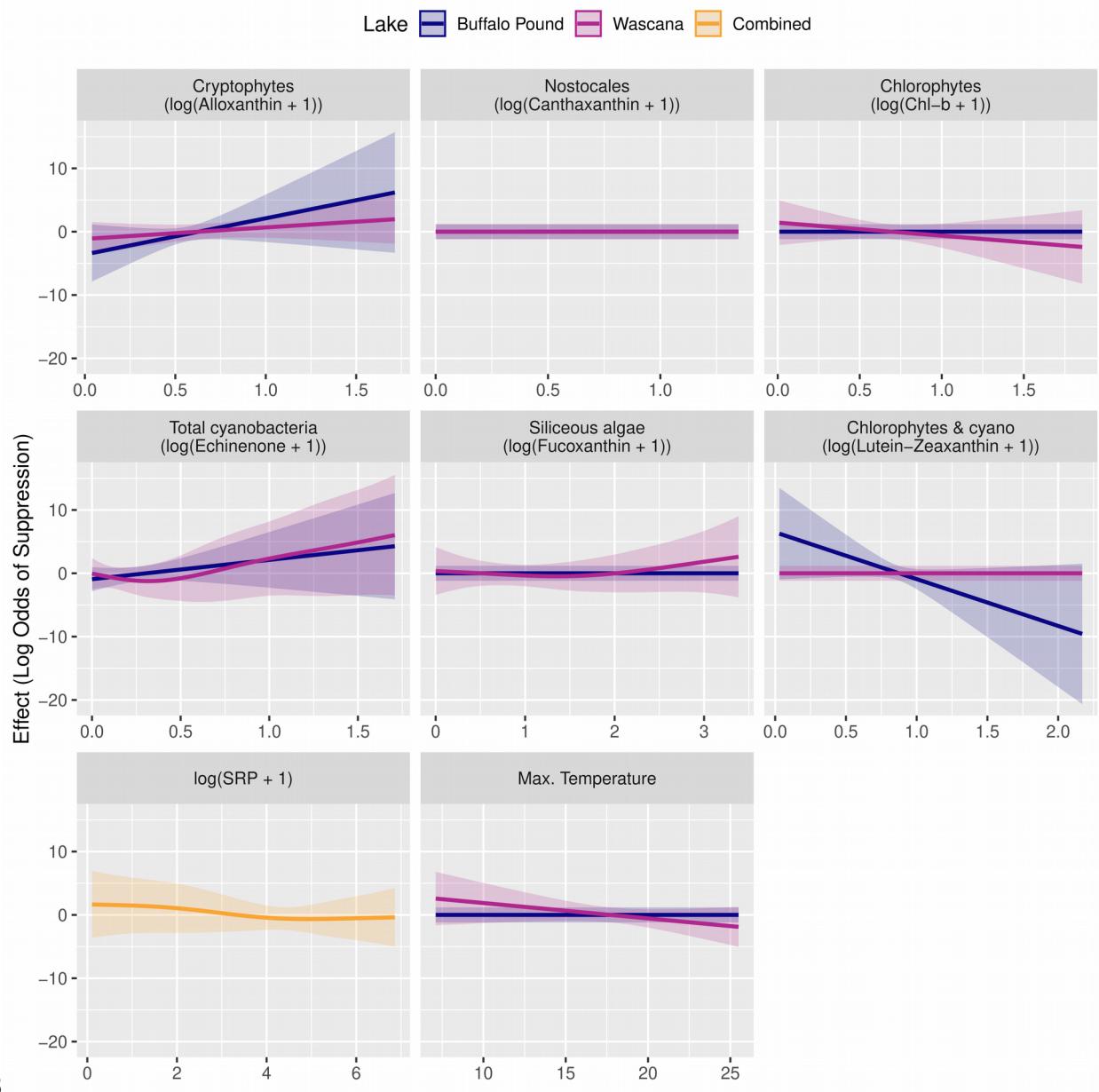


998 Fig. 7.

999



1000**Supplementary Information Figure S1.** Partial plots of significant binomial covariates in
1001bioassay response in generalized additive models developed with significant biotic and abiotic
1002parameters. The y-axis denotes the log (odds of suppression) by NH_4^+ , with increased likelihood
1003of suppression above the origin ($y > 0$), and increased likelihood of stimulation as values
1004decrease below the origin ($y < 0$). Significant by-lake covariates are show in blue (Buffalo
1005Pound) and pink (Wascana), while variables not separated by lake are shown in yellow. Dashed
1006lines represent 95% confidence interval around the fit. Abbreviations include chl B (chlorophyll
1007b), SRP (soluble reactive phosphorus), and maxTemp (maximum temperature).



1009 **Supplementary Information Table S1.** Summary of overall (i.e., combined abiotic and biotic parameters) model output of the
 1010 dichotomous response of phytoplankton abundance to NH_4^+ amendment, with significant predictors ($p < 0.05$) in bold. Units are in
 1011 nmol L⁻¹(all pigments), °C (temperature), and µg P L⁻¹ (SRP).

Variable	Lake	Effective degrees of freedom	Reference degrees of freedom	χ^2	p-value
log(fucoxanthin+1)	Buffalo Pound	2.086e-06	4	0.000	0.597
log(fucoxanthin+1)	Wascana	1.712e+00	4	3.166	0.129
log(alloxanthin+1)	Buffalo Pound	9.073e-01	4	7.114	0.004
log(alloxanthin +1)	Wascana	8.370e-01	4	3.719	0.029
log(Chl b+1)	Buffalo Pound	2.203e-06	4	0.000	0.835
log(Chl b +1)	Wascana	7.519e-01	4	2.105	0.074
log((lutein + zeaxanthin) +1)	Buffalo Pound	9.341e-01	4	10.712	<0.000
log((lutein + zeaxanthin) +1)	Wascana	2.093e-06	4	0.000	0.782
log(canthaxanthin+1)	Buffalo Pound	1.409e-05	4	0.000	0.436
log(canthaxanthin+1)	Wascana	2.649e-06	4	0.000	0.476
log(echinone+1)	Buffalo Pound	8.460e-01	4	4.155	0.024
log(echinone +1)	Wascana	1.911e+00	4	6.750	0.015
Temperature	Buffalo Pound	1.567e-06	4	0.000 0	0.771
Temperature	Wascana	8.993e-01	4	6.403	0.006
log(SRP + 1)	Both	1.369e+00	4	2.998	0.099

1012